Effect of sorghum varieties and molasses addition on prussic acid content and of silage quality

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Abstract. The study was conducted to determine the effect of sorghum varieties and molasses addition in silage making on the physical quality, pH, dry matter, organic matter (OM), Fleigh score, and prussic acid content. The study was designed using Completely Randomized Design in the factorial pattern. The first factor was sorghum varieties (super and BMR), and the second factor was the level of molasses (0% and 4% molasses). The pH of silage with molasses (4.45±0.28) was significantly lower (P<0.05) than silage without molasses addition (4.65±0.26). The DM content (16,13±1,83) and Fleigh score (49,91±12,43) were significantly higher (P<0.05) on silage with molasses addition. It can be concluded that BMR sorghum silage resulted in better physical and chemical quality than super sorghum silage. The addition molasses on sorghum silage decrease the pH but increases OM content and Fleigh score. Ensilage process decreases the prussic acid content of sorghum forage. The combination of sorghum varieties and molasses addition was significantly different.

1. Introduction
Sorghum is a forage originating from East Africa and has a high biomass production, able to survive on marginal land, resistant to drought and inundation. There are various kinds of sorghum varieties, including super sorghum and brown midrib resistance (BMR) sorghum. Super sorghum is the first sweet sorghum variety that was released by the Indonesian Agency for Agricultural Research and Development [1]. BMR sorghum is a conventional sorghum variety of mutations. Sorghum has many advantages; however, sorghum contains toxic compounds called prussic acid or cyanogenic glycosides, which can lead to a decrease in livestock productivity. Cyanogenic glycosides that get in through the livestock body will go directly to the circulatory system, which can inhibit oxygen transport into the tissues for cell respiration. Therefore cyanogenic glycosides that get in through the livestock body should be restricted 2 to 4 mg/kg [2]. A treatment to reduce the prussic acid content in sorghum is by making it into silage. The fermentation process in making silage produces lactic acid by lactic acid bacteria and reduces the activity of clostridia. Molasses (a by-product of sugar beet and sugar cane) can increase the activity of lactic acid bacteria. During the ensiling process, the toxic compounds changed into non-toxic compounds in forage sorghum. Therefore, this study was conducted to determine the effect of sorghum varieties and molasses on the physical and chemical qualities of silage. Ensiling process is expected to reduce prussic acid levels and decrease pH and provide good silage quality.
2. Material and methods

2.1 Location
The research was carried out at the Laboratory of Forage and Pasture, Faculty of Animal Science, Universitas Gadjah Mada. Analysis of prussic acid content was conducted in the Laboratory of Food Chemistry and Biochemistry, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta.

2.2 Material
The material used consisted of Super-2 and BMR sorghum harvested after 60 days and molasses. The material used to test the prussic acid content is 0.1 N NaOH, and alkali picrate pH 11. The pieces of equipment used are a chopper, 80-micron PE plastic, Willey mill with a screen diameter was 1 mm, oven with a temperature of 55°C and 105°C, beaker glass, test tube, and spectrophotometer 200.

2.3 Methods
Super and BMR sorghum planted in regosol soil, fertilized using urea fertilizer at a dose of 200 kg/ha, 100 kg KCl / ha, SP36 100 kg/ha, and 5 tons of manure/ha harvested at 60 days. The study was conducted using Split Plot design (2 x 2). Sorghum varieties as main plot (super-2 and BMR), the addition of molasses as the sub-plot (molasses 0% and 4% DM basis), each treatment replicated three times so that there were 12 bags of silage. The fermentation process was done in 21 days. Parameter observed in this research including; physical and chemical. Physical quality conducted by five panelists based on a questionnaire that has made, including color score, texture, aroma, and mold. Chemical quality includes pH value [3], dry matter content [4], a decrease of dry matter content, organic matter content [4], a decrease of organic matter content, prussic acid content [5], fleigh score [6]. Data were analyzed using ANOVA (α=0.05); significant differences between treatments were tested with Duncan’s Multiple Range Test [7].

3. Result and discussion

3.1 Physical quality of silage
The results showed that BMR sorghum silage produces a darker color (P<0.05) than super. Based on the result of the research, the score of BMR silage was 2.77±0.51, and Super silage was 1.37±0.15. The differences in the color caused by the initial color of forage sorghum. The difference in sorghum varieties has no significant effect on texture, aroma, and mold of the silage. The best quality of silage has a crumbly texture and does not clot. Silage does not clot due to the addition of soluble carbohydrates so that the pH is decreasing and can inhibit the mold [8]. In the degradation of super and BMR sorghum, mold was found in all parts of the silage. This shows that there is no oxygen in the silo and the lactic acid bacteria that can ferment carbohydrates into lactic acid. Mold needs oxygen to multiply and grow. Mold can grow at pH 3 to 8, also at temperatures of 0 to 40 °C [9]. The growth of mold is inhibited along with the increased lactic acid concentration. In order to prevent the growth of fungi, silage is vacuumed and leave no air cavity in the silo. Silage that is too dry can increase mold growth because silage will be unable to become compact.

| Parameter            | Super     | BMR       |
|----------------------|-----------|-----------|
| Silage colors*       | 1.37±0.15 | 2.77±0.51 |
| Silage texture       | 1.00±0.00 | 1.00±0.00 |
| Silage aroma         | 2.07±0.30 | 2.13±0.37 |
| silage mold          | 1.00±0.00 | 1.00±0.00 |

*significantly different P< 0.05)
The level of molasses addition changed the color of the silage, silage with 4% molasses (2.27±1.01) was of a darker color (P<0.05) than silage without molasses (1.87±0.59). The color of molasses caused the color change in silage. Molasses had a blackish brown color because when molasses added to silage it causes discoloration of the silage to become darker. Forage-based silage has good quality when it has a green color, not brown or even black[10]. Molasses addition has no significant effect on texture, aroma, and mold of the silage. The average aroma of silage produced from each treatment is acid. The acid flavor arises due to an increase in the production of lactic acid by lactic acid bacteria. The increasingly acidic flavor changes in line with the lower of silage pH.

Table 2. Physical quality of sorghum silage with molasses addition

| Parameter      | 0%       | 4%       |
|----------------|----------|----------|
| Silage colors* | 1.87±0.59| 2.27±1.01|
| Silage texture | 1.00±0.00| 1.00±0.00|
| Silage aroma   | 2.27±0.33| 1.93±0.24|
| Silage mold    | 1.00±0.00| 1.00±0.00|

*significantly different P< 0.05)

3.2 Chemical quality of silage

The result showed that BMR (4.33±0.17) has a lower pH (P<0.05) than super (4.77±0.15). The low pH produced from BMR sorghum silage is because BMR sorghum varieties have a higher soluble carbohydrate content compared to super sorghum. The higher amount of soluble carbohydrates influences the increase of lactic acid production. The increasing of lactic acid causes a decreasing of silage pH value.

The dry matter content of BMR sorghum silage was 17.14 ± 0.74 %, higher (P<0.05) than super sorghum silage which was only 14.44 ± 0.47. The differences are caused by the dry matter content of BMR sorghum in fresh conditions higher than super. The results of the study showed that the difference in sorghum varieties had no significant effect (P>0.05) on the organic matter content. BMR sorghum silage had a higher fleigh score (P<0.05) than super sorghum silage. BMR fleigh score was 66.15±8.14, then super was 43.08±6.33. The difference in the values of fleigh scores on super and BMR sorghum silage were caused by pH and silage dry matter content.

The study showed that different sorghum varieties had significant effect (P<0.05) on the prussic acid content. Super sorghum had higher prussic acid content (71.94±12.36) than BMR sorghum (55.29±9.12). The prussic acid content of sorghum plants that are safe for livestock is 200 ppm in fresh condition [2]. The prussic acid content of sorghum influenced by sorghum varieties. This is caused by the stem and leaf ratio of the two sorghum varieties. Prussic acid or cyanide is abundant in leaves compared to stems. The amount of leaf production followed the amount of prussic acid. Super sorghum production can reach 28.75 tons/ha in the dry matter [1]. Sorghum production of BMR is lower than super sorghum, which is 2.8 to 9.77 tons/ha in the dry matter[11].

Table 3. Chemical quality silage of super and BMR sorghum with molasses addition

| Parameter                | Super       | BMR         |
|--------------------------|-------------|-------------|
| pH value*                | 4.77±0.15   | 4.33±0.17   |
| Dry matter content (%)*  | 14.44±0.47  | 17.14±0.74  |
| Organic matter content (%)| 85.98±0.62 | 85.26±1.91 |
| Fleigh score*            | 43.08±6.33  | 66.15±8.14  |
| Prussic acid content (ppm)*| 71.94±12.36| 55.29±9.12  |

*significantly different P< 0.05)

The difference in molasses addition decreased (P<0.05) the silage pH value. The best pH value was produced from sorghum silage with 4% molasses (4.45±0.28) while without molasses was 4.65±0.26. The decrease in pH value occurs along with the addition of molasses. Soluble arbohydrates contained
in molasses caused is the fermentation process, which then produces lactic acid. The good pH value for silage is 4.2 to 4.5[12].

The addition of 4% molasses resulted in a higher (P<0.05) dry matter content (16.13 ± 1.83) than silage without molasses addition (15.46 ± 1.25). The dry matter content of silage increases with increasing levels of molasses addition. The addition of silage additives in the form of molasses can increase the dry matter content and lactic acid and reduce the pH value and the level of ammonia [13]. The result of silage dry matter content is still relatively low. The dry matter content to produce good silage is in the range of 30 to 40% [14]. The low dry matter content can be caused by the wilting of sorghum, which is not optimal after harvesting. The wilting of sorghum, which is not maximal, causes the water content in the plant to be high, so the dry matter content is low. Ensilage can cause a decrease in the dry matter content of sorghum.

Table 4. Chemical quality of sorghum silage with molasses addition

| Parameter                        | 0%          | 4%          |
|----------------------------------|-------------|-------------|
| pH value*                        | 4.65±0.26   | 4.45±0.28   |
| Dry matter content (%)*          | 15.46±1.25  | 16.13±1.83  |
| Organic matter content (%)       | 84.99±1.76  | 86.25±0.54  |
| Fleigh score*                    | 49.91±12.43 | 59.32±14.77 |
| Prussic acid content (ppm)*      | 59.10±11.78 | 68.13±14.65 |
| *(significantly different P< 0.05) |             |             |

The addition of molasses has no significant effect (P>0.05) on silage organic matter. This proves that the variety or molasses addition does not influence the organic matter content. The organic matter content in sorghum after silage was decreased.

The difference in molasses addition significantly affected (P<0.05) the fleigh score. Increasing the number of fleigh scores is influenced by the level of molasses addition related to decreasing pH value and an increasing of dry matter content in silage. Silage is categorized as best quality silage if it produces a fleigh score of 85 to 100, good quality 60 to 80, sufficient quality 55 to 60, medium quality 25 to 40, and low quality if less than 20 [14]. Based on the category above, super sorghum silage is classified as medium quality silage, while BMR sorghum silage was classified as good quality silage. Silage without the addition of molasses produces medium-quality silage, while silage with the addition of 4% molasses was classified as silage with sufficient quality.

Making silage can reduce the prussic acid content of sorghum. The prussic acid content in Super sorghum decrease of more than 70% from 283.59 ppm (before silage) to 71.94 ppm (after silage). The prussic acid content in BMR sorghum decrease of more than 80% from 307.03 ppm (before silage) to 55.29 ppm (after silage). The reduction of HCN, which ranges from 28.2-32.8%, is equivalent to 1.19 kg of silage (as fed) that can be consumed by cattle [15]. The enzyme can reduce the prussic acid content of more than 60%. The process of reducing prussic acid content is by wilting, cutting, and compaction of forages. Ensilage in sorghum can reduce prussic acid levels more than 50%. This is related to activate enzymes that can remove free cyanide and release in the form of gases [16].

4. Conclusion
Based on the study that has been done it can be concluded that BMR sorghum silage produces silage with better physical and chemical qualities than super sorghum silage. BMR sorghum silage has a lower pH, organic matter, prussic acid, and higher dry matter and fleigh scores than super sorghum silage. The addition of 4% molasses to super and BMR sorghum silage can reduce pH, and increase the fleigh silage score. Ensilage in making silage from super and BMR sorghum can reduce the prussic acid content more than 70%.

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