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Research Article Effects of Lactat Acid Bacteria Inoculan and Additive on Quality and Characteristics of Brown Midrib Sorghum Mutant Line Silage (Sorghum bicolor L. Moench) Riesi Sriagtula*, Imana Martaguri, Mardhiyetti, Zurmiati Department of Animal Feed and Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indo- nesia. 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. Received | June 25, 2019; Accepted | July 26, 2019; Published | December 17, 2019   *Correspondence | Riesi Sriagtula, Department of Animal Feed and Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indonesia; Email: riesisriagtula @ansci.unand.ac.id   Citation | Sriagtula R, Martaguri I, Mardhiyetti, Zurmiati (2020). Effects of lactat acid bacteria inoculan and additive on quality and characteristics of brown midrib sorghum mutant line silage (sorghum bicolor l. Moench). Adv. Anim. Vet. Sci. 8(1): 25-31. DOI | http://dx.doi.org/10.17582/journal. aavs/2020/8 .1. 25.31 ISSN (Online) | 2307-8316; ISSN (Print) | 2309-3331   INTRODUCTION F orage 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 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 rumi- nant cattle when this forage is not available, especially in winter season (Saricicek et al., 2016). to develop sustainable livestock industry. The presence of Silage is the most effective preservation to supply cattle forage in ration is to help rumen function well, reduce ac- fodder in dry season in tropical area. Nevertheless, in idosis risk, and increase consumption (Sari et al., 2015). tropical area high quality silage is difficult to obtain be- However, the availability of sustainable forage is still a cause forage has low lactat acid bacteria (LAB) and water problem because of season. In wet season the production soluble carbohydrate (WSC) (Pholsen et al., 2016). An of forage is high, but in dry season the forage cannot grow effort to improve silage quality is the use of additive in well so that production fluctuation occurs (Siregar, 1994). ensilage process to stimulate fermentation of LAB (Bu- To overcome the fluctuation of fodder forage, preservation reenok et al. 2006). Besides, forage commonly has high of forage can be done when the production level is high, by level of water content (>80%) which cause ensilage process implementing fermentation technology. One of fermenta- 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 penn- isetum purpereum 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). Sor- ghum 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 sor- ghum mutant line. Based on the explanation above, aim of this conducted research is to observe effect of LAB 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 addi- tion are treated and both are mixed evenly with sorghum stover. The mixing is put in plastic bag (silo) and com- pressed 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 val- ue 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 x DM (%) – 15) - (40 x 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) accord- ing to Steel and Torri (1997). addition and different additive on nutrition quality and to know whether it is necessary to be applied to silage of RESULTS sweet sorghum (BMR sorghum mutant line) or not. Treatment effect on nutritional content is shown in Table MATERIALS AND METHODS 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 sor- Material ghum mutant line Patir 3.7. In this research, LAB addi- In this research, whole plant (stover) sorghum consisting stem, leave, and panicle of BMR sorghum mutant line tion does not give significant effect (P>0.05) on DM in- Patir 3.7 (Sorghum bicolor L. Moench), rice bran, maize, crease.The second major finding was that no interaction Yakult® are used. Tools used are plastic bag, cutting scissors, between factor A (LAB addition) and factor B (additive chopper machine, scale, vacuum, and oven. addition) on fiber faction content. Likewise, single factor of LAB addition (factor A) shows insignificant different effect (P>0.05) on fiber fraction content of silage. None- Research Methodology theless, single factor of additive addition (factor B) gives This is an experimental research using Completely Rand- omized Design with factorial pattern. Factor A consists of significant different effect (P<0.05) on NDF and cellulose A1 = without LAB, A2 = LAB. Factor B consists of B1 content. The fiber fraction content of sorghum stover be- = without additive, B2 = rice bran, B3 = soft maize. Each fore silage and after ensilage are shown in Table 3 and 4. treatment was replicated 4 times in order to get 24 com- These experiments confirmed thatno interaction (P>0.05) between LAB inoculation and additive on pH and NF val- binations of treatment. Source of LAB is probiotics drink, ue and single factor of both

LAB inoculation and addi- Yakult® with its dosage that is 1 ml (v/w)/fresh weight based on Pholsen et al. (2016), while rice bran and maize tive gives a different effect insignificantly (P>0.05).Silage are 3% (g/g)/fresh weight based on Ridwan et al. (2005). characteristic can be seen from pH and fliegh value (NF) LAB population (Lactobacillus casei) is 11x109 CFU/ml. 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±0.71b 23.73±1.11a 23.46±1.02a 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±4.57b 53.66±2.86ab 48.72±3.12b 50.18±3.98 A2 55.19±5.13a 50.51±1.72ab 50.82±1.95ab 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 January 2020 | Volume 8 | Issue 1 | Page 27 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 con- tent 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 du- ration of certain storage period. LAB addition does not give significant effect on DM in- crease in this research, the same result is also reported by Konca et al. (2015) that LAB addition on silage of sun- flower does not increase DM content. DM content is sig- nificantly 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±1.17a 56.33±2.05ab 55.40±1.85b 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±1.45a 32.20±1.87ab 30.84±1.93b 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 pH A1 3.59±0.05 3.57±0.04 A2 Mean NF A1 A2 3.56±0.03 3.58±0.05 105.24±2.81 108.08±1.25 3.61±0.04 3.59±0.04 109.54±3.66 106.63±3.67 B3 3.61±0.05 3.60±0.04 3.60±0.04 106.51±2.11 109.15±3.09 Mean 106.46±2.61 108.09±3.73 107.83±2.83 Mean 3.59±0.05 3.59±0.04 107.10±3.25 107.94±2.92 Note: The treatments no significant influence ($P>0.05$). A1=without BAL, A2= BAL, B1=without additive, B2= rice bran, B3= corn, NF= Fliegh value. corn are added 3% for each so that it increases DM of ma- terial. 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 si- lage 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). Anoth- er factor which shows that nutritional content is different insignificantly is pH value which is also different insignifi- cantly 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 ma- terial is sorghum stover consisting stem, leave, and panicle. Sorghum stem contains high sugar called as sweet sor- ghum. 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 insignif- icantly ($P>0.05$) 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 maturi- ty stage is not effective compared to maize which is har- vested 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 quanti- fied, 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 stov- er 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 Antar- ibaba 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 Lactobacillusplantarum and Enterococcus fae- cium in sunflower silage does not improve DM, CP, EE and ash content. Treatment without additive addition (B1) produces higher NDF and cellulose content significantly ($P<0.05$) com- pared 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 in- cluding 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 cellu- lose 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 car- bohydrate 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 carbohy- drate content and increases component of cell wall. During silage process, dry matter is lost, mainly non structural car- bohydrate to be sugar as a source of energy in respiration process of plant and activity of aerobic bacteria. This ac- tivity causes non structural carbohydrate content decreases and structural carbohydrate content increases (fiber frac- tion). Beside that, respiration process and activity of aero- bic 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 proteol- ysis 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. Accord- ing 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 solu- ble carbohydrate causes production of short chain organic acid during ensilage process decreasing pH quickly and ef- ficiently 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 val- ue 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 har- vested 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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