Biostarter formulated from the rumen bacterial of buffalo effectively change the chemical composition of the rice straw

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Abstract. The use of such agricultural by-products as rice straw is important in fulfilling the needs of feed for ruminants, especially during the dry season. However, the main problem of using rice straw as feedstuff is low digestibility due to the existence of the links between lignin and cellulose or hemicellulose. This study was designed to evaluate the effects of the application of biostarter (BS) developed from rumen bacterial of buffalo on the changes of the chemical composition of the rice straw under a different duration of fermentation time. The experiment was carried out in a factorial arrangement according to a completely randomized design. The first factor was different levels of BS application, i.e. rice straw without BS (a1), rice straw + 5% BS (a2), rice straw +10% BS (a3), and rice straw + 15% BS (a4). The second factor was the duration of the fermentation time, i.e., two weeks (b1) and four weeks (b2). Each treatment combination was repeated three times, giving the total number of the experimental unit was 24. The results of the study indicated that increased levels of BS had a significant (P<0.05) effect on the concentration of crude protein, crude fiber, ash, and nitrogen-free extract (NFE) of the rice straw but no effects (P>0.05) on lipid content. The length of the fermentation period only affected (P<0.05) the crude fiber and crude protein content but no effects (P>0.05) on NFE, lipid, and ash. There was no interaction between levels of BS and the duration of the fermentation time on the chemical components of the rice straw. In conclusion, the use of BS was successfully changing the chemical composition of rice straw.

1. Introduction
One of the key factors determining the level of production of ruminant livestock is the available forage in terms of quantity, quality, and continuity. In the current situation, the main problem faced is the limited production of forage due to the decreasing area of pasture for forage production, while forage is the main energy source for ruminant animals. Therefore, an alternative source of forage that could be used as feed for the ruminant animal has to seek out. The agricultural by-product, such cereal straw, has been long known as an alternative feed for ruminant, especially during the dry season. Indonesia especially South Sulawesi, has a huge potency of agricultural and agro-industrial byproduct. This potency could be optimized as an alternative feed for ruminants [1,2]. However, the use of such type
feedstuff has some inherent problems that need to be overcome. Low digestibility, low crude protein content, high fiber content, and imbalance of mineral contents are some of the factors that may reduce its function as feedstuff [3,4]. Therefore some types of processing are required before using them as animal feeding. However, one factor that should be considered in choosing one type of processing method is that the processed feedstuff should be comparable with the conventional feed in terms of nutrition and economics [5].

Processing of low quality of feedstuff can be conducted by processing such stuff by either physical, chemical, biological process, or its combination. Each method of processing has owned its advantages and disadvantages. Compared with the chemical processing method, the biological treatment of roughages offers the advantage of using fewer chemicals and lower energy input for processing. In addition, the biological treatment of fibrous feeds has other advantages, which include an improvement in the digestibility of the lignocellulose, an improvement in feed quality, and the destruction of harmful compounds present in the feedstuffs [5]. However, the clear disadvantages are much longer times required and the losses of readily degraded constituents of the substrate straws [5,6].

Among biological treatment, white-rot fungi (WRF) are commonly used. Those fungi are known to metabolize lignin, cellulose, and other fibrous components due to the presence of the several enzymes involved in the lignin degradation process, such as ligninases, phenol oxidases [7–9]. Some of the studies reported that the use of fungal consortium consisted of Aspergillus niger and Trichoderma viride can decompose cellulose, hemicelluloses, lignin, and total carbon significantly comparing the control, indicating the potential of this method for use in a large-scale composting of the rice straw [10–12]. Application of WRF on the rice straw enables this fermented rice straw to be used up to 70% to replace elephant grass in the ration of the goat [13]. In other studies, the use of WRF could improve the nutrient quality of corn stover [14] and the quality and digestibility of cocoa pods [15].

Besides fungi, several studies have reported the use of rumen bacterial isolates to improve the quality of high fiber feedstuff. The rumen contents are characterized by a high microbial population which has the capability to degrading the particulate matter such as cellulososes. Among the ruminants raised in South Sulawesi Province, buffalo have a capability in adapting and surviving in very poor feed conditions, which showing that rumen microbes of that animal have adapted in such conditions [16]. A study comparing the diversity of buffalo and cattle giving similar feed reported that the diversity of rumen bacterial of buffalo was much higher than that of cattle which implies that this complex mixed microbial or a consortium of rumen bacterial is very potential to be used as a biodecomposer or biostarter in the fermentation process [17,18]. Another study evaluated the effect of different inoculum sources on the rice straw anaerobic digestion and concluded that rice straw inoculated with digested manure achieved the highest enzyme activities due to the synergism between cellulase and xylanase activities which was very important in the lignocellulose degradation [19]. The application of rumen microbial culture of buffalo significantly improves dry matter and organic matter digestibility of rice straw in vivo and in vitro [20,21]. Another study reported that the fermentation quality of rice straw silage improved as the impact of the application of lactic acid bacteria [22]. In the more applicative term, the use of Pennisetum purpureum inoculated by rumen bacterial of buffalo significantly increased nutrient digestibility and performance of Frisian Holstein calves [23]. Generally, the biological treatment of high fiber materials is conducted through the fermentation of that stuff to allow an ample time of the microbes to work on the substrate. The fermentation process could be carried out either in anaerobic conditions or aerobic conditions [24,25]. The purpose of this study was to assess the change of chemical composition of rice straw as an impact of the application of biostarter developed from the rumen bacterial of buffalo fermented under anaerobic conditions at the different duration of the fermentation time.

2. Materials and methods

2.1. Biostarter formulation

The rumen fluid from three adult buffalos was used as a source of inoculant for biostarter formulation. The rumen fluid was taken a right after the animals were slaughtered. The rumen fluid was directly
placed into the container prepared in advance and directly transferred into the laboratory. The rumen fluid was kept in the freezer (-30°C) before using it. The procedure in formulating the biostarter was prepared according to the procedure of Natsir et al. [17]. Media for the rumen bacterial consisted of K$_2$HPO$_4$ 1 g, MgSO$_4$ 1 g, Na$_2$CO$_3$ 1 g, (NH$_4$)$_2$SO$_4$ 2 g, Nutrient Agar 15 g, and Carboxymethyl Cellulose (CMC) 15 g. All ingredients were diluted into 1000 ml of distilled water. The mixture was autoclaved at 121°C for 10 minutes. The sterile media was poured into Petridis and let it cool down. The rumen fluid, which has been diluted up to the concentration of $10^{-9}$, was added by using a spatula. The media containing bacterial isolates then incubated at 37°C for 48 h.

For biostarter formulation, the materials consisting of molasses 150 g, urea 15 g, CaCO$_3$ 5 g, NaCl 5 g, mineral 2 g, rice brand 400 g were diluted into 500 ml of distilled water and mixed thoroughly before adding bacterial isolates prepared before hands. The mixture was incubated at the temperature of 37°C for 7 days. Following the incubation, the mixture was dried in the oven at 45°C, then grounded and kept at the room temperature before its application to the rice straw (figure.1).

![Figure 1. Biostarter formulated from rumen bacterial of buffalo](image)

2.2. Experimental design and fermentation process

The experiment was carried out in a factorial arrangement according to a completely randomized design [26]. The first factor was different levels of biostarter (BS) application on the rice straw, i.e., rice straw without BS (a1), rice straw + 5% BS (a2), rice straw +10% BS (a3), and rice straw + 15% BS (a4). The second factor was the different duration of the fermentation periods, i.e., two weeks (b1) and four weeks (b2). Each treatment combination was repeated three times, giving the total number of the experimental unit was 24.

The rice straw used in this study was obtained from the surrounding district of the study site. Prior to the fermentation, rice straw was chopped into smaller fractions (3-5 cm length). Biostarter was diluted into molasses + urea solution with the ratio 1:5 (w/w). The amount of biostarter added to the molasses urea solution was based on the treatment composition. The solution was spray homogenously over the chopped rice straw (roughly 7.5 kg for each treatment). The rice straw then placed in the black plastic bags (2.5 kg/bag) or three plastic bags per treatment. All the air from each bag was pushed from inside the bag. The anaerobic fermentation process lasted either for 2 weeks or 4 weeks. At the end of the fermentation period, the bag was opened and dried under room temperature till chemical analysis.

2.3. Parameter and laboratory analysis

At the end of the fermentation period, the fermented rice straw was observed for general physical characteristics, including the odor, color, the existence fungal. The main parameter measured in this study was the chemical components of the fermented rice straw, namely crude protein, crude fiber, lipid, ash, nitrogen-free extract (NFE). Prior to the laboratory analysis, the rice straw samples were dried in the oven at 65°C for 72 hours. Determination of the chemical components of the rice straw,
including crude protein, crude fiber, lipid, ash, and NFE were determined using the AOAC procedure [27].

2.4. Data analysis
The data were analyzed factorially using analysis of variance according to a completely randomized design. The first factor consisted of 4 levels of biostarter and the second factor consisted of 2 factors of fermentation time. The polynomial contrast was used to analyze the effects of different levels of biostarter on the chemical components of the rice straw. Data were analyzed using SPSS program [28].

3. Results and discussions
In general, the physical characteristics of the fermented rice straw across all the treatments showed a good result. This was indicated by a very little percentage or almost no fungal was observed across the treatments, even in the control treatment (given only molasses-urea solution without addition of biostarter). The odor of the fermented rice straw was acid aroma indicating a good result of the fermentation process. The color of fermented rice straw was bright brown in all treatments suggesting that the fermentation process was a success [22,25].

Table 1. Chemical composition of rice straw following the anaerobic fermentation either for 2 weeks or 4 weeks

| Level of BS | Fermentation Time | Crude protein (%) | Lipid (%) | Crude fiber (%) | Ash (%) | Nitrogen Free Extract (%) |
|-------------|-------------------|-------------------|-----------|----------------|---------|--------------------------|
| 0%          | 2 weeks           | 8.52±0.73         | 1.18±.82  | 29.81±0.67     | 26.92±0.67 | 27.88±0.79               |
|             | 4 weeks           | 8.79±0.80         | 1.03±.43  | 28.59±0.51     | 24.79±1.79 | 30.63±2.52               |
| 5%          | 2 weeks           | 8.75±0.46         | 0.33±.30  | 28.68±0.97     | 24.51±2.48 | 31.93±1.89               |
|             | 4 weeks           | 9.33±1.14         | 0.48±0.16 | 26.99±0.99     | 23.96±1.86 | 32.77±1.65               |
| 10%         | 2 weeks           | 9.07±1.12         | 0.56±0.47 | 28.34±1.00     | 26.60±2.17 | 29.69±2.18               |
|             | 4 weeks           | 9.83±0.80         | 0.45±0.13 | 27.24±0.82     | 22.24±1.59 | 33.48±1.80               |
| 15%         | 2 weeks           | 10.80±0.40        | 0.22±0.06 | 26.24±0.23     | 24.02±2.05 | 32.01±1.68               |
|             | 4 weeks           | 9.83±0.80         | 0.45±0.13 | 27.24±0.82     | 22.24±1.59 | 33.48±1.80               |

Mean: 0% 6.85±0.70, 1.11±0.46, 29.20±0.90, 25.85±1.68, 29.26±2.25
Mean: 5% 9.04±0.84, 0.41±0.23, 27.83±1.28, 24.23±1.98, 32.35±1.65
Mean: 10% 9.94±1.21, 0.39±0.35, 27.29±1.32, 25.31±2.36, 30.85±2.16
Mean: 15% 10.21±0.73, 0.81±0.41, 26.81±0.82, 22.68±2.11, 32.73±2.43

In terms of the chemical components, the average percentage of the chemical composition of the rice straw due to the anaerobic fermentation either for 2 weeks or 4 weeks is presented in Table 1. In general, the application of biostarter affected all the chemical components of the rice straw while the time of fermentation only affected selected chemical components. Except for lipid, ash, and NFE components of the rice straw, which was not followed a certain pattern of changes, the changes of the other components followed a specific pattern, either increased (crude protein) or decreased (crude fiber) as the level of biostarter applied increased from 0% (a1) to 15% (a4). The average crude protein component of the rice straw increased from 8.65% (a1) to 10.21% (a4), the crude fiber decreased from 29.20% (a1) to 26.81% (a4), while lipid, ash, and were fluctuated or did not follow an increase or decrease pattern.
Analysis of variance indicated that the level of biostarter affected the contents of crude protein (P=0.011), lipid (P=0.005), Nitrogen free extract (P=0.039), crude fiber (P<0.001), and tended (P=0.068) to affect ash content. Polynomial contrast analysis indicated that increasing levels of biostarter significantly increased (P=0.011) the crude protein, while the increased level of biostarter significantly decreased the crude fiber content (P=0.001) and the ash content (P=0.036) of the rice straw. The NFE content of the rice straw showed linear and cubic response on the increase of biostater levels. With regard to the effects of fermentation time, there were no significant effects between 2 weeks and 4 weeks on ash content (P = 0.203), NFE content (P = 0.208), and lipid content (P=0.510) of the rice straw. However, the crude protein content of rice straw fermented for 4 weeks was significantly higher (P= 0.020) than that fermented for 2 weeks, while the crude fiber content decreased significantly (P<0.001) from 2 weeks fermentation time to 4 weeks fermentation time. Further analysis indicated that there was no interaction between the different levels of biostater and the duration of the fermentation time on the chemical components of the rice straw. Therefore, the discussion in this paper was only focused on the main effect of each factor.

The magnitude of chemical component changes was variable. For the crude protein content, there was an increase of 18% when comparing the crude protein of a4 (15% BS) with that of a1 (0% BS) and the increase was only 4.5% if a1 was compared with a2 (5% BS). The crude fiber contents of the rice straw decrease as the level of biostater increase. When the magnitude of change in crude fiber was compared between a1 and a4, the decrease was around 18% but the decrease was only 4.7% if comparing a1 and a2. It is important to note that less improvement observed between control (a1) and other treatments (a2-a4) may be related to the addition of urea and molasses for the control group (a1). Adding urea for fermentation of rice straw could significantly increase the crude protein and decrease crude fiber of the rice straw [29,30]. This also implies that biostater containing bacterial isolates used in this particular study can work effectively during the fermentation if it was supported by the availability of nutrients required by the bacterial of the biostater in order to perform maximally. However, if comparing the changes in the chemical components of the rice straw between unfermented and fermented rice straw, there was a significant increase in terms of nutrient components. For example, the crude protein contents of rice straw without any treatment could be varied between 3% and 5% [30–32]. However, when the comparison was made between fermented rice straw with unfermented rice straw, the improvement of crude protein was very significant. The crude protein content of this study increased between 80% and 200%. The significant increase for crude protein contents and a significant decrease in the crude fiber of fermented rice straw indicated the effectiveness of biostater applied in this study. With regard to the fermentation period, there were no significantly different effects (P>0.05) between 2 weeks and 4 weeks fermentation period except for the crude protein and crude fiber components, in which the longer the fermentation time the higher the crude protein and the less crude fiber components of the rice straw.

The impact of the application of biostater on changing the chemical components of rice straw has been predicted. The application of biostater affected the chemical components of the rice straw in different patterns. It decreased the fiber components of the rice straw on one side, and it increased the crude protein content of the rice straw on the other hand. The fermentation under anaerobic conditions enables the microbes of the biostater to work effectively in degrading the organic components of the rice straw. The presence of microorganisms in the biostater is able to break down the lignohemicellulose and lignocellulose links of the substrate, making it more accessible for further hydrolysis to be simple components and quantitatively the percentage of crude fiber will decrease. The results of this study agree with results of previous studies which reported that anaerobic fermentation of rice straw using a consortium of bacteria including proteolytic bacteria, cellulolytic bacteria, ligninolytic bacteria, and lipolytic bacteria can improve the nutritive values of the rice straw as those bacteria could produce such enzymes as cellulase, lactase, or xylanase to hydrolyze the components of cellulose, hemicellulose, and lignin in the straw [11,19,22,24,33–35]. Other studies using fungi as biodecomposer have been reported to degrade the fiber component of the rice straw [9–11,13,15]. In terms of NFE, the pattern of change did not follow a certain pattern. The reason for that is during the
fermentation; the bacteria prefer to use NFE as energy sources for the fermentation. In addition, the change of NFE is also determined by the changes of other chemical components during the fermentation. So fluctuation of chemical components of lipid and ash directly affected the variable percentage of NFE of the rice straw as the NFE is determined based on the calculation by subtracting 100% with the percentage of crude protein, crude fiber, lipid, and ash [36,37].

4. Conclusion
The use of biostater developed from rumen bacterial of buffalo was successful in changing the chemical components of the rice straw in terms of increase crude protein and decrease of the fiber composition of rice straw fermented under anaerobic conditions either in 2 weeks or 4 weeks. The use of biostater of 5% is still effective in increasing the quality of rice straw while the fermentation period could be shortened from 4 weeks to 2 weeks without the negative impact of the chemical components of the rice straw. The finding of this study suggests that biostater formulated from rumen bacterial of buffalo has a promising prospect to be used as a biological agent to improve the quality of high fiber feedstuff such as rice straw as feeding for the ruminant animal.

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