ISBN : 978-602-14917-9-9

QUALITY OF RICE STRAW SILAGE WITH VARIOUS ADDITIVES

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Abstract. Rice is a staple food for Indonesian people. Rice straw is waste produced from rice plant when harvesting. Rice straw can be utilized as feed for livestock especially ruminant. Silage is fermentation technology for preserving roughage such as rice straw. In making silage, additive is needed to maintain lactic acid bacteria for successful fermentation. The aim of this study was to evaluate the effects of various additives (rice bran, glucose and molasses) for three different varieties of rice crop. Rice varieties used were Inpari 30, Inpari NutriZinc and Rindang. This study was conducted in a Completely Randomized Design (CDR) with 4 treatments and 3 replications. Each variety was given treatment T0 = Rice Straw + Lactic Acid Bacteria (*Lactobacillus plantarum* 1A2); T1 = T0 + Rice Bran 5%; T2 = T0 + Glucose 2%; T3 = T0 + Molasses 5%. The parameter observed were, pH, temperature, dry matter, ash, crude protein, crude fat and crude fiber. The data obtained are analyzed by analysis of variance (ANOVA) followed by Duncan Test. The results shown that the treatments significantly affect on pH, dry matter, ash, crude protein, crude fat and crude fiber. It was concluded that Inpari 30 was the best variety because it had the highest crude protein content 10.24% and rice bran was the best additive to produced best quality silage compared with other additives.

1. Introduction

Indonesia is a tropical country that has two seasons, dry and wet season. The agriculture sector can be improved throughout the year with good management. Indonesia has many lands that have a lot of potentials to plant food crops or forage. The increasing economic sector affects the usage of land to build housing or industry. The limited land to utilized as a forage field makes the local farmer have to think the best solution to fulfill the need for forage, especially in the dry season.

The staple food in Indonesia is rice and one of the waste from rice crop is rice straw. Rice straw is produced around 50% of the harvested grain production. The comparison between the weight of grain harvested with straw at harvest is generally 2:3 [1]. Statistic Indonesia [2] reported that rice production in Indonesia in 2018 amounted to 56 million tons of dry grain so that it is estimated that 84 million tons of rice straw are produced It is abundant since it is not fully utilized. Rice straw can be a solution for the local farmer as forage. Therefore rice straw has disadvantages by containing high crude fiber such as lignocellulose and silica. The feed processing technique is needed to increase the feeding value of rice straw.

The fermentation technique is a technology that can be applied to rice straw by storing roughage in anaerobic conditions. In making silage add exogenous lactic acid bacteria (LAB) and additive are commonly applied to improve the feeding value of low quality roughage. LAB are commonly used as silage inoculant to improve silage fermentation and inoculation rates of 10^5 to 10^6 CFU/g are often recommended [3,4]. There are several LAB that can be used in making silage and one of them is *Lactobacillus plantarum* 1A2. Additive play an important role in the fermentation process to

ISBN : 978-602-14917-9-9

achieve acceptable silage quality. The common additives used in silage are rice bran, glucose, and molasses. Molasses has been shown to decrease pH and ammonia levels in silage [5]. Application of *Lactobacillus plantarum* and additive is one of method to improve the fermentation process that can increase acidic conditions. Improved fermentation process can increase quality of silage. The adequate *Lactobacillus pantarum* would affected the fermentation and efficient utilization of substrate in material. The inoculant would increase lactic acid production and it will decrease the pH of silage. Then it can make fermentation process faster. So this study conducted to evaluate the quality of rice straw silage with various additives.

2. Materials and methods

2.1. Date and Place

This research was conducted from Agustus 2019 to November 2019. The location of silage making and proximate analysis at the Research Center for Biotechnology, Indonesian Institute of Sciences, Bogor.

2.2. Materials

The fresh rice straw varieties used were Inpari 30, Inpari NutriZinc and Rindang from National Research Center for Rice Plants, Sukamandi, Subang and harvested at approximately 120 days after planting. Rice bran, glucose and molasses from local market in Bogor. Innoculant of *Lactobacillus plantarum* 1A2 was from the Research Center for Biothecnology, Indonesian Institute of Sciences.

2.3. Methods

2.3.1. Ensiling

The fresh rice straw obtained after rice crop harvested at about 9 a.m and air-dried for wilting. The rice straw were chopped to length of approximately 5 cm. The chopped rice straw were mixed with inoculum of *Lactobacillus plantarum* 1A2 of $1x10^6$ CFU/g silage materials. Additives were applied in different laboratory-scale silos (about 700 g). Adding rice bran 5% for T1, glucose 2% for T2 and molasses 5% T3. T0 = Rice Straw + Lactic Acid Bacteria (*Lactobacillus plantarum* 1A2); T1 = T0 + Rice Bran 5%; T2 = T0 + Glucose 2%; T3 = T0 + Molasses 5%. The silos incubated at room temperature for 60 days.

2.3.2. Silage preparation and analysis

After 60 days incubated at room temperature, silos were opened. There were 36 samples and every sample was divided into 2 parts for prepraration. First part was oven-dried at 60^{o}C and milled to pass through a 0.5 to 1 mm aparture sieve for proximate analysis. The second part, sample was extracted for silage juice. Silage juice was made use blender with the addition of sterilized distilled water to the silage sample (1:1 w/v); the juice was filtered using sterilized double cheese clothes and placed into a 50 ml sterilized corning tube.

2.3.3. Silage quality evaluation

Silage samples were analyzed with chemical measurment using the proximate standard procedure of AOAC (2005) and followed the manufacturer's procedure of FOSS instrument (Hoganas, Sweden). Dry matter (DM) analysis was analyzed using oven (Thermo scientific), Ash analysis was analyzed using furnace (Heraeus Instrument), Crude protein (CP) was analyzed using Kjeltec^{TM} 8400 (FOSS), Crude fat (CF) was analyzed using Soxtec^{TM} 2050 (FOSS) and Crude fiber (CF) was analyzed using Fibertec^{TM} 2010 (FOSS). Other parameters were also tested including pH using Cyberscan pH310 Eutech, LAB population was determined by total plate count (TPC) using MRS agar incubated on temperature 37^{O}C and counted after 48 hours [6].

ISBN : 978-602-14917-9-9

2.3.4 Experiment design

The experiment design used in this study was a completely randomized design (CRD) with two factor. Three varieties, four treatments and three replication. Data were analyzed by using ANOVA with SPSS 16 for Windows. Significant effects of each treatment were further analyzed by using the least significant difference by Duncan Test ($P<0.05$).

2.3.5 Treatment and parameter

The treatmen in this study was T0 = rice straw + *Lactobacillus plantarum* 1A2, T1 = T0 + rice bran 5%, T2 = T0 + glucose 2%, T3 = T0 + molasses 5%. The parameters observed were pH, temperature, total plate count and nutrient content.

3. Results and discussion

Overall, the study shown the quality were good. Besides the parameters of silage, the physical characteristic of silage was assassed by the texture and color. Either texture nor color shown good quality. The texture andwas not too wet and the color was brown. There were less percentage of fungi found in the silage.

3.1 pH and temperature

The quality of silage was affected by condition inside the silo. pH and temperature play an important role on it. The condition inside the silo is illustrated from the data on the pH and temperature inside the silo presented in table 1.

Table 1. pH and temperature

Parameter	Variety	T0	T1	T2	T3	Average
pH	R	4.67±0.55	4.30±0.30	4.26±0.55	3.83±0.01	4.26±0.15^{c}
	I NZ	4.20±0.02	4.48±0.06	4.23±0.30	3.91±0.05	4.20±0.15^{b}
	I 30	5.15±0.13	4.44±0.13	4.49±0.12	4.18±0.13	4.57±0.01^{a}
	Average	4.67±0.48^{a}	4.41±0.10^{b}	4.33±0.14^{c}	3.97±0.19^{d}	
Temperature (^{O}C)	R	28.67±1.15	28.00±0.00	28.00±1.00	28.00±0.00	28.17±0.63
	I NZ	28.33±0.58	28.33±0.58	28.67±0.58	28.33±0.58	28.42±0.00
	I 30	28.33±0.58	28.00±0.00	28.00±0.00	28.00±0.00	28.08±0.33
	Average	28.44±0.19	28.11±0.19	28.22±0.38	28.11±0.19	

R = Rindang, I NZ = Inpari NutriZinc, I 30 = Inpari 30; T0 = rice straw + *Lactobacillus plantarum* 1A2, T1 = T0 + rice bran
5%, T2 = T0 + glucose 2%, T3 = T0 + molasses 5%; Different letter in the same column are significantly different ($P<0.05$)
in each parameter.

The pH after the silage harvested shown varieties and addtives significantly different ($P<0.05$) shown in table 1. The highest pH was reached by inpari 30 variety with 4.57 and the lowest was rindang with 4.26. It was influenced by microbes activity in fermentation process. Muck and Kung [7] reported that the treatment with lactic acid bacteria inoculant can result in a lower pH compared with untreated inoculant on because lactic acid produced greater. During ensiling, lactic acid produced by LAB, the highest concentration of lactic acid in silages, it contributed the most to the decline in pH [8]. Referring to it, the addition of additive increase the water soluble carbohydrate (WSC) so population of microbes especially *Lactobacillus plantarum* 1A2 so the production of lactic acid got higher and the pH decrease. The lower pH reached in the silo the higher microbes activity. The addition of additive shown that control had the highest on pH 4.67 and molasses had the lowest pH

ISBN : 978-602-14917-9-9

3.97. It occurred because there was less simple sugar to consumed by microbe so it had lower activity then silage added by molasses. Trabi et al. [9] reported the addition of glucose 2% could decreased the pH in silage compared with control.

The heat produced during silage fermentation was a normal occurrence. The measurement of temperature shown not significantly different (P>0.05). Eventhough there was no effect of varieties and treatments on temperature, the range of the temperature still on normal. Kung et al. [8] reported after the active phase of fermentation was complete, it was about 25^{O}C to 30^{O}C. This condition related how silage was made, if there were too many air trapped inside the silo an oxidation from aerobic miroorganisms the temperature would increased. It was effected the quality of silage, the higher temperature reached inside the silo it could damaged protein on substrate. The roughage should be chopped, packed and sealed tightly as soon as possible [8].

There were correlation between pH and temperature. After silage feed-out aerobic detertoration occurred. The air penetrated into silage mass result in the growth of yeast. It increased the temperature and followed by increase in silage pH.

3.2 Total plate count

Counting of microbe in sampel needed to knew the colony forming unitt. There was not significantly different of varieties and additives (P>0.05). The data on the effect of addition various additive and different varieties can be seen in table 2.

Table 2. Total plate count (TPC)

Parameter	Variety	T0	T1	T2	T3
TPC (CFU/ml)	R	9.03 x 10^5	8.55 x 10^5	5.54 x 10^5	5.28 x 10^5
	I NZ	4.08 x 10^5	4.87 x 10^6	2.51 x 10^7	4.72 x 10^5
	I 30	6.73 x 10^6	1.26 x 10^7	5.72 x 10^6	5.56 x 10^5

R = Rindang, I NZ = Inpari NutriZinc, I 30 = Inpari 30; T0 = rice straw + *Lactobacillus plantarum* 1A2, T1 = T0 + rice bran 5%, T2 = T0 + glucose 2%, T3 = T0 + molasses 5%.

The population on the table shown the addition of glucose had the highest population 2.51x10^7 CFU/ml. It was indicated glucose could provide microbes especially BAL as source of energy. The result shown that BAL could survive on rice straw even without additive. Based on data with specific of variety and additive rice straw silage like inpari 30 and inpari nutrizinc with addition of rice bran and glucose had the more population than other treatment. Adding glucose supplied LAB with enough fermentation substrate that could enhance LAB growth and accelerated lactic acid accumulation [10]. Various studies indicated that inoculant sometimes had a positive probiotic effect on ruminant performance [11]. Based on that statement, the existence of *L.plantarum* on the rice straw silage had a positive effect of probiotic and could increase on performance.

3.3. Proximate analysis

Determining nutrient content in feedstuff usually used chemical analysis known as proximate analysis. There were significantly different on the treatment. The varieties shown significantly different on ash content, crude protein and crude fiber and the various additives shown significantly different on all parameters (P<0.05). The nutrient content presented on table 3.

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Table 3. Proximate analysis

Parameter	Variety	T0	T1	T2	T3	Average
Dry Matter (%)	R	96.49±0.52	95.99±0.86	95.02±0.52	94.84±0.22	95.59±0.79
	I NZ	95.52±0.84	96.17±0.32	94.62±0.84	93.82±1.23	95.03±1.03
	I 30	95.00±0.97	95.09±0.53	94.54±1.51	92.69±3.05	94.33±1.12
	Average	95.67±0.76^{a}	95.75±0.58^{a}	94.73±0.26^{ab}	93.78±1.07^{b}	
Ash (%)	R	18.53±1.66	16.83±1.23	17.75±0.21	17.07±0.65	17.54±0.77^{b}
	I NZ	23.72±0.88	21.90±1.14	22.94±0.13	21.69±0.32	22.56±0.94^{a}
	I 30	23.58±0.25	21.34±0.93	22.25±0.76	21.69±0.31	22.21±0.98^{a}
	Average	21.94±2.96^{a}	20.02±2.78^{c}	20.98±2.82^{b}	20.15±2.67^{c}	
Crude Protein (%)	R	9.14±0.32^{bc}	9.48±0.24^{b}	9.01±0.12^{bc}	8.63±0.21^{cd}	9.06±0.35
	I NZ	6.79±0.53^{g}	8.25±0.31^{de}	7.48±0.24^{f}	7.75±0.26^{ef}	7.56±0.61
	I 30	9.37±0.79^{b}	10.83±0.35^{a}	10.42±0.27^{a}	10.36±0.37^{a}	10.24±0.62
	Average	8.43±1.43	9.52±1.29	8.97±1.47	8.91±1.33	
Crude Fat (%)	R	2.25±0.11	4.49±1.54	2.43±0.59	2.17±0.73	2.84±1.11
	I NZ	2.80±0.21	5.67±0.84	2.25±0.28	1.92±0.27	3.16±1.71
	I 30	2.01±0.39	4.91±0.10	1.72±0.46	1.67±0.30	2.58±1.56
	Average	2.35±0.40^{b}	5.02±0.59^{a}	2.13±0.37^{b}	1.92±0.25^{b}	
Crude Fiber (%)	R	26.92±0.67	21.81±1.01	22.54±0.61	23.52±2.10	23.70±2.26^{a}
	I NZ	26.76±1.34	23.17±1.63	22.97±1.14	23.12±2.04	24.01±1.84^{a}
	I 30	22.54±0.69	20.64±1.05	20.09±0.30	18.34±2.47	20.40±1.73^{b}
	Average	25.41±2.48^{a}	21.87±1.27^{b}	21.87±1.55^{b}	21.66±2.88^{b}	

R = Rindang, I NZ = Inpari NutriZinc, I 30 = Inpari 30; T0 = rice straw + *Lactobacillus plantarum* 1A2, T1 = T0 + rice bran 5%, T2 = T0 + glucose 2%, T3 = T0 + molasses 5%; Different letter in the same column are significantly different (P<0.05) in each parameter.

On dry matter content, the addition of rice bran had the highest DM in 95.75% because the dry matter content of rice bran was higher than other additives and it could increased the dry matter on silage. On the other hand added molasses will reduces the DM because molasses contained less dry matter. The ash content of rice straw silage shown inpari nutrizinc had the highest ash content 22.56% and rindang had the lowest on 17.54%. It indicated that inpari nutrizinc had higher retention of minerals that the other varieties because inpari nutrizinc rice crop variety potentially produced zinc 34.51 ppm [12]. The treatment shown that addition of rice brand had the lowest ash content 20.02% and control had the highest ash content at 21.94%. It was affected by nutrient content on additives so the percentage of ash content lower than control which had not affected by others nutrient content. As the representative of feed quality crude protein is very important.

There were interaction between variety and treatment and shown that inpari 30 with addition of rice bran had the highest crude protein content 10.83%. It guessed that inpari 30 variety already had high content of crude protein itself and by adding rice bran which had high content were accumulated. On the opposite inpari nutrizinc with no addition of additive shown the lowest crude protein content at 6.79%. It suspected that it lacked of protein content in the substrate plus it was not added by additive. Crude fat content only shown the treatment with rice bran had the highest percentage 5.02% and additive molasses shown the lowest crude fat 1.92% even statistically not different with glucose and without additive. The activity of microbes inside the silo could effected the crude fiber content. It shown that treatment without additive had the highest crude fiber content percentage 25.41%. It guessed that without addition of additive as source of energy for microbes the activity of it was lower than others that was added by additive. On the other hand treatment with additive shown similar results statistically because with the addition of additives LAB slightly broke the fiber content. Reddy and

ISBN : 978-602-14917-9-9

Reddy [13] reported ensiling rice straw with addition of substrate could increase nutrient contents, rice straw silage applied by poulty excreta had crude protein 11.78% compared with control diet 4.46%.

4. Conclusion

Varieties and additives can significantly increase crude protein on rice straw silage. the best variety was inpari 30 and the best additive was rice bran. there was no influence on temperature and total plate count by a variety of rice straw and additives.

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