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The *In Vitro* Digestibility of Complete Silage From *Sorghum bicolor* (L.) Moench Ingredient using Different Additives

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ABSTRACT

This study aimed to determine the *in vitro* nutrient digestibility of complete silage from *Sorghum bicolor* (L.) Moench using different additives. The method used was a completely randomized design containing 4 treatments and 4 replications, namely, R1: *Sorghum bicolor* (L.) Moench + *L. leucocephala* (without additives/control), R2: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + 20% rice bran + 10% rock sugar, R3: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + 20% corn meal + 10% rock sugar, R4: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + 20% pollard + 10% rock sugar. The additive material percentage was calculated based on the chopped weight of *Sorghum bicolor* (L.) Moench and *L. leucocephala*. The data obtained were assessed by the analysis of variance, and then continuously analyzed by the Duncan's multiple range test. The *Sorghum bicolor* (L.) Moench plants were harvested on 70 days after planting and withered for 3 hours to reduce the moisture content, and then chopped at 3 cm size. The chopped results were mixed with the additive materials based on the treatments and their percentages applied, and then moved to the plastic container (silo), the mixture that was moved into the silo was suppressed to make the chopped layer solid (anaerobic principal). Ensilage process was stood for 21 days. The complete silage was removed, and its *in vitro* nutrient digestibility was analyzed. The results showed that the use of additives could improve the dry matter, organic matter, N-NH_3, and VFA digestibility of complete silage from *Sorghum bicolor* (L.) Moench. It can be concluded that the additive supplementation of 20% pollard and 10% rock sugar can improve the dry matter, organic matter, N-NH_3, and VFA digestibility of complete silage from *Sorghum bicolor* (L.) Moench.

Keywords: Additive, Complete silage, *In vitro* digestibility

Introduction

One problem that quite influences the animal stock productivity is the feed availability, especially in dry land region. Feed will be abundant during short rainy season (3-4 months), while minimum feed availability occurs during long dry season (8-9 months). This condition will impact on the unstable stock productivity that is quite fluctuating along with the seasonal change. Therefore, an alternative way to deal with this problem is necessary; one of which is by utilizing the abundant feed availability during the rainy season for being used during the dry season through feed preservation (silage). Silage is fresh green materials that are preserved in an anaerobic condition. The plant that can be utilized and integrated as feed for ruminants is *Sorghum bicolor* (L.) Moench. Several advantages of *Sorghum bicolor* (L.) Moench are wide adaptive capability, drought resistance, high productivity, quite high nutrient contents, and pest resistant (Siregar *et al*., 2002). Qualified silage through an optimized fermentation process can be obtained with additive materials to accelerate the ensilage process (Riswandi *et al*., 2015), accelerate lactic acid formation, prevent from excessive fermentation, accelerate pH reduction, and supplement the deficient nutrient and green ingredients used (Hidayat, 2014). Additives are used to improve the nutrient or carbohydrate contents in feed that can fulfill the stock nutrient requirement. Feed quality can be identified based on the feed digestibility. Feed digestibility analysis method in ruminants can be measured by the *in vitro* method. The *in vitro* method is a metabolism process that occurs outside the stock body. The principal and condition are similar to the process occurred inside the stock body, including the metabolism process in rumen and abomasum.

Materials and Methods

Location, equipment, and material

This study was performed in the Faculty of Agriculture, University of Timor, while the *in vitro*

digestibility test was performed in the Laboratory of Dairy Animal Nutrition, Bogor Agricultural University. The equipments used were plastic container in 3 L capacity, cutting tools, analytical scale, writing tools, and *in vitro* digestibility test tools. The materials contained *Sorghum bicolor* (L.) Moench, *L. leucocephala*, rice bran, corn meal, pollard, and rock sugar. The silage ingredient nutrient composition can be seen in Table 1.

Methods

The method used was a completely randomized design with 4 treatments and 4 replications, namely, RI: *Sorghum bicolor* (L.) Moench + *L. leucocephala* (without additives/control), R2: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + 20% rice bran + 10% rock sugar, R3: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + 20% corn meal + 10% rock sugar, R4: *Sorghum bicolor* (L.) Moench + *L. leucocephala* + pollard 20% + 10% rock sugar. The additive material supplementation percentage was calculated based on the chopped sorghum and *L. leucocephala* weights.

Silage production procedure and data sampling

The *Sorghum bicolor* (L.) Moench plants were harvested on 70 days after planting (DAP) and withered for 3 hours to reduce the moisture content, and then chopped at 3 cm size. The chopped results were mixed with the additive materials based on the treatments and their percentages applied, and then moved to the plastic container (silo), the mixture that was moved into the sylo was suppressed to make the chopped layer solid (anaerobic principal). Ensilage process was stood for 21 days. Silo was opened for nutrient content analysis (Table 2). After 21 days, silo was opened, sampled, and moved to an oven at 60ºC for 2 days, and then continued with the *in vitro* digestibility value analysis.

Variables

The observed variable was the *in vitro* digestibility value using two phases method of Tilley and Terry (1963):

First phase:

- Weighing 0.5 gram of silage sample, then moving it into the centrifuge tube and heating it in an incubator/water bath at 39-41^{0}C.
- Preparing 4 L of buffer solution and heating in the water bath at 38-39^{0}C.
- Mixing 4 L of buffer solution and 1 L of rumen liquid flown with CO_2 gas in the stirred storage flask flown with CO_2 gas.
- Adding 50 mL of rumen liquid and buffer mixture in the centrifuge tube filled with a sample using automatic sprayer machine and directly closing it with a rubber valve plug quickly, while shaking and moving it to the water bath at 38-39^{0}C.
- Making blank following the similar way without adding the silage sample.
- Terminating the microbial activity after 48 hours by adding 5 mL of Na_2CO_3 solution (in each tube)
- Evaporating the undigested sample fraction using a centrifuge at 2500 rpm for 15 minutes.
- Filtering the supernatant liquid after 15 minutes of centrifugation carefully to the nylon fabric by vacuum pump machine.

Second phase:

- Flowing the attached particle on nylon fabric to the centrifuge tube with HCL-PEPSIN solution and putting back in the water bath without CO_2 gas flowing (aerobic situation) and valve Bunsen plug.
- Shaking the centrifuge tube twice during 48 hours of incubation
- Centrifuging the tube after 48 hours of incubation at 2500 rpm for 15 minutes.
- Filtering the pellet in the centrifuge tube using a thermal alumina filter, which was previously heated at 550^{0}C for 1.5 hours in the furnace, then measuring it. Residue attached on the filter dish.
- Drying the filter dish and residue overnight at 103^{0}C, then measuring the residue
- Burning the residue in the furnace at 550^{0}C for 1.5 hours and measuring the burnt residue along with the dish.

The dry and organic matter digestibility coefficients were calculated with the formula:

$$DMD = \frac{Initial\ DM\ (Residual\ DM - Blank\ DM)}{Initial\ DM} x\ 100\%$$

Note :
DM = Dry matter
DMD = Dry matter digestibility (%)
Initial DM = Sample weight times % dry weight/100 (g)
Residual DM = Dry weight after gas production (g)
Blank DM = Dry weight after gas production (Blank) (g)

$$OMD = \frac{Initial\ OM\ (Residual\ OM - Blank\ OM)}{Initial\ OM} x\ 100\%$$

Note:
OM = Organic matter
OMD = Organic matter digestibility (%)
Initial OM= Sample weight times % organic weight/100 (g)
Residual OM = Organic weight after gas production (g)
Blank OM = Organic weight after gas production (Blank) (g)

Total VFA production measurement

The supernatant obtained from the centrifugation results were taken 5 mL and distributed to the reaction tube. 1 mL of H_2SO_4 was added to the supernatant and preserved in the refrigerator. The preserved supernatant was taken 2 mL and distilled in the VFA destilator until

obtaining water vapor at 100 mL. After adding 3 drops of phenolphthalein indicator and titrating with 0.01 N NaOH until turning to pink color, the NaOH titration volume was notified, and the VFA concentration was calculated by the formula:

$$VFA = \frac{(Titration\ volume\ x\ NNaOH\ x\ 100)}{2\ ml\ sample} x\ Dilution$$

Note:
Dilution = Total supernatant volume and preserved NaOH per total supernatant taken (6/5) ml

N-NH_3 production measurement

The N-NH_3 content was determined using a micro diffusion method in the Conway dish. 1 mL of supernatant was placed in the left of Conway dish, and 1 mL of NaOH was placed near the right part. A small center of the dish was filled with a boric acid, methyl red indicator, and brom cresol green indicator at 1 mL. The Conway dish was closed with Vaseline cover and shaken until the supernatant was mixed with NaOH solution. The dish was stood for 2 hours at room temperature. The bonded ammonia with a boric acid was titrated with 0.005 N H_2SO_4 until turning to reddish color. The N-NH_3 content was calculated with the following formula:
N-NH_3 = (mL titration x N H_2SO4 x 1000) mM

Data analysis

The data were analyzed using an analysis of variance (ANOVA) and continued with a Duncan's multiple range test to identify a significant difference. The data analysis used *IBM SPSS 19.0 version* software.

Results and Discussion

Dry matter is an estimation of the carbohydrate level contained in feed ingredient as 50-80% of dry matter is carbohydrates. The highest average of dry matter digestibility (DMD) (Table 3) was obtained from the R4 treatment (71.34±0.65[a]), followed by R3 treatment (62.61±0.60[b]), R2 treatment (40.85±0.42[c]), and R1 treatment as the lowest average of DMD (36.62±0.75[d]). The analysis of variance results showed that the treatments applied significantly influenced (P<0.05) the *in vitro* DMD. Difference in each treatment was caused by different nutrient availability among treatments and microorganism capability to degrade the nutrient contents in the different complete silages. The DMD value in the R4 treatment indicated that the nutrient contents in this treatment, specifically protein, was also higher (Table 1), which impacted on high digestibility. The crude protein content in pollard (Table 1) also greatly influenced the DMD value. High and low feed ingredient digestibility value provides the amount of digested feed ingredient nutrient contents in the digestive tract (Indrayani *et al*., 2015). The nutrient value from this complete silage could induce the rumen microorganisms to degrade feed, as one condition that mainly influenced the feed digestibility depended on the rumen microorganism activity. This condition was strengthened by Valdes *et al*. (2015) that the fermented feed ingredient nutrient contents could also influence the microorganism activity.

The average of *in vitro* OMD in each treatment obtained 34.11±1.67[d] in control, 39.59±0.52[c] in rice bran, 61.92±0.87[b] in corn meal, and 70.36±066[a] in pollard. The continuous test showed that each treatment was significantly different. This condition was caused as DMD in the four treatments had a significant different. Most organic matters were dry matter components; therefore the organic matter digestibility was as same as the dry matter digestibility (Tillman *et al*., 1991; Suwignyo *et al*., 2016). High OMD value in the complete silage supplemented with additives describes that the feed nutrient contents are abundant, which can be

Table 1. The nutrient contents of complete silage from *Sorghum bicolor (L.)* Moench ingredient

Nutrient	Feed Ingredient				
	Sorghum	*L. leucocephala*	Rice bran	Corn meal	Pollard
Dry matter (%)	90.09	92.90	35.03	85.63	88.06
Organic matter (%)	88.82	84.08	69.74	83.16	84.56
Crude protein (%)	10.54	25.06	12.30	9.24	17.04
Crude fiber (%)	23.32	18.37	12.49	3.44	7.11
Ether extract (%)	2.55	5.51	2.25	4.64	4.72
Nitrogen-free extract	90.09	92.90	35.03	85.63	88.06

Data were obtained from the results of the Laboratory of Feed Chemistry research, Faculty of Animal Husbandry, Nusa Cendana University

Table 2. The nutrient contents of complete silage from *Sorghum bicolor (L.)* Moench ingredient using different additives

Nutrient	Treatment			
	R1	R2	R3	R4
Dry matter (%)	40.74	36.91	35.03	35.80
Organic matter (%)	69.40	70.43	69.74	70.62
Crude protein (%)	10.33	13.81	12.30	14.02
Crude fiber (%)	21.30	20.68	12.49	16.33
Ether extract (%)	2.51	2.87	2.25	2.69
Nitrogen-free extract	40.47	54.85	56.46	53.95

R1: *Sorghum bicolor (L.) Moench* + *L. leucocephala* (without additives/control), R2: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + 20% rice bran + 10% rock sugar, R3: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + 20% corn meal + 10% rock sugar, R4: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + pollard 20% + 10% rock sugar.
Data were obtained from the results of the Laboratory of Dairy Animal Nutrition research, Department of Feed Nutrition and Technology, Bogor Agricultural University (2019).

absorbed, degraded, and utilized by the consuming stock. The R2 treatment was followed by the R3 treatment, and the highest treatment was in the R4 treatment. This condition was caused by the complete nutrient availability in the silage originated from natural nutrient content in the silage ingredient (Table 1). Pollard has better nutrient quality, mainly protein than corn meal and rice bran, in addition to having a dissoluble carbohydrate that can be effectively used by the ruminants. High OMD in the R4 treatment was caused by high crude protein content, which increased the microorganism development that digested the feed ingredient (Santi *et al*., 2012). The available nonstructural carbohydrate proportion in pollard and sorghum cutting age that tends to be younger also support the lactic acid bacterial performance to grow and develop.

The ingredient nutrient content and quality will determine the feed ingredient digestibility. The amount of cell wall in old plant is quite high, which decreases the feed ingredient digestibility. In contrast, high carbohydrate and protein contents (cell components) in young plants (60-70 DAP cutting age) increases the feed ingredient digestibility. This carbohydrate and protein availabilities play a role in proliferation and fermentation processes of rumen microorganisms (Belanche *et al*., 2019), as carbohydrate is utilized as an energy source and protein source as N-source for microorganism body formation.

The additives use in complete silage was significantly difference ($P<0.05$) in N-NH_3. The continuous test results showed that the R4 treatment was significantly different from the R1, R2, and R3 treatments, while the R2 and R3 treatments were insignificantly different. This difference was caused by the nutrient availability provided by the additives (Table 1 and Table 2). The nutrient availability of this complete silage could be quickly utilized by the rumen microbes as N-source for growth and development of rumen microbes. One of the main nitrogen (N) sources utilized by microbes for protein synthesis in their bodies is ammonia (NH_3) (Ani *et al*., 2015). Ammonia in the rumen was formed as the result of feed amino acid degradation of non-protein nitrogen source. Ammonia is used to build the microbial cells. Most rumen microbes (80%) utilize NH_3 formed from the amino acid deamination process. Leng (1990) stated that ammonia was the main product of protein deamination to amino acids, and its requirement in the rumen provided most N-sources for microbial growth. This condition can be performed by optimizing the sorghum as ruminant feed. The optimum NH_3 concentration for rumen microbial proliferation requires NH_3 at about 6.0 – 17.65 mM (McDonald *et al.,* 2002). This requirement was fulfilled in all treatments and approached optimal in the R4 treatment. The rumen bacteria depends on the NH_3 concentration (Wallace *et al*., 2002), when the ammonia concentration in the rumen is low, then the bacterial activity in the rumen will be inhibited and decrease the feed degradation value.

The volatile fatty acid (VFA) is the main energy source for ruminants produced from the feed fermentation process in the rumen. The simple and complex carbohydrates are digested by the rumen microbes and altered to VFA. VFA is absorbed by the blood circulation system through gluconeogenesis process and altered by liver to blood sugar. This blood sugar will supply half of energy requirement for ruminants. Table 2 showed that the treatments obtained a significant difference ($P<0.05$) in the VFA concentration. The continuous test results showed a significant difference among treatments. The VFA concentration produced from this study was about 73.32 – 120.55, and the control treatment (without additives) produced low VFA, which did not reach a normal condition, while the R2, R3, and R4 treatments produced normal VFA concentration. According to Sutardi (1980), the VFA concentration produced by rumen microbes in normal condition was 80–160 mM. This condition shows that the carbohydrate fermentation process by rumen microbes produces energy as VFA can perform effectively. Another factor that also influences the VFA concentration is carbohydrate fermentability (Hindratiningrum *et al*., 2011; Wanapat *et al*., 2013). The control (R1) treatment only produced 73.72 ± 3.85^{d}, which means that it did not fulfill the rumen microbial requirement for growth and development. Table 3 shows that the pollard additive (R4) was significantly different and had higher VFA concentration than corn meal and rice bran additives. This condition was caused due to higher nutrient availability, especially pollard protein than corn meal and rice bran, as high amount of protein in pollard increased the growth

Table 3. The average of *in vitro* digestibility of complete silage from sorghum ingredient using different additives

Digestibility	Treatment			
	R1	R2	R3	R4
DMD (%)	36.62 ± 0.75^{d}	40.85 ± 0.42^{c}	62.61 ± 0.60^{b}	71.34 ± 0.65^{a}
OMD (%)	34.11 ± 1.67^{d}	39.59 ± 0.52^{c}	61.92 ± 0.87^{b}	70.36 ± 0.66^{a}
N-NH_3 (mM)	10.55 ± 0.21^{c}	13.39 ± 2.39^{b}	13.18 ± 0.33^{b}	15.74 ± 0.29^{a}
VFA (mM)	73.72 ± 3.85^{d}	90.32 ± 3.08^{c}	107.22 ± 3.30^{b}	120.55 ± 3.74^{a}

Data are presented in average ±SD

R1: *Sorghum bicolor (L.) Moench* + *L. leucocephala* (without additives/control), R2: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + 20% rice bran + 10% rock sugar, R3: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + 20% corn meal + 10% rock sugar, R4: *Sorghum bicolor (L.) Moench* + *L. leucocephala* + pollard 20% + 10% rock sugar.

DMI: Dry matter digestibility; OMD: Organic matter digestibility; N-NH_3: Ammonia; VFA: Volatile fatty acid.

^{a,b,c,d} Different superscripts on the same row show a significant difference ($P<0.05$).

Data were obtained from the results of the Laboratory of Dairy Animal Nutrition Research, Department of Feed Nutrition and Technology, Bogor Agricultural University (2019).

and microbial activity to degrade the complete silage, therefore the fermentation product, namely, VFA, was found to be higher. Feed ingredient types, compositions, and non-structural carbohydrate fractions closely influence the VFA content. The complete silage feed not only contained sufficient protein content, but also non-structural carbohydrate ingredient feed source, which were easily digested due to increased VFA content.

Conclusions

It is concluded that the 20% pollard and 10% rock sugar additive supplementations can improve the dry matter digestibility, organic matter digestibility, N-NH_3, and VFA of complete silage from *Sorghum bicolor* (L.) Moench ingredient.

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