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# Silage Quality of Sorghum Harvested at Different Times and Its Combination with Mixed Legumes or Concentrate Evaluated *in Vitro*

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## ABSTRACT

**The experiment was designed to evaluate the silage quality of sorghum forage varieties of Citayam and BMR 3.6 strain at different harvesting times and the effectiveness of a legumes addition as a concentrate substitute in sorghum forage silage-based diets on *in vitro* fermentability using rumen fluid of beef cattle. Experimental design for silage quality was completely randomized design with 2 x 3 factorial, i.e., forage sorghum types (Citayam and BMR 3.6) and time of harvesting the forage sorghum (85, 95, and 105 d). Experimental design for *in vitro* fermentability and digestibility was randomized block design with 2 x 2 factorial arrangement, i.e. types of ration (with 2 levels i.e., a mixture of legumes and concentrate) and types of sorghum forage silages (with 2 levels i.e., Citayam and BMR 3.6). All silages had a good odor, color, and texture. Silage of sorghum harvested at 105 d had better grades and was selected for *in vitro* studies. The treatment had no effect on pH and organic matter digestibility. BMR 3.6 based silage had greater values of $NH_3$, total VFA, rumen microbial population, methane, and dry matter digestibility. Substitution of concentrate with a mixture of legumes did not affect fermentability, microbe population and digestibility in the rumen. Silage of sorghum strain BMR 3.6 harvested at 105 d had a very good quality and mixing with legumes could replace concentrate in forage sorghum silage based diet on *in vitro* fermentability and digestibility using beef cattle rumen fluid.**

*Key words: BMR 3.6, Citayam, mixture of legume, silage, sorghum*

## ABSTRAK

**Tujuan penelitian ini ialah untuk mengevaluasi kualitas silase hijauan sorgum varietas Citayam dan galur BMR 3.6 pada umur panen yang berbeda dan mengevaluasi efektivitas campuran legum sebagai pengganti konsentrat dalam pakan berbasis silase hijauan sorgum secara *in vitro* menggunakan cairan rumen sapi potong. Rancangan percobaan untuk kualitas silase adalah rancangan acak lengkap faktorial 2 × 3, yaitu jenis hijauan sorgum (Citayam dan BMR 3.6) dan umur panen hijauan sorgum (85, 95, dan 105 hari). Rancangan percobaan untuk fermentabilitas dan kecernaan *in vitro* adalah rancangan acak kelompok faktorial 2 × 2, yaitu jenis ransum (campuran legum dan konsentrat) dan jenis silase hijauan sorgum (Citayam dan BMR 3.6). Semua silase memiliki aroma, warna, dan tekstur yang baik. Fermentabilitas silase sorgum umur panen 105 hari memiliki nilai yang lebih baik sehingga dipilih untuk percobaan *in vitro*. Perlakuan tidak berpengaruh pada pH dan kecernaan bahan organik. Pemberian BMR 3.6 meningkatkan nilai $NH_3$, VFA total, populasi mikroba rumen, produksi gas metan, dan kecernaan bahan kering. Penggantian konsentrat dengan campuran legum tidak mempengaruhi fermentabilitas, populasi mikroba, dan kecernaan di dalam rumen. Silase hijauan sorgum galur BMR 3.6 pada umur panen 105 hari memiliki kualitas yang sangat baik dan campuran legum dapat menggantikan konsentrat pada pakan berbasis silase hijauan sorgum secara *in vitro* menggunakan cairan rumen sapi potong.**

*Kata kunci: BMR 3.6, Citayam, campuran legum, silase, sorgum*

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## INTRODUCTION

Farm management should be able to establish and provide a source of protein for meeting livestock needs because it is the most important and expensive component in the ration. Protein sources commonly used in ruminants are concentrates of agricultural byproducts and plantation products. However, in Indonesia, there are areas with limited availability of concentrate, requiring enormous costs in the supply of concentrate. One of alternatives that can be used to replace the concentrate is legume forage that has high crude protein content.

Legume forage has a high crude protein content i.e., 20%-30% (McDonald *et al*., 2010) and excellent legume is used as ruminant feed. The use of seasonal and annual legume hay could improve the digestibility and synthesis of microbial nitrogen (Foster *et al*., 2009). In addition, the use of alfalfa, white and red clover legumes increased production of $NH_3$ and a mixture of grass sainfoin-legume could reduce the degradation of proteins and the production of methane (Niderkorn *et al*., 2011).

*Leucaena leucocephala* (lamtoro) leaves can be used as a source of protein and heat treatment of *L. leucocephala* could increase feed intake, nutrient digestibility, and rumen fermentability in swamp buffalo fed with ammoniated rice straw-based diet (Kang *et al*., 2012). *L. leucocephala* (tanniniferous legume) can replace *Vigna unguiculata* (low-tannin legume) in a complete feed without seriously affecting the characteristics of fermentation in the rumen (Hess *et al*., 2008). Condensed tannins (CT) of *L. leucocephala* was relatively low (15 mg CT/500 mg DM), reduced the production of $CH_4$ (methane) by 47%, but only 7% decreased in the degradation of feed dry matter (Tan *et al*., 2011). *Gliricidia* (G.) *sepium* has the potential to be utilized as ruminant feed supplement in Nigeria during the summer (Anele *et al*., 2009). *G. sepium* has a high crude protein (CP) content (23.2%), and could be given as a nitrogen supplement in Napier grass-based diet for cattle which increased lactation performances (Juma *et al*., 2006). *Indigofera* (I) *zollingeriana* has a good digestibility of nutrients for ruminants (Abdullah & Suharlina, 2010). *I. zollingeriana* has a crude protein and *in vitro* dry matter digestibility, respectively, 27.68% and 75.44% without fertilization and 31.31% and 85.50% by fertilization (Abdullah, 2010).

Sorghum (*Sorghum bicolor* L.) is a cereal plant type that has potential to be cultivated and developed in marginal and dry areas in Indonesia. Selection of sorghum as a major feed on marginal lands is the best solution in the supply of forage for ruminants. Jahanzad *et al*. (2013) stated that forage sorghum had higher productivity in the medium irrigation systems and low seed density. Sorghum also has a greater biomass than corn (Rocateli *et al*., 2012). The potential productivity of sorghum on marginal land as ruminant forages should also be supported by the types of seeds that have good quality.

Citayam and Brown midrib (BMR) 3.6 strains are genetically mutated sorghums that have superior agronomic traits. The harvest time of sorghum should be adapted to the purpose of production. There are differences in the nutrient contents of forage sorghum at the age of vegetative, early generative, until filling grain. Differences in harvest times will provide information about the nutritional values that can be used to determine a suitable harvest time of sorghum as a forage source. The abundance of sorghum production at certain harvest times needs a method of preservation to ensure the continuous availability of forage. Silage is a forage preservation method based on the lactic acid fermentation under anaerobic conditions.

Silage techniques can minimize the loss of nutrients from harvesting to storage. Lactic acid bacteria found in forages are involved in the fermentation of water-soluble carbohydrates into lactic acid and acetic acid. As a result, the pH of silage decreases and the activity of spoilage microbes can be inhibited. This condition will keep the silage remain well-preserved in the long term. Lactic acid bacteria with a population of $10^6$ CFU/g on silage will increase the stability of the silage after exposure to the air (7 d) and this condition contributes to the maintenance of nutritional value of silage from time to time (Tabacco *et al*., 2011) and inhibited the activity of undesirable microorganisms (Keles & Demirci, 2011). The addition of lactic acid bacteria such as *Lactobacillus plantarum* and water soluble carbohydrates in silage will improve silage quality (Lima *et al*., 2011) and maintain the protein during fermentation, as well as increases the growth of rumen microbes (Contreras-Govea *et al*., 2013).

Replacement of the concentrate with a mixture of legumes and sorghum forage silage is expected to be an alternative solution to the problems of ruminant livestock development on marginal lands. This study was designed to evaluate the silage quality of sorghum forage varieties of Citayam and strain of BMR 3.6 at different harvest times and the effectiveness of legume supplementation as a concentrate substitute in fermentability of sorghum forage silage-based diets *in vitro* by using rumen fluid of beef cattle as fermentation media.

## MATERIALS AND METHODS

### Silage Production

Sorghum forage Citayam and BMR 3.6 were harvested at 85, 95, and 105 days of planting. The whole sorghum forage (stem, leaf, and grain) was chopped to a theoretical length of 3-5 cm. *L. plantarum* (1A-2) inoculant ($1 \times 10^{10}$ CFU/mL) from the Indonesian Institute of Sciences (LIPI) in Cibinong was added 1% on each forage (1500 g each) and ensiled in jar silos of 1500 g. After 28 d, the silos were opened to observe physical characteristics such as silage odor, color, temperature, presence of fungi, and weight of silage. Part of silage was taken for observation of silage characteristics such as pH, proximate referring to the AOAC (2007), and the Fleigh points (Idikut *et al*., 2009). All variables of the silages were scored based on the average value of each treatment in order to obtain the best combination of silage. The best combination of silage will be selected as a source of forage in *in vitro* experiments.

### *In Vitro* Fermentation

*In vitro* fermentation and digestibility was conducted by using the method of Tilley & Terry (1963). Into each fermentation tube, 40 mL of McDougall buffer, 0.5 mg of treatment ration, and 10 mL of rumen fluid were added and the mixtures were incubated at 39°C. Fresh rumen fluid of fistulated beef cattle was obtained from Indonesian Institute of Sciences (LIPI). The treatment (Table 1) consisted of 30% isoprotein rations (concentrate or mixed legumes) and 70% sorghum forage silage for bulls with the weight of 250 kg and weight gain 0.75-1.00 kg/d containing 10.69%-11.69% CP and 57.33%-66.67% Total Digestible Nutrients (TDN) (Kearl, 1982). Samples were taken after 4 h incubation for analysis of pH, partial VFA using gas chromatography (GC) and gas production of methane (Moss *et al.*, 2000), $NH_3$ concentration with micro diffusion Conway method, microbial populations (Ogimoto & Imai, 1981), and after 48 h incubation for dry matter and organic matter digestibility analysis.

### Statistical Analysis

The experimental design for evaluating silage quality was completely randomized design with a 2 × 3 factorial, i.e., sorghum forage types (Citayam and BMR 3.6) and time of harvesting sorghum forage (85, 95, and 105 d). Experimental design for *in vitro* fermentability and digestibility was a randomized block design with 2 x 2 factorial, i.e., types of ration mixtures of legumes (*L. leucocephala, G. sepium,* and *I. zollingeriana)* and concentrates (rice brand, tofu waste, urea, and premix) and type of sorghum forage silage (Citayam and BMR 3.6) with 3 replications. The data obtained were analyzed by using analysis of variance (ANOVA). Duncan multiple range test was used to test the significant interaction. If there was significant effect of the main factor (sorghum forage, ration or silage types), the data was examined with T test. Polynomial orthogonal was used to determine the effect of harvest time (Steel & Torrie, 1997).

Table 1. Nutrient composition of experimental feed (dry matter basis) with 70% sorghum silage and 30% ration mixture

| Nutrients (%) | Citayam | | BMR 3.6 | |
|---|---|---|---|---|
| | Mixed legumes | Concentrate | Mixed legumes | Concentrate |
| Ash | 6.91 | 6.20 | 5.99 | 5.37 |
| Crude protein (CP) | 11.20 | 11.48 | 11.96 | 11.47 |
| Crude fiber (CF) | 27.99 | 29.95 | 27.40 | 27.59 |
| Ether extract (EE) | 1.51 | 3.51 | 2.06 | 3.73 |
| Nitrogen free extract (NFE) | 51.89 | 48.78 | 52.09 | 48.92 |
| Total digestible nutrient (TDN)* | 61.34 | 62.37 | 62.97 | 62.23 |

Note: * % TDN for silage= -72.943 + 4.675 (CF) – 1.28 (EE) + 1.611 (NEF) + 0.497 (CP) – 0.044 (CF)2 – 0.76 (EE)2 – 0.039 (CF) (NFE) + 0.087 (EE) (NFE) – 0.152 (EE) (CP) + 0.074(EE)2 (CP); % TDN for Ration = -133.726 + 0.254 (CF) + 19.593 (EE) + 2.784 (NEF) + 2.315 (CP) + 0.028 (CF)2 – 0.341 (EE)2 – 0.008 (CF) (NFE) - 0.215 (EE) (NFE) – 0.193 (EE) (CP) + 0.004(EE)2 (CP) Hartadi *et al*. (1980).

## RESULTS AND DISCUSSION

### Characteristics and Nutrients Quality of Silages

All silages had good odor, color, and texture. These parameters indicated that the silage fermentation was conducted very well. Based on Table 2, silage had a low pH at 3.78. Different types of sorghum and harvesting times affected the pH value. All types of sorghum showed good pH values when harvested above the age of 95 d ($P < 0.05$). The quality of silage fermentation can also be seen from the Fleigh points: different types of sorghum and harvest time affected the Fleigh points ($P < 0.05$). Fleigh points denote that values between 85 and 100, very good quality; 60 and 80, good quality; 55 and 60, moderate quality; 25 and 40, satisfying quality; <20, worthless (Idikut *et al.*, 2009). The high value of Fleigh points showed a good level of fermentation, all types of sorghum harvested over the age of 95 d had Fleigh points above 85. The content of silage CP was affected by the type of sorghum and age of harvest ($P<0.01$). The content of silage crude fiber (CF) was affected by the interaction between the type of sorghum and harvesting age ($P<0.05$). The content of TDN was affected by the type of sorghum ($P<0.01$) and harvesting age with quadratic curve ($TDN= 84.732-0.717A + 0.004A^2$) based on polynomial test ($P<0.05$).

In the beginning of fermentation process, there was a high microbial diversity until the end of the silage process. Microbial diversity was dominated by lactic acid bacteria; one is *L. plantarum*. Lactic acid bacteria contained in the silage decreased the pH of the silage (Ridwan *et al.*, 2015). A low pH value in all silages was due to the effect of the addition of lactic acid bacteria i.e., *L. plantarum*. The use of *L. plantarum* in silage fermentation improved the quality, reduced pH, increased lactic acid content, and inhibited the growth of undesirable microbes such as fungi, coliform bacteria, and clostridia after 30 and 60 days period of storage (Tohno *et al.*, 2012). Yuan *et al.* (2015) stated the addition of inoculants *L. plantarum* in the total mixed ration silage decreased the pH more than the other additives. Sorghums that were older than 95 d belong to the generative phase, a process of filling and ripening of grain. Forage sorghum silage used in this study, one of them older than 95 d, so it had grain. Grains have high carbohydrate content. The availability of carbohydrates as a substrate for lactic acid bacteria to produce organic acids especially lactic acid (Emanuel *et al.*, 2005) which cause the pH to decrease and inhibit the development of butyric bacteria. Inoculant should be given to forage sorghum with older ages to produce a better silage (Thomas *et al.*, 2013).

Citayam had lower CP than BMR 3.6. Longer harvest time decreased CP of silage. BMR 3.6 harvested at the age of 85 d had the best CP content compared with other combinations. BMR 3.6 harvested more than 85 d had the same quality as Citayam harvested at 85 d. Abdelhadi & Tricarico (2009) stated that harvesting sorghum in milk stage would increase the content of CP.

CF of BMR 3.6 was the lowest compared with other combinations. CF of Citayam was higher than the BMR

3.6. BMR 3.6 is a type of sorghum mutation that is selected for animal feed because of a lower content of CF. Based on Miron *et al*. (2005), the fraction of CF is primarily lignin. In BMR silage, the lignin was smaller than the other type of sorghum.

BMR 3.6 had greater TDN than the Citayam and this fact could be due to the content of the CF of Citayam that was higher than the other. The high content of CF was the inhibitor factor of digestibility. Harvesting sorghum at the age of more than 90-105 d after planting could increase TDN. Pereira *et al*. (2007) stated that an increase in the TDN content of sorghum silage-based rations could be related to an increase in carbohydrate content. At the age of 90 d, sorghum is in the early phases of the formation of grain that is a source of carbohydrate.

### *In Vitro* Fermentability

The average pH, ammonia ($NH_3$), and total volatile fatty acids (VFA) concentrations are presented in Table 3. The type of sorghum at harvest time of 105 days and the replacement of concentrate with mix legumes did not affect rumen pH. The concentration of $NH_3$ was only influenced by the type of sorghum silage ($P<0.01$). The concentration of VFA was also only influenced by the type of sorghum silage ($P<0.01$). Mean concentrations of partial VFA, the ratio of acetic : propionic and methane are presented in Table 4. Rations only affected the concentration of acetic acid ($P<0.05$), butyric acid ($P<0.01$), and the ratio of acetic acid: propionic acid ($P<0.05$). Sorghum types affected the production of methane gas ($P<0.05$).

Rumen pH determines the rumen condition that affects the growth of rumen microbes and rumen fermen-

Table 2. Characteristics and nutrient content of silages with different types of sorghum harvested at different time

| Variables | Sorghum types | Harvested time (d) | | | Mean of sorghum types |
|---|---|---|---|---|---|
| | | 85 | 95 | 105 | |
| pH | Citayam | 4.08±0.09$^{b}$ | 3.48±0.11$^{a}$ | 3.65±0.04$^{a}$ | 3.74±0.28 |
| | BMR 3.6 | 4.38±0.22$^{c}$ | 3.49±0.01$^{a}$ | 3.61±0.02$^{a}$ | 3.83±0.43 |
| | Mean of harvest age | 4.23±0.22 | 3.48±0.07 | 3.63±0.04 | |
| Dry matter | Citayam | 93.98±0.53 | 91.80±0.47 | 90.89±0.49 | 92.23±1.59$^{b}$ |
| | BMR 3.6 | 93.43±1.68 | 91.00±0.47 | 90.46±0.59 | 91.63±1.59$^{a}$ |
| | Mean of harvest age | 93.71±0.39 | 91.40±0.75 | 90.67±0.31 | 91.93±1.46 |
| Fleigh points | Citayam | 74.13±5.35$^{b}$ | 98.98±5.03$^{c}$ | 96.72±1.18$^{c}$ | 89.94±12.47 |
| | BMR 3.6 | 58.21±6.65$^{a}$ | 94.45±1.14$^{c}$ | 93.15±1.36$^{c}$ | 81.93±18.13 |
| | Mean of harvest age | 66.17±10.25 | 96.71±4.10 | 94.93±2.26 | |
| Crude protein (%) | Citayam | 7.56±0.29$^{C}$ | 4.77±0.33$^{A}$ | 6.35±0.47$^{B}$ | 6.23±1.40 |
| | BMR 3.6 | 9.30±0.55$^{D}$ | 7.97±0.24$^{C}$ | 7.45±0.54$^{C}$ | 8.24±0.95 |
| | Mean of harvest age | 8.43±1.23 | 6.37±2.26 | 6.90±0.78 | |
| Crude fiber (%) | Citayam | 35.04±1.24$^{d}$ | 35.40±2.46$^{d}$ | 34.56±1.45$^{cd}$ | 35.00±0.42 |
| | BMR 3.6 | 32.79±1.06$^{bc}$ | 29.65±0.79$^{a}$ | 31.95±1.77$^{b}$ | 31.46±1.63 |
| | Mean of harvest age | 33.91±1.59 | 32.52±4.07 | 33.25±1.84 | |
| TDN (%) | Citayam | 53.90±0.71 | 54.36±2.22 | 54.88±1.29 | 54.38±0.49$^{A}$ |
| | BMR 3.6 | 55.16±1.18 | 55.16±1.10 | 57.82±1.81 | 56.05±1.53$^{B}$ |
| | Mean of harvest age | 54.53±0.89 | 54.76±0.57 | 56.35±2.08 | |

Note: Means with different capital superscripts differ significantly ($P<0.01$); means with different superscripts differ significantly ($P<0.05$).

Table 3. *In vitro* fermentability of different types of sorghum silage and ration

| Variables | Rations | Types of sorghum silage | | Mean of rations |
|---|---|---|---|---|
| | | Citayam | BMR 3.6 | |
| pH | Mixed legumes | 6.70±0.00 | 6.70±0.00 | 6.70±0.00 |
| | Concentrate | 6.70±0.00 | 6.70±0.00 | 6.70±0.00 |
| | Mean types of sorghum silage | 6.70±0.00 | 6.70±0.00 | |
| $NH_3$ (mM) | Mixed legumes | 10.92±0.79 | 11.64±0.23 | 11.28±0.50 |
| | Concentrate | 10.82±0.71 | 12.22±0.82 | 11.52±0.99 |
| | Mean types of sorghum silage | 10.87±0.07$^{A}$ | 11.93±0.41$^{B}$ | |
| Total VFA (mM) | Mixed legumes | 52.20±1.92 | 59.28±4.53 | 55.74±5.00 |
| | Concentrate | 54.33±1.92 | 56.51±2.01 | 55.42±1.54 |
| | Mean types of sorghum silage | 53.27±1.51$^{A}$ | 57.90±1.96$^{B}$ | |

Note: Means in the same row with different superscripts differ significantly ($P<0.01$).

tation products. Rumen buffering capacity is supported by bicarbonate and phosphate salts that are able to maintain the pH at a level of 6-7. Results of *in vitro* study by Amer *et al*.( 2012) on the two types of sorghum silage have no effect on rumen pH (6:49 to 6:53).

$NH_3$ concentration on BMR 3.6 was greater than that of the Citayam. CP and TDN contents of the BMR 3.6 were higher and lower in CF than Citayam. $NH_3$ production (6-21 mM) depends on the solubility of dietary protein, the amount of dietary protein, the length of the feed in the rumen, and rumen pH (McDonald *et al*., 2010). The process of protein degradation into amino acids occurs outside the cell, whereas the process of amino acids degradation into ammonia occurs in the microbial cells. Addition of high contents of free amino acids from grass silages increased the concentration of $NH_3$ (Gresner *et al*., 2015).

For ruminants, the main source of energy is VFA originating from the fermentation of carbohydrate by microbes in the rumen. Most of the materials are digested in the rumen and produce short chain fatty acids called VFA that are absorbed from the rumen wall to the circulation. Total VFA concentration of BMR 3.6 was higher indicating that the sorghum silage was more easily degraded in the rumen, but the total VFA produced was still below normal level (70-150 mM) (McDonald *et al*., 2010). Total VFA of sorghum silage reported by Amer *et al*. (2012) is also below normal i.e., 44.7-58.5 mM.

Molar proportions of VFA are 0.65 acetic acid, 0.21 propionic acid, and 0.14 butyric acid depending on the type of feed consumed by the cattle. Acetic acid is produced in large quantities, about 20-50 mol/d and propionic acid is usually produced one-third of acetic acid (McDonald *et al*., 2010). Rations and sorghum types had no effect on the concentration of propionic acid. The concentration of acetic acid in mixed legumes was higher than in concentrates. Fibrous feed would produce more acetic acid proportion while feed containing more easily fermentable carbohydrate such as concentrate would produce more propionic acid. The level of acetic acid found in this study was below standard, while the level of propionic acid was above the standard. Concentrate part of the ration produced higher butyric acid than mixed legumes because the content of CF and NFE of concentrate was lower than mixture of legumes, but the average concentration of butyric acid was still below normal. Fermentation of forage produces a larger ratio of A:P than that of concentrate. Mixed legumes yielded a greater ratio of A:P than concentrate.

BMR 3.6 produced more methane gas than the Citayam. Moss *et al*. (2000), stated that the acetic acid and butyric acid were precursor of $CH_4$ production, but the formation of propionic acid could reduce the production of $CH_4$ by re-channeling hydrogen gas in the rumen. The estimate of methane gas produced was relatively lower because propionic acid production was twice higher than normal. High concentrations of propionic acid could also be due to the content of lactic acid in the sorghum forage silage and bacteria *Propionibacterium* contained in the rumen. Propionic acid can be produced from sugar through lactic acid as an intermediate by *Propionibacterium* species. *Propionibacterium* can utilize lactic acid as a substrate faster than glucose (Tyree *et al*., 1991). Chen *et al*. (2012) suggested that the use of fibrous material plants as bioreactors, such as bagasse sugarcane, enhanced the production of propionic acid by *Propionibacterium freudenreichii*. The sorghum stem has similarities with bagasse sugarcane.

**Dynamics of Rumen Microbes**

Ration ($P<0.05$) and the type of sorghum ($P<0.05$) had significant effect on the population of protozoa in the rumen (Table 5). There was an interaction between rations and the type of sorghum for total bacterial population ($P<0.05$).

Tabel 4. Molar proportion of VFA, A:P ratio, and methane with different types of sorghum silage and ration

| Variables | Rations | Types of sorghum silage | | Mean of rations |
|---|---|---|---|---|
| | | Citayam | BMR 3.6 | |
| Acetic acid | Mixed legumes | 47.77±1.10 | 47.42±1.31 | 47.60±0.24[b] |
| (% mM) | Concentrate | 46.19±2.06 | 45.47±2.28 | 45.83±0.51[a] |
| | Mean types of sorghum silage | 46.98±1.12 | 46.45±1.38 | |
| Propionic acid | Mixed legumes | 31.04±0.37 | 30.39±0.19 | 30.72±0.46 |
| (% mM) | Concentrate | 31.09±0.70 | 30.48±0.56 | 30.78±0.43 |
| | Mean types of sorghum silage | 31.06±0.04 | 30.43±0.06 | |
| Butyric acid | Mixed legumes | 13.57±0.14 | 13.92±0.28 | 13.74±0.25[A] |
| (% mM) | Concentrate | 14.83±0.44 | 15.32±0.84 | 15.07±0.35[B] |
| | Mean types of sorghum silage | 14.20±0.89 | 14.62±0.99 | |
| Ratio A: P | Mixed legumes | 1.54±0.05 | 1.56±0.03 | 1.55±0.02[b] |
| | Concentrate | 1.49±0.10 | 1.49±0.01 | 1.49±0.00[a] |
| | Mean types of sorghum silage | 1.51±0.04 | 1.53±0.05 | |
| Methane | Mixed legumes | 9.61±0.64 | 10.98±0.43 | 10.29±0.97 |
| (mM) | Concentrate | 9.86±1.03 | 10.28±0.90 | 10.07±0.30 |
| | Mean types of sorghum silage | 9.73±0.18[a] | 10.63±0.49[b] | |

Note: Means with different capital superscripts differ significantly ($P<0.01$); means with different superscripts differ significantly ($P<0.05$).

Giving mixed legumes improved protozoa population as compared to concentrates and BMR 3.6 improved protozoa population as compared to Citayam. All rations containing BMR 3.6 had better total bacterial population than those containing Citayam. The high population of protozoa and bacteria produced by BMR 3.6 in this study was due to the higher digestibility of BMR 5.6 than that of Citayam (Table 5). Total bacteria and protozoa in the normal range were $10^9$-$10^{10}$ CFU/mL and $10^6$ cells/mL. Fermentability level and digestibility of the ration are determined by the activity and the dynamics of microbes in the rumen. Better conditions in the rumen, improves dynamics and microbial activity in the rumen (McDonald *et al*., 2010).

Concentrate decreased protozoa population as compared with mixed legumes. This result could be related to the higher crude fat content in rations using concentrate (Table 1). The high crude fat in concentrate was contributed by the rice bran which had high fat content. Parrado *et al*. (2006) stated that the extract of rice bran had a fat component of 30% with oleic and linoleic acid as the major components. Abubakr *et al*. (2013) stated that the use of oils and fatty acids would be toxic to rumen protozoa. In line with the results of his research, palm byproduct lowered the population of protozoa. Wanapat & Khampa (2006) stated the addition of fat from palm oil was able to reduce the population of the rumen protozoa.

### Dry and Organic Matters Digestibilities

Type of sorghum had effect on dry matter digestibility (P<0.05), and all treatments had no effect on organic matter digestibility (Table 6). BMR 3.6 had a greater digestibility as compared with Citayam. The increase in digestibility of BMR 3.6 was due to the lower lignin content as compared to Citayam. In BMR silage, the fraction of CF was primarily lignin and the lignin was lower as compared to the other type of sorghum (Miron *et al*., 2005). Carmi *et al*. (2006) stated that in all cases a decrease in lignin content in plant organs increased the dry matter digestibility. The high *in vitro* digestibility of BMR sorghum forage silage could be seen from the high degradation of nutritional content and the low of lignin content (Miron *et al*., 2007).

Sorghum silage digestibility was still relatively lower; it could be due to the proportion of diet containing sorghum forage silage form the age of 105 d (ages of maturation) that was up to 70%. Zhang *et al*. (2015) stated the addition percentage of sweet sorghum silage in the feed decreased dry and organic matter digestibility. The addition of sweet sorghum silage up to 60%-80% combined with legume alfalfa silage 40%-20% resulted *in vitro* dry and organic matter digestibilities ranged from 49%-52% and 55%-58%, respectively.

At the ages of maturation, the process of cellulose synthesis is in progress thereby increasing cellulose content. This increase relates to the establishment of a secondary walls, rich in cellulose in the stem and leaf tissue (Carmi *et al*., 2006), resulting in lower digestibility of forage. In the study of Di Marco *et al*. (2009), the digestibility of sorghum forage silage of BMR harvested at 110 d after planted was 57% after 48 h of incubation; the result was still relatively low.

Table 5. Rumen microbial populations treated with sorghum silage type and ration

| Variables | Rations | Types of sorghum silage | | Mean of rations |
|---|---|---|---|---|
| | | Citayam | BMR 3.6 | |
| Protozoa log cell/mL | Mixed legumes | 5.25±0.33 | 5.28±0.30 | 5.26±0.02[b] |
| | Concentrate | 5.20±0.32 | 5.25±0.30 | 5.22±0.04[a] |
| | Mean types of sorghum silage | 5.22±0.04[a] | 5.27±0.02[b] | |
| Bacteria log CFU/mL | Mixed legumes | 9.29±0.05[a] | 10.46±0.62[b] | 9.88±0.83 |
| | Concentrate | 9.35±0.04[a] | 10.35±0.58[b] | 9.85±0.71 |
| | Mean types of sorghum silage | 9.32±0.04 | 10.41±0.08 | |

Note: Means in the same row with different superscripts differ significantly (P<0.01).

Tabel 6. *In vitro* dry and organic matter digestibility with different types of sorghum silage and ration

| Variables | Rations | Types of sorghum silage | | Mean of rations |
|---|---|---|---|---|
| | | Citayam | BMR 3.6 | |
| IVDMD (%) | Mixed legumes | 51.34±6.07 | 55.69±4.73 | 53.51±3.07 |
| | Concentrate | 50.74±0.75 | 55.04±0.68 | 52.89±3.04 |
| | Mean types of sorghum silage | 51.04±0.42[a] | 55.36±0.45[b] | |
| IVOMD (%) | Mixed legumes | 43.91±6.88 | 46.24±6.88 | 45.08±3.07 |
| | Concentrate | 43.90±1.00 | 46.71±1.00 | 45.30±3.04 |
| | Mean types of sorghum silage | 43.90±0.42 | 46.48±0.45 | |

Note: Means in the same row with different superscripts differ significantly (P<0.01). IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility

## CONCLUSION

Based on silage quality, *in vitro* fermentability and digestibility studies by using rumen fluid of beef cattle, sorghum forage silage strain of BMR 3.6 harvested at 105 d had a very good quality of silage and mixed legumes could replace concentrate on sorghum forage silage-based diets. Mixed legumes did not influence the fermentability, microbial activity, and digestibility in the rumen.

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