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Degradation of Rice Straw Lignocellulosic Compounds by Lignocellulolytic Bacteria Inoculant from Bali Cattle Rumen Fluidand Termites

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Abstract:
Background. *Optimizing the use of rice straw which is the main waste of food crops as feed, is a vital step in developing an integrated livestock-farming business with the "SIMANTRI" pattern.The high lignocellulosic is the main limiting factor for use of rice straw as feed.Utilization of lignocellulolytic bacteria as starter inoculants for rice straw is a strategy developed in this study.*
Material and Method. *Lignocellulolytic bacteria isolated from the rumen fluid of Bali cattle (R) and termites (T) use in this study, namely; R_1=Pseudomonas aeruginosa strain BR_9LS, R_2=Bacillus subtilis strain BR_4LG, R_3=Bacillus subtilis strain BR_2CL, R_4=Paenibacillus dendritiformis strain BR_3XY, and T_1=Aneurinibacillus sp. strain BT_4LS, T_2=Aneurinibacillus sp. strain BT_5LG, T_3=Bacillus sp. strains BT_3CL and T_4=Bacillus sp. strain BT_8XY. Eleven (11) inoculants were produced in this study were 9 inoculants of lignocellulolytic bacteria (BR_{1234}; BT_{1234}; $BR_{12}T_{34}$; $BR_{13}T_{24}$; $BR_{14}T_{23}$; $BR_{23}T_{14}$; $BR_{24}T_{13}$; $BR_{34}T_{12}$; and $BR_{1234}T_{1234}$), and 2 inoculants control (inoculants without lignocellulolytic bacteria/BR_0T_0 and inoculants of 10% bali cattle rumen fluid/BRL).*
Result.*The study showed that the use of superior lignocellulolytic bacteria inoculants from rumen fluid of Bali cattle and termites and inoculant of bali cattle rumen fluid (BRL) were able to significantly increase the degradation of lignocellulosic compounds in rice straw as indicated by a decrease in the content of NDF, ADF, ADL, cellulose, hemicellulose, insoluble lignin, and silica, had increased of crude protein content, metabolite products and dry matter and organic matter in vitro digestibility compared than$RSBR_0T_0$.*
Conclusion.*The inoculant formula $BR_{23}T_{14}$ is the best inoculant for produces the best quality fermented rice straw*

Keywords: *Lignocellulolytic Bacteria, Inoculants, Bali Cattle Rumen Fluid, Termites, Lignocellulosic*

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I. Introduction

Rice straw is the main food crop agricultural waste that will be used as feed in the SIMANTRI (acronym for Integrated Agricultural System, in Indonesian "SistemPertanianTerintegrasi") program which is the flagship program of the agricultural sector in Bali, Indonesia [1].In this system, agricultural waste will be used as feed, while animal waste, especially livestock manure (feces and urine) will be used as fertilizer/compost to be used in agriculture and biogas as an alternative energy source.The success of the*Simantri* program is strongly influenced by the quality and effectiveness of each business subsystem [1].

Utilization of rice straw as animal feed on the one hand will increase the diversification of animal feed sources, but on the other hand rice straw is known to have low nutritional quality and digestibility as a result of the high content of lignocellulosic compounds [2, 3].Howard *et al.* [2] revealed that rice straw contains 18% lignin, 32.1% cellulose and 24% hemicellulose.Utilization of rice straw without processing can result in a decrease in livestock productivity and even cause livestock businesses to experience losses [4].This is due to feed conversion that is not optimal, only 35-42% of crude fiber from rice straw can be digested by bali cattle and/or etawah/PE crossbreed goats.Even Wahyudi*et al.* [5] revealed that only 10-35% of energy from crude fiber can be utilized by ruminants.

Increasing the degradation of lignocellulosic compounds of rice straw is an important strategy in optimizing the development of the SIMANTRI pattern of agriculture-livestock business. Complete degradation of lignocellulosic compounds can only be carried out by a consortium of lignocellulolytic microorganisms capable of producing lignocellulase enzyme complexes consisting of lignase enzyme complexes (especially lignin-peroxidase/Li-P, manganese-peroxidase/Mn-P and laccase/Lac), cellulase enzyme complex (endo-β-glucanase, exoglucanase, and β-glucosidase), and hemicellulase enzyme complexes (xylanase and mannanase) [2, 6]. In nature, lignocellulolytic microbes are abundant in agricultural land, peat soil, digestive tract of

ruminants, body cells and digestive tract of termites and various other sources of bacteria [7, 8, 9, 10, 11] . The rumen contents of bali cattle and termites are a source of microbes, especially lignocellulolytic bacteria that have the potential to be used as inoculants to break down lignocellulosic compounds from agricultural waste including rice straw [1, 4]. Mudita *et al.*[4] successfully screened superior lignocellulolytic bacteria originating from the rumen fluid of Bali cattle and termites that have high degradation ability of lignocellulosic compounds, namely *Pseudomonas aeruginosa* BR_9LS, *Bacillus subtilis* BR_4LG, *Bacillus subtilis* BR_2CL, *Paenibacillusdendritiformis* BR_3XY, *Aneurinibacillus sp.* BT_4LS,*Aneurinibacillus sp.* BT_5LG, *Bacillus sp.* BT_3CL,and *Bacillus sp.* BT_8XY.

Utilization of superior lignocellulolytic bacteria from the rumen fluid of Bali cattle and termites which have high substrate degradation ability of lignocellulosic is very potential to be used as starter inoculant for various local power fermentations from waste including rice straw. The synergistic formulation of superior lignocellulolytic bacterial isolates from the rumen fluid of Bali cattle and termites will produce a high quality fermentation starter. But on the other hand the combination of various bacteria is also at risk of causing competition between microbes which actually reduces the performance of the microorganisms themselves [12]. In view of this, research on the formulation of superior lignocellulolytic bacterial isolates from the rumen fluid of Bali cattle and termites in increasing the degradation of lignocellulosic compounds in rice straw is important to support the optimization of the development of Simantri-based farm-livestock businesses based on local resources from agricultural waste.

II. Material And Methods

Study Location: The research was carried out at the Nutrition and Animal Feed Laboratory, Faculty of Animal Husbandry, Udayana University, Denpasar.

Material Study. This study using rice straw as the object of research and superior lignocellulolytic bacteria isolates from rumen fluid of Bali cattle and termites as a source of microbes for the fermentation starter inoculant.

Lignocelliulolytic Bacteria. The isolates of lignocellulolytic bacteria used in the rumen fluid of Bali cattle consisted of *Pseudomonas aeruginosa strain* BR_9LS (R_1), *Bacillus subtilis strain* BR_4LG (R_2), *Bacillus subtilis strain* BR_2CL (R_3) and *Paenibacillusdendritiformis strain* BR_3XY (R_4), while the superior termite bacterial isolates consisted of *Aneurinibacillus sp. strains* BT_4LS (T_1), *Aneurinibacillus sp. strains* BT_5LG (T_2), *Bacillus sp. strains* BT_3CL (T_3) and *Bacillus sp. strain* BT_8XY (T_4).

Inoculant Media. The media used in the manufacture of inoculants in this study were media consisting of a combination of synthetic nutrient sources (proanalysis) and natural ingredients with the composition and nutritional content presented in Table 1.

The inoculant media was made by mixing all the inoculant media materials until homogeneous and assisted also by the heating process to boiling for ± 15 minutes and then filtered. Then the inoculant media solution was sterilized in an autoclave at 121^{o}C T for 15 minutes. After the inoculant media cooled (T 39^{o}C), the media was ready to be used in inoculant production. The research was carried out at the Nutrition and Animal Feed Laboratory, Faculty of Animal Husbandry, Udayana University, Denpasar, using rice straw as the object of research and superior lignocellulolytic bacteria isolates from rumen fluid of Bali cattle and termites as a source of microbes from the fermentation starter inoculant.

Table 1 The composition and Nutrient Content of Inoculant Medium

No	Medium Ingredients	Composition
1	Thioglycollate Fluid Medium/TFM (g)	1
2	Rumen Fluid Supernatan(ml)	10
3	Molases (g)	50
4	Urea Fertilizer (g)	1
5	Tannic Acid	0,25
6	Carboxymethylcellulose/CMC	0,25
7	Xylanose	0,25
8	Rice Straw Meal (g)	0,25
9	Cassava Meal (g)	0,25
10	Rice brani (g)	0,25
11	Salt/NaCl (g)	0,25
12	Multi Vitamin-Mineral “Pignox” (g)	0,15
13	Fresh Water	hingga volumenya 1 liter
Nutrient Contents [1]		
1	Phosphor//P (ppm)	144,81
2	Calcium/Ca (ppm)	736,07
3	Zincum/Zn (ppm)	5,80
4	Sulfur/S (ppm)	158,15
5	Soluble Protein (g/ml)	0,0075

*Note: [1)]*Analysis Results from Lab. Analytic, Udayana University, Bukit Jimbaran

Research Inoculant. The inoculants produced in this study were 9 inoculants formulated from superior lignocellulolytic bacteria rumen fluid for bali cattle and termites cultured on inoculant medium and 2 control inoculants, namely inoculants produced using only inoculant medium (BR_0T_0) and inoculants produced using 10% liquid. fresh rumen cultured on inoculant medium (BRL). The types and composition of the inoculants produced are presented in Table 2.

The production of inoculants was carried out by mixing 10% of microbial culture (according to treatment) with 90% of the inoculant medium under anaerobic conditions (while flowing with CO2), then incubated at 39^{o}C for 5-7 days. After the incubation period, the inoculants are ready for use.

Table 2. Composition and Formulation of Research Inoculants

inoculaants Formula[1]		Inoculant Medium (ml)	Bali Cattle Rumen Fluid Bacteria Culture/BR[2] (ml)				Termites Bacteria Culture/BT[3] (ml)				Rumen Fluid (ml)
			R_1	R_2	R_3	R_4	T_1	T_2	T_3	T_4	
1.	BR_0T_0	1000	-	-	-	-	-	-	-	-	-
2.	BR_{1234}	990	2,50	2,50	2,50	2,50	-	-	-	-	-
3.	BT_{1234}	990	-	-	-	-	2,50	2,50	2,50	2,50	-
4.	$BR_{12}T_{34}$	990	2,50	2,50	-	-	-	-	2,50	2,50	-
5.	$BR_{13}T_{24}$	990	2,50	-	2,50	-	-	2,50	-	2,50	-
6.	$BR_{14}T_{23}$	990	2,50	-	-	2,50	-	2,50	2,50	-	-
7.	$BR_{23}T_{14}$	990	-	2,50	2,50	-	2,50	-	-	2,50	-
8.	$BR_{24}T_{13}$	990	-	2,50	-	2,50	2,50	-	2,50	-	-
9.	$BR_{34}T_{12}$	990	-	-	2,50	2,50	2,50	2,50	-	-	-
10.	$BR_{1234}T_{1234}$	990	1,25	1,25	1,25	1,25	1,25	1,25	1,25	1,25	-
11.	BRL	990	-	-	-	-	-	-	-	-	10

Note:: [1]Inoculant Formula in this research, BR0T0(Inoculant formulated without superior lygnocellulolytic bacteria isolate; BR_{1234}; BT_{1234}; $BR_{12}T_{34}$; $BR_{13}T_{24}$; $BR_{14}T_{23}$; $BR_{23}T_{14}$; $BR_{24}T_{13}$; $BR_{34}T_{12}$; and $BR_{1234}T_{1234}$ (Inoculant formulated by superior lignocellulolytic bacteria from bali cattle rumenfluid and/or termites); BRL(inoculant fomulated by bali cattle fluid fresh); [2]Superior lignocellulolytic bacteria from bali cattle rumen fluid (R_1=Pseudomonas aeruginosa strain BR_9LS, R_2=Bacillus subtilis strain BR_4LG, R_3=Bacillus subtilis strain BR_2CL and R_4=Paenibacillus dendritiformis strain BR_3XY), [3]Superior lignocellulolytic bacteria isolates from termites (T_1=Aneurinibacillus sp. strain BT_4LS, T_2=Aneurinibacillus sp. strain BT_5LG, T_3=Bacillus sp. strains BT_3CL and T_4=Bacillus sp. strain BT_8XY).

Rice Straw as Object Study. The rice straw used in this study is part of the stalks of freshly harvested rice straw with the nutrients content presented in Table 3.

Rice straw fermentation was carried out anaerobically using the produced fermentation starter inoculant (according to the treatment). The fermentation technique was carried out by adding 1 kg (DM) of rice straw with 1 liter of inoculant solution consisting of 10 ml of inoculant solution (according to treatment), 10 ml of molasses and 980 ml of clean water. Then mixed in such a way until homogeneous. The fermentation process was carried out using a black plastic bag as a silo and fermented for 2 weeks under an-aerobic conditions. After 2 weeks the fermented rice straw was opened to be used for further research activities.

Table 3 The Nutrients Content of Rice Straw Research

No	Nutrients[1]	Nutrien Content (%)
1	Dry Matter (% fresh basis)	85,3787
2	Dry Matter (% DW basis)	95,7195
3	Organic Matter (%)	77,8330
4	Anorganic Matter/Ash (%)	22,1670
5	*Crude Protein*/CP	2,7088
6	*Neutral Detergent Fiber*/NDF	82,2408
7	*Acid Detergent Fiber*/ADF	57,1597
8	*Acid Detergen Lignin*/ADL	25,6721
9	Cellulose	31,4876
10	Hemicellulose	25,0811
11	Lignin insoluble	15,2515
12	Silica	10,4205

Note: Result of Feed and Nutrition Laboratory of Faculty of Animal Husbandry, Udayana University

Study Design. The study was carried out with a Completely Randomized Design/CRD with 11 treatments and 3 replications, so that in total there were 33 experimental units.

The treatments were:

1. $RSBR_0T_0$=The rice straw fermented by inoculant without superior lignocellulolytic bacteria (fermentation with medium inoculant; control treatment)
2. $RSBR_{1234}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely*Pseudomonas aeruginosa strain BR_9LS, Bacillus subtilis strain BR_4LG, Bacillus*

subtilis strain BR_2CL and*Paenibacillus dendritiformis strain BR_3XY*

3. $RSBT_{1234}$=The rice straw fermented by inoculant formulated using superior bacteria from termites, namely*Aneurinibacillus sp. strain BT_4LS, Aneurinibacillus sp. strain BT_5LG, Bacillus sp. strain BT_3CL* and*Bacillus sp. strain BT_8XY*
4. $RSBR_{12}T_{34}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Pseudomonas aeruginosa strain BR_9LS,*and *Bacillus subtilis strain BR_4LG, and superior bacteria from termites, namely Bacillus sp. strain BT_3CL* and *Bacillus sp. strain BT_8XY.*
5. $RSBR_{13}T_{24}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Pseudomonas aeruginosa strain BR_9LS* and*Bacillus subtilis strain BR_2CL, and superior bacteria from termites, namely*Aneurinibacillus sp. strain BT_5LG* and *Bacillus sp. strain BT_8XY.*
6. $RSBR_{14}T_{23}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Pseudomonas aeruginosa strain BR_9LS* and*Paenibacillus dendritiformis strain BR_3XY, and superior bacteria from termtes, namely: Aneurinibacillus sp. strain BT_5LG andBacillus sp. strain BT_3CL.*
7. $RSBR_{23}T_{14}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Bacillus subtilis strain BR_4LG* and *Bacillus subtilis strain BR_2CL*, and superior bacteri from termites, namely*Aneurinibacillus sp. strain BT_4LS* and *Bacillus sp. strain BT_8XY*
8. $RSBR_{24}T_{13}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Bacillus subtilis strain BR_4LG* and*Paenibacillus dendritiformis strain BR_3XY,* and superior bacteria from termites, namely*Aneurinibacillus sp. strain BT_4LS* and*Bacillus sp. strain BT_3CL*
9. $RSBR_{34}T_{12}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Bacillus subtilis strain BR_2CL* and*Paenibacillus dendritiformis strain BR_3XY,* and superior bacteria from termites, namely*Aneurinibacillus sp. strain BT_4LS* and*Aneurinibacillus sp. strain BT_5LG*
10. $JBR_{1234}T_{1234}$=The rice straw fermented by inoculant formulated using superior bacteria from bali cattle rumen fluid, namely *Pseudomonas aeruginosa strain BR_9LS, Bacillus subtilis strain BR_4LG, Bacillus subtilis strain BR_2CL* and*Paenibacillus dendritiformis strain BR_3XY,* and superior bacteria from termites, namely*Aneurinibacillus sp. strain BT_4LS, Aneurinibacillus sp. strain BT_5LG, Bacillus sp. strain BT_3CL* and*Bacillus sp. strain BT_8XY*
11. RSBRL=The rice straw fermented by inoculant formulated using 10% rumen fluid.

Variable Measure. The variables observed in this study were the nutrient content of rice straw silage (concentration of dry matter, organic matter, inorganic matter/ash, crude protein, lignocellulosic fiber fraction, such as NDF, ADF, ADL, cellulose, hemicellulose, insoluble lignin, and silica, silage metabolites product (pH, totally VFA, N-NH_3), and dry matter and organic matter in vitro digestibility of rice straw silage.

Procedure Analysis.

Evaluation of Nutrients Content of Rice Straw

The nutrients content of rice straw observed in this study was the content of dry matter/BK, organic matter/BO, inorganic matter, crude protein/PK, and cell wall components/crude fiber fraction consisting of Neutral Detergent Fiber/NDF, Acid Detergent Fiber /ADF, Acid Detergent Lignin/ADL, cellulose, hemicellulose, insoluble lignin and silica.

Before the nutrient content analysis was carried out, the feed sample was prepared by drying the sample at 70^{o}C in a Drough Force Oven for 48 hours using an aluminum cup with a known weight, and then milling the sample with a 1 mm diameter sieve. This finely ground sample is used for nutrient content analysis. Evaluation of the dry matter content, inorganic matter and organic matter of the sample was carried out by proximate analysis based on the Weende method (AOAC, 2005) [13, 14]. The dry matter content of the feed sample was determined by heating the sample at a temperature of 105 - 110^{o}C oven for 9 - 12 hours until the sample weight was constant. The inorganic matter content (ash) was determined by burning the feed sample in a kiln at 600^{o}C for 3 hours until the sample turned to ash which was marked with a grayish white color without any black spots. The organic matter content of the sample is the difference between the dry matter of the sample and the inorganic matter. Meanwhile, the crude protein content of the samples was analyzed using the semi-micro Kjeldahl method using the VapodestKjeldatherm from Gerhardt [15, 16, 17].

Determination of the content of fiber fractions/cell wall components of feed samples (NDF, ADF, ADL, cellulose, hemicellulose, in-soluble lignin and silica) was carried out by means of Van Soest analysis (AOAC Official Method 973.18) [18, 19]. Neutral Detergent Fiber/NDF which is a reflection of all feed cell wall components or insoluble feed components (lignocellulosic and inorganic/insoluble minerals) were analyzed by cooking/heating with boiling neutral detergent solution for 1 hour followed by washing. rinsing using hot

aquadest, alcohol and acetone. Undissolved organic matter is a component of neutral detergent fiber/NDF from the tested feed samples. Acid detergent fiber/ADF (acid detergent fiber/SDA) is part of the cell wall of the feed that is difficult to dissolve, which is a reflection of cellulose, lignin and dissolved inorganic materials, determined by heating the feed sample for 1 hour using an acidic detergent solution with the main composition Cetyltrimethylammonium Bromide (CTAB) and followed by rinsing using hot distilled water, alcohol and acetone. The dry residue obtained from the oven at a temperature of 105^{o}C for 6 hours is the ADF component of the sample. Hemicellulose concentration was calculated from the difference between NDF and ADF concentrations. Determination of acid detergent lignin/ADL which is a continuation of the process of determining acid detergent fiber/ADF is carried out by soaking the dry residue of ADF for 3 hours using 72% H_2SO_4 to cover all residues and accompanied by stirring every hour. After three hours, the residue was washed with hot distilled water until it was free from acid (pH 7) and continued with drying in an oven at 105^{o}C for 6 hours. The resulting dry residue weight is a reflection of the ADL content of the sample. The difference between the dry weight of the ADF residue and the dry weight of the ADL residue is the cellulose component of the sample. Meanwhile, the content of insoluble lignin and silica was determined by burning the dry residue of ADL in a kiln at 600^{o}C for 3 hours. The ash obtained is the silica content of the sample, while the burnt organic matter is the insoluble lignin component of the sample.

Evaluation of Rice Straw Metabolite Product

Metabolite products from rice straw fermented that were evaluated in this study were pH, production of totally VFA and N-NH_3. Measurement of pH/acidity degree of fermented rice straw using pH meter, for the first, the samples was carried out by first soaking 50 grams of feed silage (feed fermented by research inoculants) with 100 ml of distilled water in a closed bottle container and left for 24 hours at a cold temperature (refrigerator). Then filtered and measured the pH of the sample using a digital pH meter WTW pH3210.

The totally VFA production was analyzed by steam distillation method following the General Laboratory Procedure [20]. The analysis activity was carried out by first filtering the silage sample that had been prepared and taking 2.5 ml of the liquid portion of the sample silage and putting it into a distillator tube, then adding 0.5 ml of 15% H_2SO_4. Furthermore, it was distilled on Vapodestequipment using the VFA50 method (specifically the total VFA method) [16] and the results of the distillation were accommodated using an erlenmeyer which had been filled with 2.5 ml of 0.5 N NaOH to accommodate 150 ml. The distillate was titrated with 0.1002N HCl with 1 drop of Phenolphthaline/PP as an indicator until the end point of the titration. The same was done for blanks without samples. The total VFA concentration is calculated by the formula:

$$\text{Totally VFA (mM)} = \frac{\text{(Volum titran blanco – Volum titran sample) x N HCl x 1000}}{\text{volum sampel (ml)}}$$

The N-NH3 concentration of silage samples was analyzed using the phenol hypochlorite method with the aid of a spectrophotometer (Department of Dairy Science, 1966) [21, 22]. The analysis was carried out by first making a curve and an equation for the standard ammonia regression line with normality 1 on a spectrophotometer with a wavelength of 640 nm. In this study the resulting regression equation is y = 3.8236x - 0.0835 (R^2 = 0.994). In addition, a 10% phenol solution, 0.5% sodium nitroprusside solution and an oxidizing solution were also prepared. Sample analysis was carried out by inserting 5 ml of sample solution (the result of sample preparation using the Lana method, 1998) into a spectrophotometer tube. Furthermore, 0.2 ml of phenol solution, 0.2 ml of sodium solution, nitroprusside and 0.5 ml of oxidizing solution were added. The absorbance reading of the sample was carried out after 5 minutes of adding the oxidizing solution using the cuvette of the spectrophotometer and then the N-NH3 concentration of the sample was determined based on the regression equation formula from the standard.

Evaluation of Dry Matter and Organic Matter In- Vitro Digestibility of Rice Straw

Evaluation of dry matter and organic matter digestibility in vitro from rice straw was carried out using the Minson & Mc Leod method [22, 23]

Analytical activities were carried out by first preparing 0.25 grams of the milled sample into an in-vitro tube. Then it was added with 25 ml of rumen fluid which had been mixed with buffer solution in a ratio of 1: 4. The sample mixture with rumen fluid was then incubated in a shaking bath for 48 hours at 39oC and every 6 hours was shaken to remove the gas formed. After incubation, the sample was centrifuged at 3000 rpm for 15 minutes and the supernatant was sucked/discarded with the help of a vacuum pump and then rinsed with distilled water 2 times by centrifuging the rinsed sample. Furthermore, the residue obtained was added to 25 ml of pepsin solution in HCl and incubated again for 48 hours at 39^{o}C in a shaking bath and shaken every 6 hours. Then

again centrifuged and rinsed with distilled water 2 times and the residue obtained was transferred to a porcelain dish and dried in the oven and continued in the ashing process to obtain a dry residue and ash residue.

Statistical Analysis. The resulting data will be analyzed with variance/analysis of variance (Anova). If there are results that are significantly different (P<0.05) between treatments, the analysis is continued with the Honestly Significant Difference test [24].

III. Resultand Discussion

Utilization of selected lignocellulolytic bacteria from the rumen fluid of Bali cattle and termites, namely *Pseudomonas aeruginosa strain BR$_9$LS* (R_1), *Bacillus subtilis strain BR$_4$LG* (R_2), *Bacillus subtilis strain BR$_2$CL* (R_3) and *Paenibacillusdendritiformis strain BR$_3$XY* (R_4), while the superior termite bacterial isolates consisted of *Aneurinibacillus sp. strains BT$_4$LS (*T_1*), Aneurinibacillus sp. strains BT$_5$LG* (T_2), *Bacillus sp. strains BT$_3$CL* (T_3) and *Bacillus sp. strain BT$_8$XY* (T_4)as rice straw starter was able to produce quality silage with higher crude protein content and significantly different (P<0.05) (except treatment $RSJBR_{1234}$ and $RSBT_{1234}$; P>0.05) compared to fermented rice straw silage without lignocellulolytic bacteria/$RSBR_0T_0$ inoculants, and with dry matter content, organic and inorganic matter content was not significantly different (P>0.05). Rice straw fermentation using rumen fluid inoculants of Bali cattle/RSBRL also produced rice straw silage with higher nutrient content and significantly different from $RSBR_0T_0$ (Table 4).

Table 4 Nutrients Content of Rice Straw Fermented by Formula Inoculant Research

Fermented Rice Straw[1]	Nutrients Content				
	Dry Matter (% fresh basis)	Dry Matter (% DW basis)	Organic Matter (% DM basis)	Anorganic Matter (% DM basis)	Crude Protein (% DM basis)
$RSBR_0T_0$[2]	15.509^{def4}	95.531^{a}	78.029^{a}	21.971^{a}	3.387^{a}
$RSBR_{1234}$	15.392^{de}	95.592^{a}	79.062^{a}	20.938^{a}	3.640^{ab}
$RSBT_{1234}$	14.808^{b}	95.574^{a}	78.037^{a}	21.963^{a}	3.661^{ab}
$RSBR_{12}T_{34}$	15.345^{cd}	95.607^{a}	78.480^{a}	21.520^{a}	3.786^{b}
$RSBR_{13}T_{24}$	15.539^{ef}	95.654^{a}	78.089^{a}	21.911^{a}	3.863^{b}
$RSBR_{14}T_{23}$	14.749^{b}	95.520^{a}	79.171^{a}	20.829^{a}	3.897^{b}
$RSBR_{23}T_{14}$	15.857^{g}	95.115^{a}	79.712^{a}	20.288^{a}	4.317^{c}
$RSBR_{24}T_{13}$	15.582^{f}	95.478^{a}	79.189^{a}	20.811^{a}	4.245^{c}
$RSBR_{34}T_{12}$	15.199^{c}	95.721^{a}	79.061^{a}	20.939^{a}	4.233^{c}
$RSBR_{1234}T_{1234}$	14.046^{a}	95.695^{a}	78.373^{a}	21.627^{a}	4.219^{c}
RSBRL[3]	15.385^{de}	95.277^{a}	78.599^{a}	21.401^{a}	4.242^{c}
SEM[5]	0.034	0.212	0.485	0.485	0.061

Note: [1)]Rice straw fermented by formula inoculant research which formulated by lignocellulolytic bacteria isolated from bali cattle rumen fluid ® and termites (T), such as: R_1=*Pseudomonas aeruginosa strain BR$_9$LS*. R_2=*Bacillus subtilis strain BR$_4$LG*. R_3=*Bacillus subtilis strain BR$_2$CL*. R_4=*Paenibacillusdendritiformis strain BR$_3$XY*. R_{1234}=Kombinasi R_1;R_2;R_3 and R4. T_1=*Aneurinibacillus sp. strain BT$_4$LS*. T_2=*Aneurinibacillus sp. strain BT$_5$LG*. T_3=*Bacillus sp. strain BT$_3$CL*. T_4=*Bacillus sp. strain BT$_8$XY*. [2)]$RSBR_0T_0$=Rice straw fermented by inoculant without lignocellulolytic bacteria (only by inoculant medium).[3)]RSBRL=Rice straw fermented by 10% fresh rumen liquor. [4)]The same superscript on same colom shows non-significant differences *(P>0.05),* [5)] *SEM=Standard Error of The Treatment Means*

Table 4 shows that the utilization of superior lignocellulolytic bacteria isolated from the rumen fluid of Bali cattle and/or termites was able to produce rice straw silage with a crude protein content of 7.45 – 27.46% higher and significantly different (P<0.05; except $RSBR_{1234}$ and $RSBT_{1234}$) compared to that produced by inoculants. BR_0T_0 which has a crude protein content of 3.387%. Silage $RSBR_{23}T_{14}$ is a rice straw silage with the highest crude protein content of 4.317% which is not significantly different (P>0.05) with the formula of rice straw silage $RSBR_{24}T_{13}$,RSBRL,$RSBR_{34}T_{12}$ and $RSBR_{1234}T_{1234}$. This shows the high supply of protein derived from microbial body cells (lignocellulolytic bacteria) from the inoculant used as a response to the synergism of the lignocellulolytic bacterial inoculant formula, especially $BR_{23}T_{14}$ which results in high growth and population of inoculant bacteria so that the supply of microbial protein is also high which will increase rice straw silage protein content [25, 26, 27]. Beck *et al.* [26] revealed that microbial body cells are composed of various important macro and micro nutrients, such as proteins, carbohydrates, fats, and various inorganic materials. Even Zeibick*et al* [27] added that more than 50% of bacterial biomass is composed of protein. Meanwhile, there was no significant difference (P>0.05) in the dry matter, organic matter and inorganic matter content, indicating that there was no lost nutrients (nutrient leaching) in the ensilage process and even quantitatively, the dry matter and organic matter content tended to increase (Table 4).

The use of microbial culture inoculants, both superior lignocellulolytic bacteria and Bali cattle rumen fluid inoculants in rice straw ensilases, was proven to be able to breakdown the lignocellulosic fiber fraction of rice straw as indicated by the presence of lignocellulosic fiber components, both NDF (neutral detergent fiber). ADF (acid detergent fiber), ADL (acid detergent lignin), cellulose, hemicellulose, insoluble lignin and lower silica respectively 18.34 – 27.91%; 21.51 – 30.46%; 22.49 – 35.07%; 20.69 – 26.71%; 11.12 – 22.10%; 17.56 – 28.72% (Table 5.2.10) compared to the lignocellulosic fiber content of rice straw before fermentation.

Fermentation using inoculants without bacterial culture (BR_0T_0) also reduced the lignocellulosic fiber fraction of rice straw, but with a lower percentage compared than fermentation using superior lignocellulolytic bacterial culture inoculants of bali cattle rumen fluid and/or termites, each by 11.87%. 13.01%. 14.20%. 12.03%. 9.26%. 15.09% and 12.91% of the content of NDF, ADF, ADL, Cellulose, hemicellulose, lignin and silica in rice straw (Table 5).

The decrease in the content of the lignocellulosic fiber fraction from rice straw was a response to the activity of the lignocellulase enzyme produced by lignocellulolytic bacteria found in the research inoculants. The more effective and synergistic the lignocellulase activity of the inoculant, the higher the lignocellulosic fiber conversion ability produced [2, 6]. Lignocellulase enzyme activity such as ligninase. endoglucanase. Exoglucanase and xylanase produced by superior lignocellulolytic bacteria inoculants of bali cattle rumen fluid and/or termites and Bali cattle rumen fluid inoculants have remodeled lignocellulosic fibers from rice straw as indicated by a decrease in lignocellulosic fiber content from rice straw silage, either NDF, ADF, ADL, cellulose, hemicellulose, insoluble lignin and silica (Table 5) and an increase in the production of simple compounds such as VFA and N-NH_3 accompanied by a decrease in the pH of the resulting silage (Table 6).

Table 5. The Lignocellulose Fraction on Fermented Rice Straw by Research Inoculant

Fermented Rice Straw[1]	Lignocellulose Fraction (%)						
	NDF	ADF	ADL	Cellulose	Hemi-cellulose	*insoluble* Lignin	Silica
$RSBR_0T_{01}$[2]	72.481^{f4}	49.724^{d}	22.025^{e}	27.698^{b}	22.758^{h}	12.950^{f}	9.076^{g}
$RSBR_{1234}$	67.157^{e}	44.864^{c}	19.898^{d}	24.966^{a}	22.293^{g}	11.307^{e}	8.590^{fg}
$RSBT_{1234}$	66.775^{e}	44.513^{bc}	19.541^{cd}	24.972^{a}	22.262^{g}	11.137^{de}	8.404^{def}
$RSBR_{12}T_{34}$	66.120^{de}	44.128^{bc}	19.235^{bcd}	24.893^{a}	21.992^{f}	10.772^{cde}	8.462^{ef}
$RSBR_{13}T_{24}$	66.133^{de}	44.359^{bc}	19.719^{cd}	24.640^{a}	21.774^{e}	10.395^{bcd}	8.324^{cdef}
$RSBR_{14}T_{23}$	63.675^{cd}	42.166^{ab}	18.143^{abc}	24.023^{a}	21.509^{d}	10.097^{bc}	8.046^{bcde}
$RSBR_{23}T_{14}$	59.286^{a}	39.749^{a}	16.670^{a}	23.079^{a}	19.537^{a}	9.242^{a}	7.428^{a}
$RSBR_{24}T_{13}$	60.791^{ab}	40.815^{a}	17.236^{a}	23.580^{a}	19.975^{b}	9.622^{ab}	7.614^{ab}
$RSBR_{34}T_{12}$	61.345^{abc}	41.338^{a}	17.674^{ab}	23.663^{a}	20.008^{b}	9.801^{ab}	7.873^{abc}
$RSBR_{1234}T_{1234}$	62.565^{bc}	42.179^{ab}	18.178^{abc}	24.001^{a}	20.386^{c}	10.018^{abc}	8.160^{cdef}
RSBRL[3]	61.789^{abc}	41.535^{a}	17.651^{ab}	23.884^{a}	20.254^{c}	9.726^{ab}	7.926^{abcd}
SEM[5]	0.506	0.485	0.336	0.435	0.035	0.167	0.105

Note: [1)]Rice straw fermented by formula inoculant research which formulated by lignocellulolytic bacteria isolated from bali cattle rumen fluid ® and termites (T), such as: R_1=*Pseudomonas aeruginosa strain BR$_9$LS.* R_2=*Bacillus subtilis strain BR$_4$LG.* R_3=*Bacillus subtilis strain BR$_2$CL.* R_4=*Paenibacillusdendritiformis strain BR$_3$XY.* R_{1234}=Kombinasi R_1;R_2;R_3 and R4. T_1=*Aneurinibacillus sp. strain BT$_4$LS.* T_2=*Aneurinibacillus sp. strain BT$_5$LG.* T_3=*Bacillus sp. strain BT$_3$CL.* T_4=*Bacillus sp. strain BT$_8$XY.* [2)]$RSBR_0T_0$=Rice straw fermented by inoculant without lignocellulolytic bacteria (only by inoculant medium).[3)]RSBRL=Rice straw fermented by 10% fresh rumen liquor. [4)]The same superscript on same colom shows non-significant differences *(P>0.05),* [5)] *SEM=Standard Error of The Treatment Means*

Table 5 also shows that the lignocellulolytic bacterial inoculant formula $BR_{23}T_{14}$ produced rice straw silage with the lowest lignocellulosic content, including NDF, ADF, ADL, cellulose, hemicellulose, insoluble lignin and silica. This is a very positive signal indicating that the formula is an inoculant formula with the best synergistic bacterial consortium so that it can significantly reduce the lignocellulosic content of rice straw. This also shows the combination of the four bacteria, namely Bacillus subtilis strain BR_4LG. Bacillus subtilis strain BR_2CL from the rumen fluid of Bali cattle and Aneurinibacillus sp. BT_4LS strain. and Bacillus sp. strain BT_8XY bacterial isolate from termites is able to produce lignocellulase enzyme activity, namely ligninase, endoglucanase, exoglucanase and xylanase which are able to work simultaneously in a sustainable manner so that lignocellulosic compounds, both lignin, cellulose and hemicellulose from rice straw can be better degraded so that their concentration in silage is lower. This condition was also significantly supported by the highest yield of rice straw silage metabolites (Table and the highest in vitro dry matter and organic matter digestibility of rice straw silage (Table 7).

Chandra *et al.*[28] revealed that in an anaerobic environment, lignolytic microbes (bacteria) with their ligninase enzymes (lignin peroxide/Li-P, manganese peroxidase/Mn-P, versatile peroxidase/VP, lakase/Lac, and dye-decolorizing peroxidase/DyPs as well as various other types of ligninase enzymes) will remodel lignin compounds to form hydroxyl/phenol compounds (aromatic alcohols), carboxyl (including VFA), amines (including NH_3), organic minerals (organomethallic), CO_2, H_2O and CH_4, while cellulolytic and hemicellulolytic microbes and supported by non-sacarolytic bacteria with individual and/or multi-enzyme cellulosic activity which will degrade cellulose and hemicellulose to form simple sugars (glucose.xylosa.mannosa.dll) which will be fermented immediately to form organic acids, H_2, CO_2 and CH_4.

In this study, specifically for lignin compounds which are the main factor in the low degradation/digestibility of rice straw, which previously contained ADL of 25.672% and insoluble lignin of 15.252% (Table 3) by lignocellulolytic bacteria, rumen fluid of Bali cattle and/or termites and microbial culture of Bali cattle rumen fluid from the inoculants used was able to reduce by 22.49 - 35.07% and 25.86 - 39.40%,

respectively (Table 5) and produce VFA and N-NH_3 respectively by 85.537 - 108.967 mM and 5,459 - 8,797 mM with the acidity of silage 3.923 – 4.358 (Table 6). The inoculants formula $BR_{23}T_{14}$which produce using *Bacillus subtilis strain BR$_4$LG* and *Bacillus subtilis strain BR$_2$CL.* and so *Aneurinibacillus sp. BT$_4$LS strains. and Bacillus sp. strain BT$_8$XY* was able to produce rice straw silage with the lowest ADL and insoluble lignin content of 16.670% and 9.242% (Table 5) and produced 108.967 mM VFA and 8.797 mM N-NH_3 (Table 6).

The presence of *Bacillus subtilis strain BR$_4$LG* which has been known to have high lignolytic ability with the highest ligninase specific activity (Mudita *et al*., 2019) and is supported by the presence of *Bacillus subtilis strain BR$_2$CL, Aneurinibacillus sp. strains BT$_4$LS,* and *Bacillus sp. strain BT$_8$XY* which is also capable of producing ligninase enzymes. such as lignin peroxidase. manganese peroxidase/MnP. Laccase/Lac, dandye-decolorizing peroxidase/DyP [28, 29] thus supporting the production of high lignin turnover.

The presence of various extracellular ligninase enzymes (*Li-P, Mn-P., Lac., DyP*) will remodel lignin compounds including those found in rice straw during the fermentation/ensilase (anaerobic) process to form hydroxyl group compounds (phenol). carboxyl groups (including VFA). amino groups (including NH_3). and organic mineral compounds (*organomethallic/organotins*) [28].*Lignin peroxidase/Li-P* is the main catalyst in the reform of lignin compounds. will oxidize non-phenolic aromatic components which are the main constituents (±90%) of the lignin structure by means of electron transfer, disassembly of aromatic rings and the breakdown of various lignin chain bonds, especially the Cα-Cβ bonds of lignin molecules which are the main pathway for lignin reform [6, 29]. *Manganese peroxidase/Mn-P* will oxidize Mn^{2+} to Mn^{3+} and H_2O_2 which play a role in the cleavage of phenolic components from lignin, while *laccase/Lac* will break down phenolic compounds, oxidizing aromatic amines. and other compounds through the reduction of molecular oxygen to H_2O and the formation of free radicals [6, 30]. The presence of various ligninase enzymes produced by inoculants of rumen lignocellulolytic bacteria in Bali cattle and/or termites, especially in the formula $RSBR_{23}T_{14}$ resulted in a significant decrease in the concentration of lignin (ADL and insoluble lignin) (Table 5) and the highest total VFA and N-NH_3 and with low pH (Table 6).

Table 6. Metabolite Product and pH of Fermented Rice Straw by Research Inoculant

Fermented Rice Straw[1]	pH and Metabolite Product of Fermented Rice Straw		
	pH	N-NH_3 (mM)	VFA Total (mM)
$RSBR_0T_0$[2]	4.335[b4]	3.896[a]	80.064[a]
$RSBR_{1234}$	4.065[a]	5.459[b]	85.537[ab]
$RSBT_{1234}$	4.064[a]	5.631[b]	85.552[ab]
$RSBR_{12}T_{34}$	4.080[a]	6.672[bc]	88.543[bc]
$RSBR_{13}T_{24}$	4.279[b]	7.181[cd]	91.195[bc]
$RSBR_{14}T_{23}$	4.082[a]	8.142[de]	92.729[c]
$RSBR_{23}T_{14}$	3.923[a]	8.797[e]	108.967[d]
$RSBR_{24}T_{13}$	4.024[a]	8.403[de]	108.451[d]
$RSBR_{34}T_{12}$	4.013[a]	8.535[e]	108.213[d]
$RSBR_{1234}T_{1234}$	4.358[b]	7.563[cde]	106.529[d]
RSBRL[3]	4.036[a]	8.357[de]	108.021[d]
SEM[5]	0.032	0.257	1.324

Note: [1)]Rice straw fermented by formula inoculant research which formulated by lignocellulolytic bacteria isolated from bali cattle rumen fluid ® and termites (T), such as: R_1=*Pseudomonas aeruginosa strain BR$_9$LS.* R_2=*Bacillus subtilis strain BR$_4$LG.* R_3=*Bacillus subtilis strain BR$_2$CL.* R_4=*Paenibacillusdendritiformis strain BR$_3$XY.* R_{1234}=Kombinasi R_1;R_2;R_3 and R4. T_1=*Aneurinibacillus sp. strain BT$_4$LS.* T_2=*Aneurinibacillus sp. strain BT$_5$LG.* T_3=*Bacillus sp. strain BT$_3$CL.* T_4=*Bacillus sp. strain BT$_8$XY.* [2)]$RSBR_0T_0$=Rice straw fermented by inoculant without lignocellulolytic bacteria (only by inoculant medium).[3)]RSBRL=Rice straw fermented by 10% fresh rumen liquor. [4)]The same superscript on same colom shows non-significant differences *(P>0.05),* [5)] *SEM=Standard Error of The Treatment Means*

High concentrations of N-NH_3 from fermented rice straw (silage) produced by inoculants of superior lignocellulolytic bacteria in the rumen of bali cattle and/or termites ($RSBR_{1234}$; $RSBT_{1234}$; $RSBR_{12}T_{34}$; $RSBR_{13}T_{24}$; $RSBR_{14}T_{23}$; $RSBR_{23}T_{14}$; $RSBR_{24}T_{13}$; $RSBR_{34}T12$; $RSBR_{1234}T_{1234}$) and RSBRL was compared with rice straw silage $RSBR_0T_0$ (5,459 – 8,797 mM Vs 3,896 mM) (Table 6) other than as a response to the high level of degradation of lignin compounds from rice straw which were able to release amino/amine groups (-NH_2). also as a result of the overhaul of the protein from the rice straw itself as well as the protein of the dead microbial (bacteria) body.

The breakdown of cellulose and hemicellulose from fermented rice straw using superior lignocellulolytic bacteria inoculants of bali cattle rumen fluid and/or termites and Bali cattle rumen fluid inoculants also progressed well as indicated by a decrease in the neutral content of detergent fiber/NDF. acid detergent fiber/ADF. cellulose and hemicellulose respectively 18.34 – 27.91%. 21.51 – 30.46%. 20.69 – 25.71% and 11.12 – 22.10% (Table 5) of pre-fermented rice straw which contained 82.241% NDF. ADF 57.160%. cellulose 31.488% and hemicellulose 25.081% (Table 3). Rice straw fermentation using inoculants without culture/superior lignocellulolytic bacteria/BR_0T_0 was also able to reduce the cell wall fiber fraction (NDF, ADF,

cellulose and hemicellulose) of rice straw by 11.87% respectively 13.01%, 12.03% and 9.26% (Table 5 vs. Table 3).

In this research. The breakdown of fiber fractions especially cellulose and hemicellulose from rice straw during the fermentation process is the result of the activity of cellulase enzyme complexes (*endoglucanase, exoglucanase* and *glucosidase*), hemicellulase enzyme complexes, especially xylanase *(endoxylanase, exoxylanase, xylosidase*) produced by cellulolytic and hemicellulolytic bacteria [31] and supported by non-cellulolytic/non-hemicellulolytic enzymes, including *glucose-6-phosphatase, phosphoglucose isomerase, xylose isomerase, xylose kinase, pyruvate decarboxylase, alcohol dehydrokinase,* and other saccarolytic enzymes produced by non-cellulolytic bacteria [28]. On the research inoculants, Cellulase enzyme complex breakdown cellulose complexes, both amorphous and crystalline components of rice straw cellulose into glucose. The xylanase enzyme complex will remodel xylanose into its constituent compounds (xylose, mannose, arabinose, glucose, galactose), while non-cellulase and non-hemicellulase enzymes will ferment glucose, xylose, and mannose. arabinose and galactose into H_2 and CO_2, organic acids (acetic, propionic, butyric) so that the total VFA production from rice straw silage was high, namely 80,064 – 108,967 mM (Table 6).

The high effectiveness of rice straw fermentation by superior lignocellulolytic culture/bacteria inoculants was also shown by the production of rice straw silage which had a high acidity (low pH) of 3,933 – 4,335. Rice straw silage $RSBR_{23}T_{14}$, $RSBR_{34}T_{12}$, $RSBR_{24}T_{13}$, RSBRL, $RSBR_{1234}$, $RSBT_{1234}$, $RSBR_{12}T_{34}$, $RSBR_{14}T_{23}$ produced rice straw silage with a pH of 5.84 – 9.51% lower ($P<0.05$) than rice straw silage $RSBR_0T_0$ which had a pH of 4.335 (Table 6). This is a response to the activity of lignocellulolytic bacteria from the inoculants used which are proven to be able to breakdown lignocellulosic compounds in rice straw into hydrogen, CO_2 and organic acids which result in an increase in the concentration of hydrogen ions (H^{2+}) so that the degree of acidity of rice straw silage increases (pH drops).

Table 7. Dry Matter and Organic Matter In-Vitro Digestibility of Fermented Rice Straw by Research Inoculant

Fermented Rice Straw[1]	Dry Matter and Organic Matter In-Vitro Digestibility	
	DM Dig. (%)	OM Dig. (%)
$RSBR_0T_0$[2]	44.976[a4]	47.461[a]
$RSBR_{1234}$	46.760[ab]	50.113[b]
$RSBT_{1234}$	47.084[b]	50.076[b]
$RSBR_{12}T_{34}$	49.151[c]	52.045[bc]
$RSBR_{13}T_{24}$	50.175[cd]	53.509[cd]
$JRSBR_{14}T_{23}$	51.820[de]	54.988[de]
$RSBR_{23}T_{14}$	55.109[g]	57.932[g]
$RSBR_{24}T_{13}$	54.174[fg]	57.021[fg]
$RSBR_{34}T_{12}$	53.094[ef]	56.591[efg]
$RSBR_{1234}T_{1234}$	50.957[d]	55.045[def]
RSBRL[3]	52.957[ef]	56.566[efg]
SEM[5]	0.356	0.401

Note: [1)]Rice straw fermented by formula inoculant research which formulated by lignocellulolytic bacteria isolated from bali cattle rumen fluid ® and termites (T), such as: R_1=*Pseudomonas aeruginosa strain BR9LS.* R_2=*Bacillus subtilis strain BR4LG.* R_3=*Bacillus subtilis strain BR2CL.* R_4=*Paenibacillusdendritiformis strain BR3XY.* R_{1234}=Kombinasi R_1;R_2;R_3 and R4. T_1=*Aneurinibacillus sp. strain BT4LS.* T_2=*Aneurinibacillus sp. strain BT5LG.* T_3=*Bacillus sp. strain BT3CL.* T_4=*Bacillus sp. strain BT8XY.* [2)]$RSBR_0T_0$=Rice straw fermented by inoculant without lignocellulolytic bacteria (only by inoculant medium).[3)]RSBRL=Rice straw fermented by 10% fresh rumen liquor. [4)]The same superscript on same colom shows non-significant differences *(P>0.05),* [5)] *SEM=Standard Error of The Treatment Means*

Utilization of superior lignocellulolytic bacteria in the rumen of Bali cattle and/or termites as well as Bali cattle rumen fluid inoculants as a starter in the fermentation process proved to be able to produce rice straw silage with dry matter digestibility/DMD and higher organic matter digestibility/OMD in vitro ($P<0.05$) 3.97–22.53% and 5.51-22.06%, respectively, compared to rice straw silage produced using inoculants without culture/superior lignocellulolytic bacteria/$RSBR_0T_0$ which had DMD 44.975% and OMD 47.461% (Table 7). The table also shows that rice straw silage $RSBR_{23}T_{14}$; $RSBR_{24}T_{13}$ and $RSBR_{34}T_{12}$ have the best dry matter and organic matter digestibility.

The fermentation process uses lignocellulolytic bacteria inoculants, especially the inoculant formula with code $BR_{23}T_{14}$, $BR_{24}T_{13}$ and $BR_{34}T_{12}$ which are known to have qualities, especially high lignocellulase enzyme activity, will be able to accelerate the breakdown of complex compounds into simpler components and loosen the chain bonds that make up complex compounds so as to facilitate the work of substrate-degrading enzymes resulting in increased nutrient digestibility [2]. This is significantly seen from the presence of lignocellulosic fiber fraction in fermented rice straw products which is negatively correlated with dry matter digestibility and organic matter from rice straw silage. The results showed that the NDF content of rice straw silage had a high negative correlation with dry matter and organic matter digestibility, each of which followed the equation $Y= -0.827X + 103.8$ ($R^2=0.914$) and $Y= -0.872X + 109.9$ ($R^2 =0.938$). In the presence of cellulose,

dry matter and organic matter digestibility of rice straw silage had a high negative correlation which followed the equations Y= -2.362X + 108.4 (R^2=0.792) and Y= -2.525X + 115.6 (R^2=0.834) . The hemicellulose content of rice straw silage has a high negative correlation with dry matter and organic matter digestibility which follow the equations Y= -2.693X + 107.5 (R^2=0.879) and Y= -2.828X + 113.6 (R^2=0.893). while for the concentration of insoluble lignin, dry matter and organic matter digestibility of rice straw silage had a high negative correlation that followed the equation Y= -2.966X + 81.59 (R^2=0.897) and Y= -3.150X + 86.71 (R^2=0.933) (Figure 1 - 2).

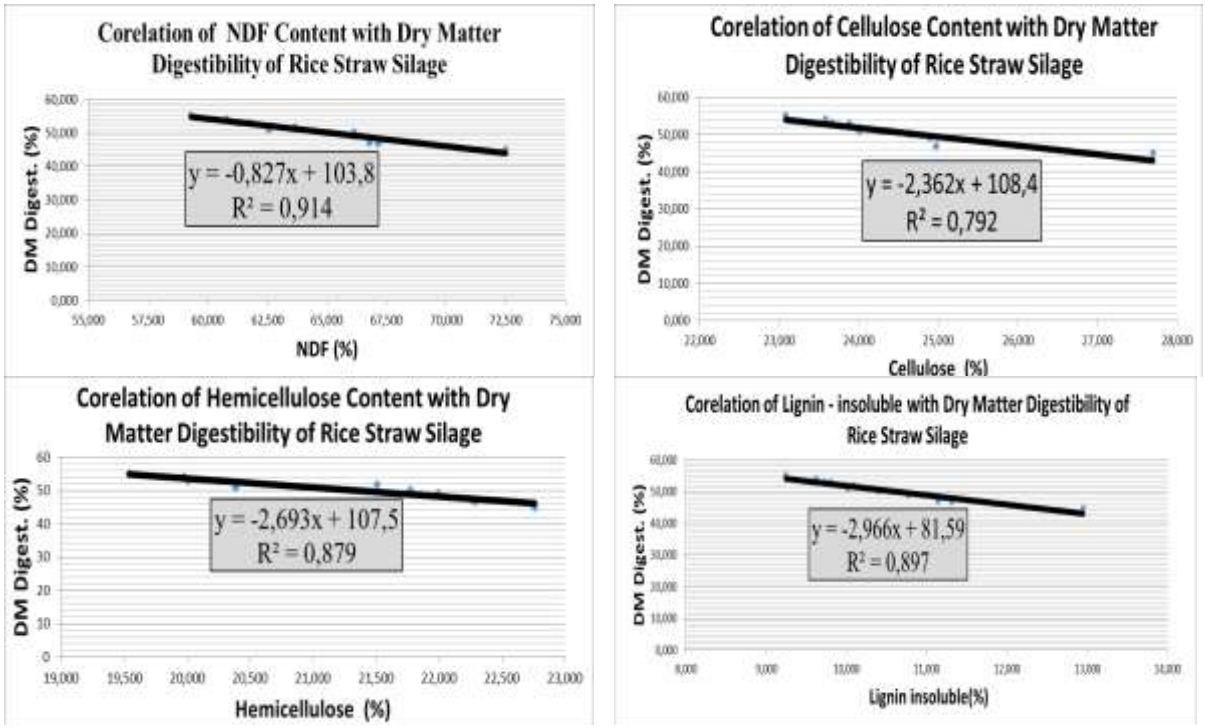

Figure 1.Correlation of Lignocellulosic Fiber Content with Dry Matter in-vitro Digestibility of Fermented Rice Straw by Research Inoculants

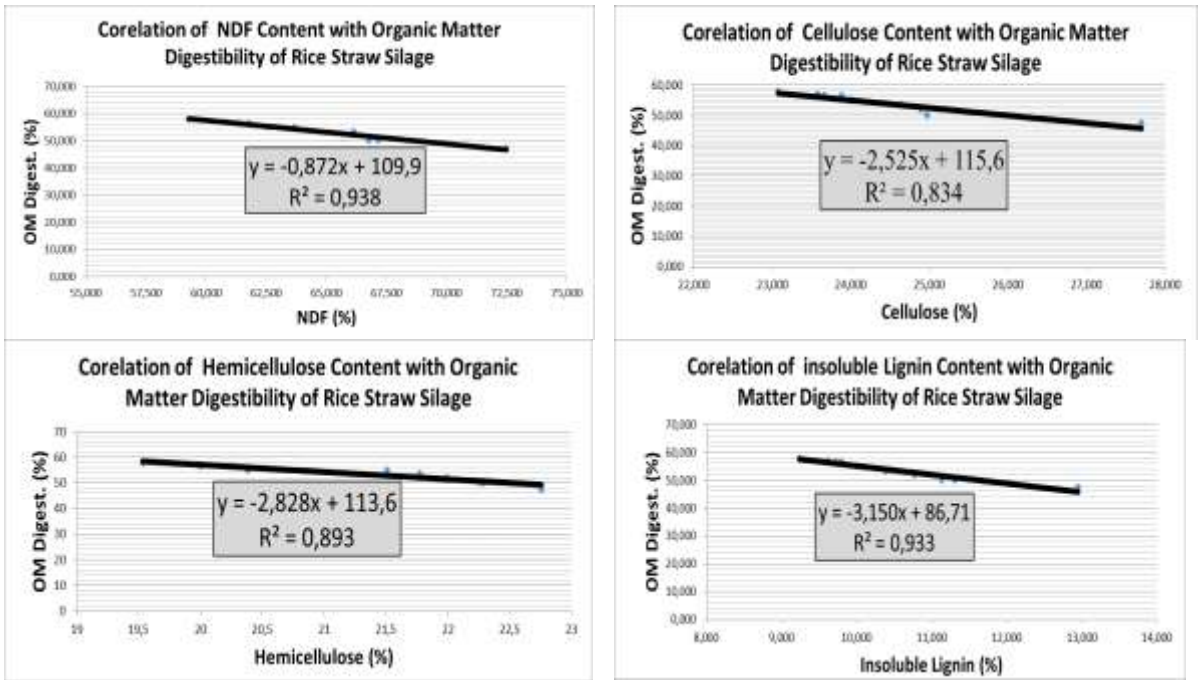

Figure 2.Correlation of Lignocellulosic Fiber Content with Organic Matter *in-vitro*Digestibility of Fermented Rice Straw by Research Inoculants

Figures 1 and 2 clearly show that the presence of lignocellulosic fiber will greatly affect the nutrient digestibility of a feed ingredient. The lower the lignocellulosic fiber content of a feed ingredient, the higher the digestibility of dry matter and organic matter. So that efforts to utilize feed ingredients from agricultural waste such as rice straw must be accompanied by efforts to reduce the lignocellulosic fiber content of these feed ingredients. Utilization of inoculants containing lignocellulolytic bacteria that are able to work synergistically will produce quality feed silage that can be used as a source of nutrients for livestock. In this research, formula inoculant $BR_{23}T_{14}$is believed to be best bacterial inoculants with the most synergistic activity so that they are suitable for optimizing the use of agricultural waste such as rice straw as animal feed.

IV. Conclusion

Based on the results of the study, it can be concluded that the use of superior lignocellulolytic bacteria inoculants from the rumen fluid of Bali cattle and termites was able to increase the breakdown of lignocellulosic compounds in rice straw to produce high quality rice straw silage with high nutrient content, metabolite production and digestibility of dry matter and organic matter in vitro. The inoculant formula BR23T14 (an inoculant formulated using Bacillus subtilis strain BR4LG and Bacillus subtilis strain BR2CL from Bali cattle rumen fluid and Aneurinibacillus sp. strain BT4LS and Bacillus sp. strain BT8XY from termites) was the best inoculant capable of producing rice straw silage with high levels of the highest turnover of lignocellulosic fibers.

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