IN VITRO RUMINAL FERMENTATION AND DEGRADABILITY OF RICE HUSK ON RICE BRAN SUBSTITUTION

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Abstract

Rice bran is a by-product of the rice milling process and has been well used as livestock feed. Rice bran is often adulterated with rice husk. The objective of this study was to evaluate the *in vitro* ruminal fermentation characteristics of rice bran with various compositions of rice husk and assess the relationship between rice husk addition and rice bran quality. The experiment was arranged in a completely randomized design with rice husk addition as a factor and three replications. Data of proximate value, gas production, ruminal degradability, and volatile fatty acid production were analyzed by analysis of variance. Moreover, significant effects of each treatment in the *in vitro* fermentation were further analyzed by Duncan's multiple range test ($P<0.05$). It was shown that the addition of rice husk to rice bran could increase acetic acid level, but it reduced potential gas production, gas production rate, organic matter and dry matter digestibility, and propionic acid level. Interestingly, the linear regression of dry matter digestibility, organic matter digestibility, and potential gas production showed the high adjusted R^2 values. Moreover, this study also revealed that 10% of rice husk substitution on rice bran could significantly reduce the dry matter digestibility.

Keywords: rice bran, rice husk, digestibility, *in vitro* ruminal fermentation

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Introduction

Rice is a staple food for more than half of the world's population and mostly produced and consumed in Asia (Wani *et al.,* 2012; Zou & Yang, 2019). An increase in rice consumption is driven mainly by population growth and by the increased per capita consumption. An increase in rice production is followed by the increased by-product. The main by-products of rice are rice straw, rice husk, and rice bran. The rice kernel mainly consists of 70% endosperm, 20–21% rice husk, 6–8% rice bran, and 1% rice germ of the total seed weight (Zou & Yang, 2019). Rice husk, the largest quantity of rice kernel by-product, mainly contains about 80% organic substance and 20% inorganic materials. Currently, most rice husk is underutilized or left unused because it has low nutritive value (1.92-5.63% crude protein, 37.33-53.63% crude fiber, and 302.33 kcal/kg gross energy) (Telew *et al.*, 2013; Amrullah *et al*., 2019).

Rice bran is a by-product of the rice milling process which has economic value. It contains nutrients of protein, fiber, amino acids, minerals, vitamins, and antioxidants (Younas *et al.,* 2011). Rice bran has a long history of use in livestock feed as a source of protein and energy. Rice bran contains approximately 9.5-12.1% crude protein, 10.6-23.5% crude fiber, and 3165-3563 kcal/kg gross energy (Supriyati *et al.,* 2015; Hardini, 2010; Warren & Farrell, 1990; Oliveira *et al.,* 2011). Several studies have been carried out to evaluate rice bran as cattle feed (Abrar *et al.,* 2016; Wakabayashi *et al.,* 2013; Gadberry *et al.,* 2004).

Animal feeds account for 60–70% of the variable production costs in intensive livestock systems. In recent years, the price of rice bran has grown rapidly due to the high demand. Hence, rice bran is often adulterated with rice husk to get a lower price.

Presumably, a high content of rice husk will decrease the nutritive value of rice bran, because of the differences in nutrient content. A

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low-quality feed may decrease livestock productivity. However, the information about how much the content of rice husk in rice bran can be tolerated to maintain the quality of feed is not clear yet. Therefore, the objective of this study was to evaluate the fermentation characteristics of rice bran with various rice husk composition and assess the relationship between the compositional changes of rice husk with the quality of rice bran. The ruminal fermentation, including digestibility as well as proximate analysis of rice husk addition to rice bran were performed in this study.

Materials and Methods

Materials

Rice bran and rice husk were obtained from local suppliers. The materials were milled into 18 mesh flour and stored in polyethylene bags at 4 °C prior to further analysis.

Methods

The experiment was arranged in a completely randomized design with rice husk addition as a factor and 3 replications. The treatments were arranged according to the following experimental treatments (dry matter basis).

R1: 100% Rice bran
R2: 90% Rice bran : 10% Rice husk
R3: 80% Rice bran : 20% Rice husk
R4: 70% Rice bran : 30% Rice husk
R5: 60% Rice bran : 40% Rice husk
R6: 50% Rice bran : 50% Rice husk
R7: 40% Rice bran : 60% Rice husk
R8: 30% Rice bran : 70% Rice husk
R9: 20% Rice bran : 80% Rice husk
R10: 10% Rice bran : 90% Rice husk
R11: 100% Rice husk

The samples were analyzed for proximate analysis consist of dry matter content (Method No.930.15), ash content (Method No 930.05), crude protein content (Method No 978.04), crude fiber content (Method No 930.10), and crude fat content (Method No 930.09) (AOAC, 2005) and *in vitro* fermentation. For a confirmation study, different rice bran from another local supplier was used to test the equation to predict *in vitro* fermentation variable.

In vitro ruminal fermentation

In vitro ruminal fermentation technique of Theodorou *et al.,* (1994) was followed with some modification. Ruminal fluid was collected from three rumen-fistulated Ongole crossbred cattle before morning feeding. Animal care procedures throughout the study followed protocols approved by the Ethical Clearance Committee of Indonesian Institute of Sciences (Number 9879/WK/HK/XI/2015). Rumen fluid was mixed in equal proportion, and filtered through four-layered cheesecloth, and immediately taken to the laboratory. Samples from each treatment were added to the serum bottle as much as 0.5 g. Then, added by 50 ml of rumen-McDougall buffer solution (1:2 v/v), purged with oxygen-free CO_2. Serum bottles sealed with a butyl rubber stopper and an aluminium crimp seal and transferred into an incubator at 39°C.

Gas production was measured at 2, 4, 6, 8, 10, 12, 24, and 48 h of incubation. After 48-h of incubation, samples were taken for the measurements of pH value. Rumen-buffer mixture was separated by centrifugation (6,000rpm, 10 min) for volatile fatty acid (VFA) analysis. The VFA was analyzed using gas chromatography (GC 8A, Shimadzu Corp., Kyoto, Japan, with capillary column type containing 10% of SP-1200, 1% of H_3PO_4 on 80/100 Cromosorb WAW, and nitrogen as the gas carrier). Incubation was continued by mixing 50mL pepsin-HCl with the substrate collected from centrifugation. Samples of dry matter digestibility (DMD) and organic matter digestibility (OMD) were taken after 48-h incubation. Thereafter, the substrate was separatedby vacuum filtration using Whatman™ filter papers No. 41. Dry matter and organic matter digestibility were calculated as the DM and OM which disappeared from the initial weight inserted into the tube, respectively.

Confirmation study

Confirmation study was performed to determine the difference between the estimated data values of *in vitro* parameters calculated by equations obtained from regression analysis with the observed data values from laboratory testing. Rice brans were obtained from different local suppliers. Samples were tested for proximate and *in vitro* analysis. The results of *in vitro* rumen fermentability parameter obtained through calculations using equations

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and laboratory analysis were analyzed using a completely randomized design with 3 replications.

Statistical analysis

All data were analyzed by SPSS 23 for windows. Data of proximate, gas production, rumen feed disappearance, and volatile fatty acid production were analyzed by using analysis of variance (ANOVA). Significant effects of each treatment were further analyzed by Duncan's multiple range test ($P<0.05$). Data of gas production were adjusted at the model proposed by Ørskov & McDonald (1979) as $p = a + b(1 - e^{-ct})$ that P is the gas produced at time *t*, '*a*' is the gas produced by the soluble fraction, '*b*' is the gas produced by the insoluble but slowly fermenting fraction, '*c*' is constant gas production rate, '*t*' is the time of fermentation. Kinetic parameters of Ørskov'sequation were obtained by non-linear regression procedure. The correlation between the proximate variable and the rumen fermentation variable was obtained by the correlation coefficient Pearson analysis procedure. Moreover, the regression equation of proximate variable for estimating rumen fermentation variable estimation was obtained by linear regression procedure.

Results

The nutrient composition of rice bran with various ratios of rice husk addition is shown in Table 1. A pure rice bran (R1) consisted of 91.37% dry matter, 90.59% organic matter, 10.8% crude protein, 11.53% ether extract, and 12.69% crude fiber. Moreover, rice husk (R11) used in this experiment was composed of 11.66% moisture, 20.69% ash, 1.04% crude protein, 2.06% ether extract, and 38.41% crude fiber.

Table 1. The effect of rice husk addition on proximate composition of rice bran

Treatments	Dry Matter (%)	Organic matter (%)	CP (%)	CF (%)	EE (%)	NFE (%)
R1	91.37	90.59^{a}	10.80^{k}	12.69^{a}	11.53a	55.55^{e}
R2	89.58	89.91^{ab}	9.20^{j}	14.26^{a}	10.30b	56.15^{e}
R3	89.59	88.67^{ab}	8.47^{i}	17.62^{b}	9.33b	53.25^{de}
R4	89.19	87.10^{ab}	7.46^{h}	19.31^{b}	8.63c	51.69^{cde}
R5	90.07	86.60^{ab}	6.31^{g}	22.60^{c}	7.17d	50.52^{bcd}
R6	90.61	85.35^{ab}	5.51^{f}	24.63^{cd}	5.54d	49.66^{bcd}
R7	89.77	84.03^{bc}	4.37^{e}	28.27^{de}	5.16e	46.23^{b}
R8	89.69	85.88^{ab}	3.36^{d}	26.98^{e}	4.03f	50.51^{cde}
R9	89.64	85.64^{ab}	2.32^{c}	31.96^{f}	3.25g	48.11^{bc}
R10	89.88	84.64^{abc}	1.52^{b}	34.07^{f}	2.68h	46.38^{b}
R11	88.34	79.31^{c}	1.04^{a}	38.41^{g}	2.06i	37.80^{a}
P-value						
Linier	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Quadratic	>0.05	>0.05	<0.05	>0.05	<0.05	<0.05

CP: crude protein, EE: Ether Extract; CF: Crude Fiber, NFE: Nitrogen Free Extract

$^{a-c}$ Means with different superscripts within columns significantly differed ($p<0.05$).

It seemed that the increase in rice husk content in rice bran did not affect significantly its moisture content. This finding was in agreement with no significant difference between R1 and R11 on dry matter (Table 1). It is also shown that the addition of rice husk to rice bran could reduce crude protein, nitrogen-free extract, and ether extract compositions, while the ash content and crude fiber were increased significantly ($p<0.05$).

The gas production during 48 hours of incubation is presented in Figure 1. The result of this experiment showed that the gas was still being produced until 48 hours of incubation time in all treatments. This phenomenon indicated that the fermentable substrate remained present until 48 hours of incubation. Figure 1 also shows that the increase in rice husk composition could reduce gas production.

Moreover, the fermentation characteristics of rice bran are presented in Table 2. It was demonstrated that the substitution of rice husk on rice bran decreased significantly ($p<0.05$) potential gas production, gas production rate,

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and organic matter digestibility (OMD). The substitution of 10% rice husk on rice bran seemed not to decrease significantly the OMD to some extent.

Table 2. The effect of rice husk addition in rice bran on the *in vitro* ruminal fermentation

Treatment	a+b (mL)	c (mL/h)	pH (%)	DMD (%)	OMD (%)
R1	111.37^{k}	0.066^{e}	6.71	61.53^{k}	67.20^{j}
R2	100.27^{j}	0.058^{d}	6.76	58.15^{j}	64.76^{j}
R3	93.24^{i}	0.057^{d}	6.72	54.33^{i}	60.36^{i}
R4	90.03^{h}	0.056^{d}	6.72	49.95^{h}	54.77^{h}
R5	85.33^{g}	0.053^{c}	6.73	46.17^{g}	50.84^{g}
R6	71.96^{f}	0.053^{c}	6.76	40.33^{f}	45.64^{f}
R7	64.31^{e}	0.053^{c}	6.71	34.95^{e}	40.39^{e}
R8	52.90^{d}	0.052^{c}	6.81	29.73^{d}	33.50^{d}
R9	42.32^{c}	0.053^{c}	6.71	23.02^{c}	28.16^{c}
R10	31.73^{b}	0.048^{b}	6.73	18.72^{b}	23.14^{b}
R11	28.16^{a}	0.038^{a}	6.59	13.61^{a}	18.99^{a}
P-value					
Linier	<0.05	<0.05	>0.05	<0.05	<0.05
Quadratic	<0.05	>0.05	>0.05	<0.05	<0.05

a+b: potential gas production, c: gas production rate, DMD: dry matter digestibility, OMD: organic matter digestibility
a-k: Means with different superscripts within columns significantly differed (p<0.05).

Table 3.The effect of rice husk addition on partial volatile fatty acid (%mM) and total volatile fatty acid (Mmol/ml) of rice bran in rumen fermentation

Treatment	C2	C3	C4	Iso-C4	C5	Iso-C5
R1	52.60^{a}	26.11^{c}	11.56^{a}	3.79^{ab}	4.09	1.85
R2	54.09^{ab}	19.33^{ab}	14.51^{cd}	4.28^{abc}	4.42	3.37
R3	55.79^{b}	20.48^{b}	12.15^{ab}	6.06^{d}	3.75	1.77
R4	55.18^{b}	19.75^{ab}	12.56^{ab}	5.83^{d}	4.17	2.50
R5	54.90^{b}	19.26^{ab}	14.63^{cd}	3.21^{a}	5.70	2.30
R6	54.45^{ab}	18.69^{ab}	15.18^{cd}	4.11^{abc}	5.62	1.95
R7	55.06^{b}	18.80^{ab}	15.77^{d}	4.38^{abc}	4.12	1.87
R8	55.63^{b}	16.91^{a}	14.47^{cd}	5.06^{bcd}	5.91	2.03
R9	56.04^{b}	17.42^{a}	13.59^{bc}	4.90^{bcd}	5.61	2.45
R10	56.02^{b}	17.68^{ab}	13.61^{bc}	5.26^{cd}	5.53	1.90
R11	55.92^{b}	17.05^{a}	14.36^{cd}	6.14^{d}	4.20	2.33
P-value						
Linier	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05
Quadratic	>0.05	<0.05	<0.05	>0.05	>0.05	>0.05

C2: acetic acid, C3: propionic acid, C4: butyric acid, Iso-C4: Isobutyric acid,C5: valeric acid, Iso-C5: Iso-valeric acid, T.VFA: total volatile fatty acid
$^{a-c}$ Means with different superscripts within columns significantly differed (p<0.05).

However, decreases in dry matter digestibility (DMD), gas production rate, and potential gas production by 5.49%, 12.12%, and 9.96%, respectively were observed. Additionally, the effect of rice husk addition to rice bran was likely to lead to the formation of high production of acetic acid, but low level of propionic acid (Table 3).

In terms of crude protein and ether extract contents of rice bran, they had shown positive correlations with DMD, OMD, propionic acid and iso valerate concentrations, potential gas production, and also gas production rate but had shown negative correlations with acetate and butyrate concentrations (Table 4).

However, the crude fiber content had shown positive correlations with butyrate and acetate concentrations, while its negative correlations with DMD, OMD, propionic acid concentration, potential gas production, and gas production rate were observed. The ash content was negatively correlated with DMD, OMD, acetate, butyrate, and valerate concentrations, potential gas production, and gas production rate (Table 4).

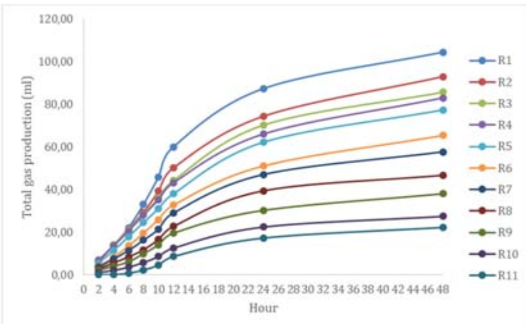

Figure 1. Gas production during 48 hours of incubation

As presented in Table 4, the correlation matrix between rice bran nutrition and rumen fermentation variables is used to describe which parameters correlate. Variables that have correlations were analyzed by the regression methods to get equations (Table 5). These equations can be used to estimate rumen fermentation variables (DMD, OMD, acetate, propionic, butyrate concentrations, potential gas production, and gas production rate) from rice bran nutrient variables (ash, crude protein, crude fiber, and ether extract). In this study, the models of *in vitro* ruminal fermentation parameter estimation by rice bran nutrients were fitted to the linear regression models. The linear regressions to estimate DMD, OMD, and potential gas production had high adjusted R^2 values by 0.977 ($P<0.05$), 0.973 ($P<0.05$), and 0.976 ($P<0.05$), respectively (Table 5).

The equations for estimating DMD, acetate and propionate concentrations, potential gas production, and gas production rate were significantly different ($P<0.05$). According to a confirmation study (Table 6), there were differences between the estimated value by the calculated equation and the observed value by the laboratory analysis on DMD and propionate concentration.

Table 4. Matrix correlation between proximate and rumen fermentation variables

Variable	Treatment	Moisture	Ash	CP	CF	EE
DMD	-.995**	-.146	-.650**	.987**	-.972**	.979**
OMD	-.995**	-.163	-.648**	.987**	-.965**	.980**
C2	.563**	.269	.416*	-.579**	.562**	-.555**
C3	-.701**	-.159	-.284	.712**	-.675**	.711**
C4	.382*	.021	.355*	-.398*	.357*	-.416*
Iso-C4	.308	.340	.277	-.281	.278	-.267
C5	.235	-.168	-.350*	-.236	.227	-.268
Iso-C5	-.125	-.105	-.109	.109	-.110	.152
a+b	-.992**	-.155	-.652**	.987**	-.963**	.976**
C	-.880**	-.180	-.723**	.866**	-.887**	.851**
pH	-.199	.212	-.018	.164	-.285	.134

CP: crude protein, EE: Ether Extract, CF: Crude Fiber, C2: acetic acid, C3: propionic acid, C4: butyric acid, Iso-C4: Isobutyric acid, C5: valeric acid, Iso-C5: Isovaleric acid, a+b: potential gas production, c: gas production rate, DMD: dry matter digestibility, OMD: organic matter digestibility
**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

Discussion

Protein from rice bran is well used for cattle because it is consist of a higher lysine content as compared to oat, maize, and wheat protein (Juliano, 1985; Wang *et al.*, 2015; Amagliani *et al.*, 2017; Mota *et al.*, 2016). Rice husk used in this experiment consisted of 11.66% moisture, 20.69% ash, 1.04% crude protein, 2.06% ether extract, and 38.41% crude

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fiber. Rice husk ash is composed mainly of silica, which values range from 87.4% to 91.4% (Alvarez *et al.,* 2014; Valverde *et al.,* 2007; Van *et al.,* 2014).

The fermentable substrate decreasesas the incubation time increases and leads to a decreasein gas production rate (Hungate, 1966; Jayanegara & Sofyan, 2008). In the early time of incubation, the treatments which consisted of higher rice bran ratios showed the higher gas production. It could be due to the presence of readily fermentable substrate in rice bran. The decreased *in vitro* rumen fermentability parameter could be the result of the decreased protein and the increased fiber in rice bran. A lower degradability of rice bran caused by a higher rice husk addition is in line with the observation of lower gas production in this study. The substitution of 10% rice husk (R2) significantly decreased DMD, but it did not effect OMD. It is suggested that the increases in fiber and silica content from rice husk were not enough to significantly reduce OMD.

The increase of rice husk portion will increase the fiber content. Fiber in rice husk consists of 28.6%-41.5% cellulose, 14.0%-28.6% hemicellulose, and 20.4%-33.7% lignin (Muhammad *et al.,* 2013; Quispe *et al.,* 2017). Ash content mainly consists of silica (Vlaev *et al.,* 2003).

Table 5. Regression equation of proximate for rumen fermentation variable estimation

Variable	Equation	Adjusted R^2	S_e	P-Value
DMD	$Y= 30.475 + 3.888X_1 - 0.532X_2 + 0.104X_3 - 0.162X_4$	0.977	2.41	<0.05
OMD	$Y= 24.89 + 4.099X_1 - 0.292X_2 + 0.127X_3 + 0.375X_4$	0.973	2.69	<0.05
C2	$Y= 54.695 – 0.525X_1 + 0.032X_2 + 0.008X_3 – 0.370X_4$	0.259	1.17	<0.05
C3	$Y= 5.774 + 0.956X_1 + 0.127X_2 + 0.264X_3 +0.215X_4$	0.529	1.92	<0.05
C4	$Y= 18.546 + 0.267X_1 - 0.112X_2 + 0.068X_3 –0.685X_4$	0.111	1.42	>0.05
a+b	$Y= 38.16 + 8.435X_1 - 0.475X_2 + 0.205X_3 - 0.859X_4$	0.976	4.62	<0.05
C	$Y= 0.082 + 0.0001X_1 - 0.001X_2 + 0.0001X_3 - 0.001X_4$	0.813	0.003	<0.05

X_1:Crude Protein, X_2:Crude Fiber, X_3:Ash, X_4:Ether Extract, S_e: Standard error

Table 6. Comparison between estimation by equation and laboratory analysis on *in vitro* fermentation parameter

Variables	Estimated value by equations	Observed value by laboratory experiments	SEM
DMD (%)	60.37	61.86	0.24
OMD (%)	67.77[a]	65.11[b]	0.64
C2 (%mM)	45.23[a]	52.01[b]	1.52
C3 (%mM)	22.44	24.57	0.64
C4 (%mM)	11.78[a]	13.64[b]	1.71
a+b(ml)	105.73[a]	101.24[b]	1.14
c (ml/jam)	0.060[a]	0.080[b]	0.004

*Rice bran was consist of: moisture: 9.46%, Crude Protein: 9.66%, Crude fiber:10.85%, Extract ether: 13.09%, Ash: 12.28%

C2: acetic acid, C3: propionic acid, C4: butyric acid, Iso-C4: Isobutyric acid,C5: valeric acid, Iso-C5: Isovaleric acid, a+b: potential gas production, c: gas production rate, DMD: dry matter digestibility, OMD: organic matter digestibility

Lignocellulose and silica are difficult to degrade in the rumen (Fonnesbeck *et al.,* 1981). The effect of rice husk addition to rice bran leads to the formation of high production of acetic acid but the decreased propionic acid concentration (Table 3). The increase in acetic acid production was likely due to the increased crude fiber content and the decreased protein content in rice bran. Moreover, it has been reported that the increase in dietary fiber can elevate acetate production (Valadares *et al.,* 1999).

Similar findings were also reported by Olfaz *et al.,* (2018) and Jayanegara *et al.,* (2009). There were positive relationships between ether extract and crude protein with

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potential gas production, gas production rate, and OMD. According to Fonnesbeck *et al.*, (1981) and De Boever *et al.* (2005), the increase in nutrient digestibility will be in line with the increases in carbohydrates and crude protein contents, but it is negatively correlated with the increase in fiber content. Sari *et al.* (2018) and Fidriyanto *et al.*(2019) also reported that DMD and OMD was decreased with the increase in fiber content. It seemed that the negative correlation could be attributed to the ash composition in rice bran. It consists of inorganic matters of mineral and silica (Satter *et al.*, 2014; Rosniyana *et al.*, 2007). Because ash is difficult to digest, a higher ash content in feed will reduce digestibility. The findings of rice husk addition in this study supported the fact that rice husk addition to rice bran may contribute to the reduction of nutritive value, *in vitro* gas production, and rumen degradability.

Adulteration of rice bran with rice husk seemed to decreasethe quality and rumen degradability of rice bran. Moreover, the increased rice husk content could reduce protein content, gas production, and digestibility. The results showed that 10% of rice husk substitution on rice bran significantly reduced DMD. The relationship and regression equation of nutrient and *in vitro* ruminal fermentation was found in this study. It is apparent that the equation can be used to estimate *in vitro* ruminal fermentation from rice bran nutrients.

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