Evaluation of parboiled rice by-product as a ruminant feed: \textit{in vitro} digestibility and methane production

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\textbf{Abstract.} Solid waste generated by parboiled rice industry are rice bran, rice husk and broken rice. Broken rice can be used for cattle feed as energy source. The aim of this study was to evaluate the effects of parboiling process on the \textit{in vitro} rumen fermentation. The experiment was arranged in a completely randomized block design with 2 rice varieties (LIPI Go 1 and Sintanur) as blocks, 2 different soaking periods (30 and 60 minutes) as treatments and 3 replications. Parboiling treatment was significantly (P<0.05) increased crude protein, organic matter, and crude fiber, but decreased ether extract and solubility. LIPI Go 1 produced higher (P<0.05) gas (11.18%), but lower (P<0.05) gas production rate (52.38%) and methane (10.27%). Soaking rice grain for 30 and 60 minutes decreased rumen dry matter digestibility by 6.13\% and 8.90\% and increased post rumen dry matter digestibility by 10.41\% and 10.43\%, respectively. Moreover, 3.55\% and 6.84\% from total samples of parboiled rice treated by 30 minutes and 60 minutes soaking periods were digested in the ileum. As ruminant feed, parboiled rice decreased methane and digestibility in rumen, and increased the digestibility in post rumen and ileum.

\section{Introduction}

\textit{Parboiling} is the \textit{hydrothermal} treatment of rice grain to improve the physical, chemical, and nutritional value. Parboiling process is consisting of three steps i.e. immersing, gelatinization and dehydration [1]. The annually production of parboiled rice around the world is 130 million tons [2]. Parboiled rice is widely consumed and has increased significantly in recent decades because it contains more nutritional components compared with non-parboiled rice [3].

The main solid waste generated by parboiled rice industry are rice bran, rice husk and broken rice. The parboiled rice by product that can be used as animal feed are rice bran and broken rice. In a recent study, Fidriyanto \textit{et al.} [4] was investigated the used of parboiled rice bran as ruminant feed using \textit{in vitro} rumen fermentation analysis. Broken rice can be used for cattle feed as source of energy. Recently, the used of rice grain as animal feed for dairy and beef cattle and other livestock animals has been increasing. Rice grain starch is digested more rapidly than corn grain starch in the rumen, so that it can be used as corn grains substitute in both dairy cattle and beef cattle feed [5,6]. Replacing corn grains with rice grains at about 70\% in the total mixed ration (TMR) were not give negative effect on \textit{in vitro} rumen fermentation [7]. However, there is no report yet on the use of parboiled rice as ruminant feed.
In this study, 2 varieties of rice grain were parboiled using different soaking time. Therefore, the objective of this study was to investigate the effects of parboiling rice grain process on in vitro rumen fermentation.

2. Material and methods

2.1. Material
Two varieties of rice grain i.e. LIPI GO1 and Sintanur were used in this study. Rice grain of LIPI GO1 were obtained from Laboratory of Agronomy for Evaluation of Product Biotechnology, Research Center for Biotechnology, Indonesian Institutes of Sciences, while Sintanur was obtained from Indonesian Center for Rice Research, Ministry of Agriculture Republic Indonesia.

2.2. Methods

2.2.1. Parboiled rice preparation
One kg of rice grain was soaked into 60°C (1:2) water for 30 and 60 minutes. Thereafter, rice grain was put into cheesecloth and steamed at 121°C 1 atm for 15 minutes by autoclave. Thereafter, the samples were dried in a cabinet drier at 40°C for 24 h. The dried sample was hulled to get rice. Rice was milled into 18 mesh flour and stored in polyethylene bags at 4°C prior to analysis.

2.2.2. Chemical analysis
Sample were analyzed for Carboxyl content [8], starch [9], Solubility [10], and proximate. Proximate analysis consisted 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), crude fat content (Method No 930.09) [11] and nitrogen free extract calculated by difference (%NFE = 100 - [%moisture + %Crude fiber + %Crude protein + %Ether extract + %Ash]).

2.2.3. In vitro rumen fermentation
Three steps in vitro ruminal fermentation was used in this experiment. In vitro rumen fermentation (first and second steps) was carried out with all treatments as described by Theodorou et al. [12] with modification. Rumen fluid for in vitro fermentation was obtained from two rumen fistulated Ongole crossbred cattle before morning feeding, mixed, filtered through a double layer of cheesecloth, pooled in prewarmed bottles, sealed and immediately transported to the laboratory.

First step of in vitro rumen fermentation was conducted using 100ml serum bottle glass, filled with 75 ml mixture of rumen fluid and Mc’Dougal buffer (1:2 ratio). The bottle was flushed with CO2 gas for 30 s to get anaerobic conditions and closed immediately. Samples were incubated in a water bath incubator at temperature of 39°C. Gas production was measured at 2, 4, 8, 10, 12, 24 and 48 h incubation. Methane production was measured at 24 and 48h incubation. After 48h incubation, pH of rumen fluid was measured. Total volatile fatty acid (VFA) was determined using the method of Chatterjee et al. [13], and N-NH3 using the method of Chaney and Marbach, [14].

Rumen dry matter (R-DMD) and organic matter digestibility (R-OMD) were measured after 48 hours of incubation. Briefly, at the end of 48 h incubation, rumen fluid samples were centrifuged at 378 x g for 10 min and the precipitated sample was washed with distilled water. In the second step, samples were mixed with 75 mL pepsin-HCl solution (containing 2 g L\(^{-1}\) pepsin and 17.8 mL L\(^{-1}\) HCl) and incubated at 39°C for 48h. Thereafter, pepsin-HCl solution was separate using Whatman™ papers no 41 (CAT No.1441-125). Post Rumen dry matter (PR-DMD) and organic matter digestibility (PR-OMD) were measured. The ileum in vitro ruminal fermentation was carried out with all treatments as described by Calsamiglia and Stern [15] with modification. In the third step, Samples were mixed with 0.5ml IN NaOH and 75 mL pancreatin solution (containing 0.5 M KH\(_2\)PO\(_4\) buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin) and incubated at 39°C for 48h. Thereafter, pancreatin solution was separated using Whatman™ papers no 41 (CAT No.1441-125). Ileum dry matter (I-DMD) and organic matter digestibility (I-OMD) were measured.
2.2.4. **Statistical Analysis.**

The experiment was arranged in a completely randomized block design with 2 varieties of rice (LIPI Go 1 and Sintanur) as blocks, 2 different soaking periods (30 and 60 minutes) as treatments and 3 replications. Data were analyzed by one-way analysis of variance using SPSS 23 (SPSS, Inc., IBM, Chicago). Significant effects of treatments were determined by Duncan's multiple range test method. Significant differences were accepted if P<0.05. Data of gas production were adjusted at the model proposed by Ørskov & McDonald [16] which were defined 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.

3. **Results and discussion**

Brief description about rice varieties used in this study as follows, Sintanur is aromatic rice and LIPI Go 1 is upland rice. The chemical composition of parboiled rice is shown in Table 1. Parboiling treatment significantly (P<0.05) increased crude protein, and crude fiber, but decreased ether extract, ash content, nitrogen free extract (NFE), and solubility. The parboiling process affects the physical and chemical properties of rice grains, such as water absorption, soluble solid loss, and protein solubility. During soaking, the pigments and nutrients such as vitamins and protein migrate from the bran to the endosperm [17].

In this study, parboiling did not affect carbonyl content and starch. The decreasing of NFE and solubility of parboiled rice were related to heat moisture treatment (HMT). Nitrogen free extract represents the soluble carbohydrate in the feed. Heat-moisture treatments will turn starch into resistant starch type 3 (RS3), produced slightly crosslinks within amorphous regions of starch granules, developed starch chain interactions and dissociation of a few double helices that can decreased starch swelling and solubility [18-20]. Resistant starch type 3 is produced in two steps: gelatinization, which is a disruption of the granular structure by heating with excess of water and retrogradation, which is recrystallization of the starch molecules upon dehydration [21,22].

### Table 1. Chemical composition of parboiled rice with different soaking periods and varieties

| Variable          | DM (%) | Ash (%) | EE (%) | CP (%) | CF (%) | NFE (%) | Carboxyl contents (%) | Starch (%) | Solubility (%) |
|-------------------|--------|---------|--------|--------|--------|---------|-----------------------|------------|----------------|
| Types of Rice     |        |         |        |        |        |         |                       |            |                |
| Sintanur          | 90.51a | 0.78b   | 1.25a  | 8.89a  | 1.81a  | 77.78a  | 0.26                  | 73.74      | 12.50b         |
| LIPI GO1          | 91.11b | 0.60a   | 0.99b  | 8.58b  | 2.25b  | 78.68b  | 0.25                  | 76.17      | 11.07a         |
| P-Value Soaking   | <0.05  | <0.05   | <0.05  | <0.05  | <0.05  | >0.05   | >0.05                 | >0.05      | <0.05          |
| periods mean      |        |         |        |        |        |         |                       |            |                |
| 0 Minutes         | 90.76a | 0.76a   | 1.22c  | 8.31a  | 1.63a  | 78.830  | 0.26                  | 76.37      | 13.81c         |
| 30 Minutes        | 91.07b | 0.70b   | 1.14b  | 8.86b  | 1.70b  | 78.66b  | 0.27                  | 74.58      | 9.97a          |
| 60 Minutes        | 90.60c | 0.62c   | 0.99a  | 9.03b  | 2.75b  | 77.192  | 0.25                  | 73.83      | 12.21b         |
| P-Value           | >0.05  | <0.05   | <0.05  | <0.05  | <0.05  | >0.05   | >0.05                 | >0.05      | <0.05          |

DM: dry matter, OM: organic matter, EE: ether extract, CP: crude protein, CF: Crude Fiber, NFE : nitrogen free extract

* Means with different superscripts within columns significantly differed (p<0.05).

Compared with Sintanur, LIPI Go 1 produced gas 11.18% significantly higher (P<0.05) but gas
production rate and gas methane were significantly lower (P<0.05) by 52.38% and 10.27% production from \textit{in vitro} fermentation, respectively. Soaking rice grain at 60°C for 30 minutes significantly decreased potential gas production by 2.76%. Parboiling treatments significantly (p<0.05) decreased methane production and increased pH of rumen compared with control. Soaking rice grain for 30 and 60 minutes decreased TVFA by 4.34% and 12.49%, respectively. Decreased rumen digestibility of starch is desirable to prevent from acidosis and to increase glycogenic substrates in post rumen [23]. Decreased of methane production in this experiment was related to resistant starch content of parboiled rice. Resistant starch also had beneficial effect in lowering methane production from \textit{in vitro} rumen fermentation. The highest pH was observed in 60 minutes soaking treatment. However, Parboiling process did not affect gas production rate.

\textbf{Table 2.} Gas production kinetic of parboiled rice with different soaking periods and varieties

| Variable | a+b (ml) | c (ml/h) | Methane (%) | pH | TVFA (mmol/g) | NH3 (mg/ml) |
|----------|----------|----------|-------------|----|---------------|-------------|
| Types of Rice mean |          |          |             |    |               |             |
| Sintanur | 209.08\textsuperscript{a} | 0.064\textsuperscript{a} | 12.89\textsuperscript{a} | 6.54 | 80.96\textsuperscript{b} | 18.22 |
| LIPI Go1 | 232.46\textsuperscript{b} | 0.042\textsuperscript{b} | 11.69\textsuperscript{b} | 6.53 | 76.23\textsuperscript{a} | 15.06 |
| P-value  | <0.05    | <0.05    | <0.05       | >0.05 | <0.05         | >0.05       |
| Soaking periods mean |          |          |             |    |               |             |
| 0 Minutes | 223.72\textsuperscript{b} | 0.052    | 13.96\textsuperscript{b} | 6.51\textsuperscript{a} | 84.20\textsuperscript{b} | 19.86 |
| 30 Minutes | 217.71\textsuperscript{a} | 0.058    | 11.58\textsuperscript{a} | 6.54\textsuperscript{b} | 79.87\textsuperscript{a} | 15.91 |
| 60 Minutes | 220.88\textsuperscript{ab} | 0.055    | 11.33\textsuperscript{a} | 6.56\textsuperscript{c} | 71.71\textsuperscript{a} | 14.16 |
| P-Value  | <0.05    | >0.05    | <0.05       | <0.05 | <0.05         | >0.05       |

\textsuperscript{a,b} Means with different superscripts within columns significantly differed (p<0.05).

Gas production kinetic of sintanur and LIPI Go 1 are shown in figure 1. The difference between Gas production kinetic of sintanur and LIPI Go 1 probably due to the ratio of amylose and amylopectin. Sintanur is aromatic rice and LIPI Go 1 is upland rice which has 18.46% and 25.58% of amylose content, respectively [24,25]. Starch digestibility of parboiled rice is depending on the degree of gelatinization and retrogradation of rice starch. Rice can be gelatinized by steaming. During parboiling process, rice moisture content during soaking and extent of heating during steaming increase the degree of starch gelatinization [26]. Gelatinization is the collapse of H bonds within the starch granule during hydrothermal treatment that will cause irreversible changes in granular swelling, native crystallite melting, loss of birefringence and starch solubilization [27]. This leads to the dissociation of crystalline regions in starch, leading to higher starch availability to human digestive enzymes [28]. Retrogradation is the recrystallization of amorphous phases created by gelatinization [29]. Retrogradation of amylose will results in the formation of type 3 resistant starch (RS3) [30]. RS3 is heat stable, melts above 120°C, and resistant to amylase digestion [31]. In contrast, retrograded amylopectin is more reversible due to the low melting point (46–65°C) of these crystallites, and therefore it is more digestible by amylase compare with retrograded amylose.
Digestibility of in vitro R-DMD and R-OMD significantly decreased (P<0.05) in parboiling treatment with all soaking periods (Table 3). Soaking rice grain for 30 and 60 minutes decreased R-DMD by 6.13% and 8.90% respectively. The lowest R-DMD and R-OMD was observed in 60 minutes soaking period’s treatment with 81.60% and 91.42%, respectively. Ruminal fermentation generates volatile fatty acids (VFA) and provides energy for microbial protein synthesis [32]. The low digestibility of R-DMD and R-OMD are probably due to increasing resistant starch content. As animal feed, rice is source of carbohydrate which can be easily degraded and used by rumen microbe. According to Oh et al. [33] 75.15% of rice DM will be digested in the rumen within 12 hours. Parboiling process increased rice hardness, crude fiber, and resistant starch. Increasing of rice hardness during parboiling process is caused by starch gelatinization, leading to an increase in fracture resistance [34]. It has been previously reported that parboiling can increase the hardness of rice [35]. Moreover, the parboiling process has also been demonstrated to induce retrogradation of the gelatinized amylopectin and cause the formation of some amylose-lipid compounds, which are also difficult to be digested [36].

Table 3. Digestibility of parboiled rice with different soaking periods and varieties

| Variable                | R-DMD % | R-OMD % | PR-DMD % | PR-OMD % | I-DMD % | I-OMD % |
|-------------------------|---------|---------|----------|----------|---------|---------|
| **Types of Rice mean**  |         |         |          |          |         |         |
| Sintanur                | 87.90b  | 96.10b  | 93.69a   | 96.99    | 98.40   | 99.21   |
| LIPI GO1                | 82.27a  | 89.42a  | 95.25b   | 96.59    | 98.65   | 99.47   |
| P-Value                 | <0.05   | <0.05   | >0.05    | >0.05    | >0.05   | >0.05   |
| **Soaking periods mean**|         |         |          |          |         |         |
| 0 Minutes               | 89.57b  | 94.88b  | 97.29c   | 98.83c   | 99.06   | 99.67   |
| 30 Minutes              | 84.08a  | 91.99b  | 94.49b   | 96.49b   | 98.04   | 99.06   |
| 60 Minutes              | 81.60a  | 91.43a  | 91.63a   | 95.05a   | 98.47   | 99.29   |
| P-Value                 | <0.05   | <0.05   | <0.05    | <0.05    | >0.05   | >0.05   |

R-DMD: rumen dry matter digestibility, R-OMD: rumen organic matter digestibility, PR-DMD: post rumen dry matter digestibility, PR-OMD: post rumen organic matter digestibility, I-DMD: ileum dry matter digestibility, I-OMD: ileum organic matter digestibility.

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

Parboiled rice decreased digestibility in the rumen and increased the digestibility in the post-rumen. Digested parboiled rice dry matter in post rumen treated with 30 minutes and 60 minutes of soaking periods were 10.41% and 10.03%, respectively. Soaking grain for 30 minutes and 60
minutes increased post rumen organic matter digestibility by 4.50% and 3.62%, respectively. Moreover, 3.55% and 6.84% from total sample of parboiled rice treated by 30 minutes and 60 minutes soaking periods was digested in the ileum, respectively. Starch digestion in the small intestine implies greater efficiency of energy compared with ruminal digestion due to reduced methane production and higher efficiency of metabolizable energy utilization [37]. Undigested DM of parboiled rice with 30 minutes and 60 minutes soaking periods were 1.96% and 1.53%. According to Harson [38] about 35 to 50% of undigested starch in the small intestine is degraded in the hindgut.

4. Conclusion
Parboiling treatment increased the proportion of crude protein, organic matter, and fiber, but decreased ether extract and solubility of rice. As ruminant feed, parboiled rice did not only decrease methane and digestibility in the rumen on in vitro rumen fermentation, but also increased efficiency of nutrient utilization by increasing digestibility in post rumen compared to untreated rice.

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