



# Effects of Lactat Acid Bacteria Inoculan and Additive on Quality and Characteristics of Brown Midrib Sorghum Mutant Line Silage (*Sorghum bicolor* L. Moench)

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**Abstract** | This study was aimed to observe the effects of lactic acid bacteria (LAB) inoculation and additives on quality and the characteristics of whole crop brown midrib sorghum. The experiment was carried out experimentally using a completely randomized factorial design with 4 replications. Factor A is A1 = without LAB, A2 = addition of BAL. Factor B consists of B1 = without additives, B2 = rice bran, B3 = corn. Parameters observed were the characteristics and quality of silage including pH, fleigh point (FP), dry matter content (DM), crude protein (CP), crude fiber (CF), crude fat (EE) and ash. Data were analyzed based on the analysis of variants according to the Duncan Multiple Range Test (DMRT). The results showed that there was no interaction ( $P > 0.05$ ) between the addition of LAB and additives to pH, FP, DM, CP, CF, EE and, ash while the single additive factor had a significantly different effect ( $P < 0.05$ ) of DM on whole crop BMR sorghum mutant silage. From this study it can be concluded that in general the addition of LAB inoculants and additives produced the same characteristics and quality of silage, however, the addition of rice bran and corn produced the higher levels of DM silage than without LAB and additives.

**Keywords** | Additives, Brown midrib, Sorghum, LAB, Yakult.

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## INTRODUCTION

Forage is basic feed of ruminant cattle consumed more than 70% of total ration (Abdullah, 2014). Forage must be included in ruminant ration because of lower forage production cost than concentrate and more eco-friendly to develop sustainable livestock industry. The presence of forage in ration is to help rumen function well, reduce acidosis risk, and increase consumption (Sari et al., 2015). However, the availability of sustainable forage is still a problem because of season. In wet season the production of forage is high, but in dry season the forage cannot grow well so that production fluctuation occurs (Siregar, 1994). To overcome the fluctuation of fodder forage, preservation of forage can be done when the production level is high, by implementing fermentation technology. One of fermenta-

tion implementations is ensilage process to produce silage. Silage is a preservation procedure of forage in anaerobic condition to make healthy fodder forage needed by ruminant cattle when this forage is not available, especially in winter season (Saricicek et al., 2016).

Silage is the most effective preservation to supply cattle fodder in dry season in tropical area. Nevertheless, in tropical area high quality silage is difficult to obtain because forage has low lactat acid bacteria (LAB) and water soluble carbohydrate (WSC) (Pholsen et al., 2016). An effort to improve silage quality is the use of additive in ensilage process to stimulate fermentation of LAB (Bureenok et al. 2006). Besides, forage commonly has high level of water content (>80%) which cause ensilage process is not successful because butyric acid becomes the main

fermentation product (Pholsen, 2016). Consequently, it is necessary to do wilting way and addition of additive and LAB in ensilage process. Hartadi et al. (2005) said that the addition of additive like rice bran containing carbohydrate content as nitrogen free extract (NFE) 48.7%, can preserve the quality of forage. Ridwan et al. (2005) reported that the addition of rice bran 1 - 5% in the production of *pennisetum purpureum* silage has an effect on increasing quality of silage.

Sorghum (*Sorghum bicolor* L. Moench) commonly becomes silage because it produces high-dry matter and is tolerant to drought. Sorghum is cereal crop producing seed and sugar on stem, and it also produces forage. It is better to harvest sorghum in *soft dough* phase because it has moisture around 60-70% (Gerik et al. 2003). Silage of sorghum in the form of *whole plant* (stem, leave, panicle) has lower quality than silage of *maize stover*, because the sorghum which is used is conventional variety containing higher lignin (8%) (Miller and Stroup, 2003), which influences bacteria performance in ensilage process. *Brown midrib* sorghum is a mutation result which has lower lignin content (6%) and has sugar brix on stem around 13,37% (Sriagtula et al. 2016). Sorghum grain has plentiful starch and sugar on stem, and it is available carbohydrate (non structural carbohydrate) as energy for LAB in ensilage process. As a result, additive addition in the form of fermentable carbohydrate maybe unimportant in ensilage process of whole plant BMR sorghum mutant line. Based on the explanation above, aim of this conducted research is to observe effect of LAB addition and different additive on nutrition quality and to know whether it is necessary to be applied to silage of sweet sorghum (BMR sorghum mutant line) or not.

## MATERIALS AND METHODS

### MATERIAL

In this research, whole plant (stover) sorghum consisting stem, leave, and panicle of BMR sorghum mutant line Patir 3.7 (*Sorghum bicolor* L. Moench), rice bran, maize, Yakult® are used. Tools used are plastic bag, cutting scissors, chopper machine, scale, vacuum, and oven.

### RESEARCH METHODOLOGY

This is an experimental research using Completely Randomized Design with factorial pattern. Factor A consists of A1 = without LAB, A2 = LAB. Factor B consists of B1 = without additive, B2 = rice bran, B3 = soft maize. Each treatment was replicated 4 times in order to get 24 combinations of treatment. Source of LAB is probiotics drink, Yakult® with its dosage that is 1 ml (v/w)/fresh weight based on Pholsen et al. (2016), while rice bran and maize are 3% (g/g)/fresh weight based on Ridwan et al. (2005). LAB population (*Lactobacillus casei*) is  $11 \times 10^9$  CFU/ml.

## PROCEDURE OF SILAGE PRODUCTION

BMR sorghum mutant line Patir 3.7 is harvested in *soft dough* phase (90 days after sowing/DAS), then it is cut by using chopper machine. The matter is wilted one night in order to reduce the water content. LAB and additive addition are treated and both are mixed evenly with sorghum stover. The mixing is put in plastic bag (silo) and compressed by using vacuum pump. Plastic bag is tied tightly and stored 21 days. After that, it is harvested and tested about silage quality and characteristic involving nutritional content (DM, CP, CF, EE and ash), fiber fraction content (ADF, NDF, Cellulose, hemicellulose, lignin, and silica) and pH, NF value.

## PROCEDURE OF VARIABLE MEASUREMENT

Quality of silage nutrition is observed by using proximate analysis and AOAC method (1980), while fiber fraction content is measured according Van Soest (1991). pH value is measured by taking 10 g sample of silage which is soaked in 50 ml of aquadest. Then, it is stirred and kept for 15 minutes. pH value is measured by using pH metre. NF value is calculated by using formula  $NF = 220 + (2 \times DM (\%) - 15) - (40 \times pH)$  (Idikut et al., 2009).

## DATA ANALYSIS

Data are analyzed by using analysis of variance (ANOVA) using SPSS 16 software, then significant effect would test by using Duncan Multiple Range Test (DMRT) according to Steel and Torri (1997).

## RESULTS

Treatment effect on nutritional content is shown in Table 2. This study has shown there is no interaction ( $P > 0.05$ ) of LAB inoculation and additive addition on nutritional content (DM, CP, CF, EE and ash) of silage of BMR sorghum mutant line Patir 3.7. In this research, LAB addition does not give significant effect ( $P > 0.05$ ) on DM increase. The second major finding was that no interaction between factor A (LAB addition) and factor B (additive addition) on fiber fraction content. Likewise, single factor of LAB addition (factor A) shows insignificant different effect ( $P > 0.05$ ) on fiber fraction content of silage. Nonetheless, single factor of additive addition (factor B) gives significant different effect ( $P < 0.05$ ) on NDF and cellulose content. The fiber fraction content of sorghum stover before silage and after ensilage are shown in Table 3 and 4. These experiments confirmed that no interaction ( $P > 0.05$ ) between LAB inoculation and additive on pH and NF value and single factor of both LAB inoculation and additive gives a different effect insignificantly ( $P > 0.05$ ). Silage characteristic can be seen from pH and fliegh value (NF) shown in Table 5.

**Table 1:** Nutrient content of sorghum stover before silage (% DM basis)

Nutrients	%
DM	25.22
CP	10.10
CF	20.43
EE	3.95
Ash	5.66
NFE	59.87

DM=dry matter, CP=crude protein, CF=crude fiber, EE+ether extract, NFE=nitrogen free extract.

**Table 2:** Nutrients content of BMR sorghum mutant line Pator 3.7 silage

Nutrients (%)		B1	B2	B3	Mean
DM	A1	21.99±0.78	23.69±1.12	22.95±0.55	22.88±1.06
	A2	22.57±0.53	23.79±1.34	23.97±1.20	23.49±1.16
	Mean	22.24±0.71 <sup>b</sup>	23.73±1.11 <sup>a</sup>	23.46±1.02 <sup>a</sup>	
CP	A1	7.91±1.19	7.96±1.22	8.98±1.38	8.29±1.22
	A2	8.52±2.93	11.21±0.59	8.15±1.60	9.36±2.18
	Mean	8.27±2.18	9.58±1.95	8.51±1.45	
CF	A1	30.07±0.58	28.46±2.31	29.78±1.72	29.44±1.70
	A2	26.26±2.37	29.07±1.85	28.12±1.32	28.13±1.88
	Mean	28.80±2.28	28.76±1.97	28.95±1.68	
EE	A1	3.99±0.89	4.82±1.45	5.17±0.58	4.66±1.07
	A2	3.04±0.88	3.84±0.47	4.57±1.14	3.97±0.98
	Mean	3.68±0.94	4.33±1.13	4.87±0.89	
Ash	A1	6.70±1.47	5.36±3.48	8.33±0.78	6.79±2.38
	A2	3.53±2.14	5.16±3.02	6.02±3.93	5.19±3.12
	Mean	5.64±2.21	5.29±3.05	7.18±2.89	
NFE	A1	49.03±4.57 <sup>b</sup>	53.66±2.86 <sup>ab</sup>	48.72±3.12 <sup>b</sup>	50.18±3.98
	A2	55.19±5.13 <sup>a</sup>	50.51±1.72 <sup>ab</sup>	50.82±1.95 <sup>ab</sup>	51.57±2.97
	Mean	51.08±5.28	51.86±2.65	49.77±2.66	

Lowercase letter in the same line indicates significant influence (P<0.05). DM=dry matter, CP=crude protein, CF=crude fiber, EE+ether extract, NFE=nitrogen free extract

A1=without BAL, A2= BAL, B1=without additive, B2= rice bran, B3= corn.

**Table 3:** Fiber fraction content of sorghum stover before silage (% DM basis)

Fiber fraction	%
ADF	26.15
NDF	31.28
Hemicellulose	29.29
Cellulose	20.09
Lignin	4.24
Silica	1.82

ADF=acid detergent fiber, NDF= neutral detergent fiber.

## DISCUSSION

Composition of sorghum stover which is used to produce silage is shown in Table 1. DM content of sorghum stover

harvested in soft dough phase (90 DAS) is categorized as good forage to be silage matter if it has DM content more than 20% (Antaribaba et al., 2009). The study shows that storage factor during ensilage process of sorghum stover causes reduction in CP and NFE content while CF content increases. The result is similar to Feyissa et al. (2014), that is CP, IVOMD and ME content of forage decrease and fiber fraction content increases before storage until duration of certain storage period.

LAB addition does not give significant effect on DM increase in this research, the same result is also reported by Konca et al. (2015) that LAB addition on silage of sunflower does not increase DM content. DM content is significantly higher (P<0.05) in treatment with rice bran (B2) and corn (B3) addition. In this treatment, rice bran and

**Table 4:** Fiber fraction on BMR sorghum mutant line Patir 3.7 silage

Fiber fraction (%)		B1	B2	B3	Mean
ADF	A1	39.23±1.95	38.50±3.23	37.49±0.67	38.40±2.13
	A2	37.64±3.17	38.26±1.63	37.29±1.67	37.78±1.98
	Mean	38.55±2.44	38.38±2.37	37.40±1.08	
NDF	A1	58.34±1.30	56.38±1.69	56.58±0.43	57.10±1.46
	A2	57.73±1.12	56.28±2.65	53.48±1.89	56.14±2.41
	Mean	58.03±1.17 <sup>a</sup>	56.33±2.05 <sup>ab</sup>	55.40±1.85 <sup>b</sup>	
Hemicelulose	A1	20.91±3.33	16.87±2.47	17.92±2.88	18.57±3.18
	A2	19.53±2.87	18.45±3.48	17.28±4.68	18.65±3.16
	Mean	20.22±2.97	17.66±2.92	17.70±3.07	
Celulose	A1	32.46±0.97	31.94±2.35	31.11±2.64	31.84±2.00
	A2	34.48±1.20	32.45±1.58	30.48±0.62	32.47±1.97
	Mean	33.32±1.45 <sup>a</sup>	32.20±1.87 <sup>ab</sup>	30.84±1.93 <sup>b</sup>	
Lignin	A1	5.59±1.09	5.27±1.31	5.47±1.33	5.44±1.11
	A2	4.97±0.61	4.48±0.47	4.27±0.99	4.60±0.68
	Mean	5.28±2.21	4.87±1.00	4.87±1.23	
Silica	A1	1.18±0.16	1.30±0.21	1.06±0.58	1.19±0.31
	A2	1.21±0.47	1.33±0.16	1.71±0.71	1.36±0.42
	Mean	1.19±0.33	1.32±0.17	1.32±0.65	

Lowercase letter in the same line indicates significant influence (P<0.05). A1=without BAL, A2= BAL, B1=without additive, B2= rice bran, B3= corn, ADF=acid detergent fiber, NDF=neutral detergent fiber.

**Table 5:** Characteristic of silage BMR sorghum mutant line BMR Patir 3.7

Parameter		B1	B2	B3	Mean
pH	A1	3.59±0.05	3.57±0.04	3.61±0.05	3.59±0.05
	A2	3.56±0.03	3.61±0.04	3.60±0.04	3.59±0.04
	Mean	3.58±0.05	3.59±0.04	3.60±0.04	
NF	A1	105.24±2.81	109.54±3.66	106.51±2.11	107.10±3.25
	A2	108.08±1.25	106.63±3.67	109.15±3.09	107.94±2.92
	Mean	106.46±2.61	108.09±3.73	107.83±2.83	

Note: The treatments no significant influence (P>0.05). A1=without BAL, A2= BAL, B1=without additive, B2= rice bran, B3= corn, NF= Fliedge value.

corn are added 3% for each so that it increases DM of material.

There is no significant difference (P>0.05) of single factor of LAB with additive on nutritional content of sorghum silage because fermentable carbohydrate content in the silage matter is quite high. BMR sorghum mutant Patir 3.7 is sweet sorghum because of its quite high sugar content on stem, that is 13% Brix (Sriagtula et al. 2016). Another factor which shows that nutritional content is different insignificantly is pH value which is also different insignificantly in all treatment combination. Low pH supported by high lactat acid concentrate is important to preserve low DM loss, inhibit protein and other nutrient degradation in silage (Amer et al., 2012), so that nutritional content in silage of BMR sorghum mutant stover is not different in

this research.

Additive addition in B2 and B3 produces quality of silage which is not different significantly (P>0.05) because material is sorghum stover consisting stem, leave, and panicle. Sorghum stem contains high sugar called as sweet sorghum. Panicle of sorghum in soft dough phase producing seed (milky stage) is a source of starch. Sugar and starch are fermentable carbohydrate (*fermentable sugar*) and a part of *water soluble carbohydrate* (WSC). So, addition of rice bran and corn in this research produces quality of silage which is as good as the control group. Long et al. (2006) states that sugar content in stem of sorghum is an important factor to produce good sorghum silage.

Treatment of LAB inoculation produces nutritional con-



tent and silage characteristic which are different insignificantly ( $P > 0.05$ ) to control group (without LAB). The research result is in line with [Comino et al. \(2014\)](#), that is LAB addition in silage of maize stover in late maturity stage is not effective compared to maize which is harvested at an early age. For this reason, population natural LAB contained in late maturity forage before preservation is high, so that a great ensilage process occurs and effect of LAB inoculation in ensilage decreases. In this research, LAB population of fresh sorghum stover is not quantified, yet it is expected that LAB of sorghum stover is quite high when it is harvested in late maturity stage (soft dough phase). Besides, [Muller \(2009\)](#) said that later harvest stage will increase amount of yeast, fungus and LAB in forage before preservation. Natural LAB contained in silage of sorghum stover have great activity because sorghum stover has sugar on stem. [Sriagtula et al. \(2016\)](#) asserts that BMR sorghum stover Patir 3.7 which is harvested in soft dough phase containing sugar on stem in the amount of 13 % Brix. It is supported by [Jones et al. \(2004\)](#) statement that sugar is primary food of LAB and low sugar content in the material will inhibit LAB activity. LAB in Yakult (*L. casei*) addition in silage of sorghum stover does not give significant effect because substrate difference. *L. casei* is not effective on substrate with high crude fiber, inline [Antaribaba et al. \(2009\)](#) claims that inoculan *L. casei* addition is not effective in improving silage fermentation quality of forage. Furthermore, [Koc et al. \(2009\)](#) reports that LAB inoculation of *Lactobacillus plantarum* and *Enterococcus faecium* in sunflower silage does not improve DM, CP, EE and ash content.

Treatment without additive addition (B1) produces higher NDF and cellulose content significantly ( $P < 0.05$ ) compared to treatment with rice bran addition (B2) and corn (B3). For this reason, additive addition causes an increase of DM content of the material from 22.24% in control to 23.73 (B2) and 23.46 (B3) ([Table 2](#)). Dry matter contains organic matter including fiber fraction so that change of DM content will influence other nutritional contents including fiber fraction. Factor influencing NDF content in this research is cellulose. Low NDF content on B2 and B3 treatments has a correlation to the decrease of cellulose content on treatment of B2 and B3 ([Table 4](#)). [Fariani and Akhadiarto \(2012\)](#) also explain that one factor which influences NDF value is cellulose. [Guerrero et al. \(2010\)](#) also states that loss of DM, increasing fiber content, and decreasing CP content are common phenomena in the process of forage storage.

The increase of fiber fraction content after ensilage than before ensilage, is related to decrease of water-soluble carbohydrate content (non fiber carbohydrate) like starch, so fiber fraction content increases because of loss of non fiber component. Moreover, [Borgatti et al. \(2012\)](#) suggest that

ensilage in sugar cane causes decrease of soluble carbohydrate content and increases component of cell wall. During silage process, dry matter is lost, mainly non structural carbohydrate to be sugar as a source of energy in respiration process of plant and activity of aerobic bacteria. This activity causes non structural carbohydrate content decreases and structural carbohydrate content increases (fiber fraction). Beside that, respiration process and activity of aerobic bacteria in the early ensilage produces heat and causes reaction of non enzymatic inducing nutritional component like CP is insoluble and bound with lignin ([McCormick et al., 2011](#)). This increases ADF, NDF, cellulose and lignin in this research. In contrast, [Amer et al. \(2012\)](#) that CP fraction changes during ensilage because protein proteolysis becomes non protein compound by protease enzyme.

pH value in this research is around 3.58-3.60 lower than [Amer et al. \(2012\)](#), that pH of sorghum silage is 3.8. pH value in this research is categorized as ideal value. According to [Ferreira et al. \(2011\)](#), pH value which is lower than 4.0 indicates fermentation of lactat acid which inhibits the growth of unexpected microorganism to ensure the quality of product.

In this research, the ideal pH value in all combinations of treatment shows availability of soluble carbohydrate in the material which meets the need to produce lactat acid, so additive addition does not give significant effect ( $P > 0.05$ ). [Junior et al. \(2015\)](#) mentions that the availability of soluble carbohydrate causes production of short chain organic acid during ensilage process decreasing pH quickly and efficiently in the silage material.

*Flieg* value (NF) gives information about quality of silage based on pH and DM value of silage. [Idikut et al. \(2009\)](#) writes that NF ( $> 85$ ) produces silage with excellent quality, 60 - 80 is good, 40 - 60 is good enough, 20 - 40 is average and NF  $< 20$  is not good. In this research, NF is more than 100, it is also found by [Idikut et al. \(2009\)](#). High *fleigh* value is caused by high DM value of silage and low pH value of silage. [Saricicek et al. \(2016\)](#) mentions that fleig value which is more than 100 shows the super quality of silage.

## CONCLUSION

LAB inoculan and additive addition produce silage with characteristic and quality which are as same as the silage without LAB and additive addition. However, rice bran and corn addition produce higher DM content, and lower NDF and cellulose compared to silage of BMR sorghum stover without LAB and additive addition which is harvested in soft dough phase. LAB inoculan and additive addition for BMR sorghum mutant silage was not needed in BMR sorghum mutant Patir 3.7 silage.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS CONTRIBUTION

Riesi Sriagtula and Imana Martaguri designed the experiments, performed the experiments and performed statistical analysis. Mardhiyetti and Zurmiati drafted and revised the manuscript and consent to publish in AAVS.

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