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In vitro rumen fermentability kinetics of parboiled rice bran

R. Fidriyanto*, R. Ridwan, Rohmatussolihat, W. D. Astuti, N. F. Sari, E. B. M. Adi, E. S. Mulyaningsih and Y. Widyastuti
Research Center for Biotechnology, Indonesian Institute of Sciences,
Jl. Raya Bogor km 46, Cibinong, Bogor, West Java 16911 - Indonesia
**Corresponding E-mail :rusli.sbh@gmail.com*

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ABSTRAK

Produk beras pratanak telah diproduksi secara luas oleh industri di negara-negara berkembang. Peningkatan produk beras pratanak menyebabkan meningkatnya limbah dedak. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh proses pembuatan beras pratanak terhadap nutrisi dedak dan mengevaluasi nilai nutrisi dan karakteristik dedak padi pratanak dalam fermentasi rumen menggunakan metode kecernaan secara in vitro. Percobaan dilakukan dengan rancangan acak lengkap faktorial dengan 2 faktor dan 3 ulangan Faktor pertama adalah jenis dedak padi yang berasal dari 4 varietas tanaman padi yang terdiri dari LIPI GO1, LIPI GO2, LIPI GO4, dan Sintanur. Faktor kedua adalah waktu perendaman berbeda terdiri dari 0 menit (kontrol), 30 menit dan 60 menit. Data dianalisis dengan menggunakan ANOVA dan signifikansi dari masing-masing perlakuan dianalisis lebih lanjut dengan menggunakan uji Duncan multiple range (P <0,05). Parameter kinetika dihitung dengan persamaan Ørskov melalui analisa regresi non-linear. Hasil penelitian menunjukkan bahwa kualitas dedak padi pratanak dipengaruhi secara signifikan oleh proses parboiling seperti periode perendaman dan varietas padi. Komposisi kimia, produksi gas rumen, dan kecernaan dedak pratanak berbeda-beda tergantung pada varietas padi. Perendaman selama 30 menit dan 60 menit menurunkan bahan organik, protein kasar, dan meningkatkan serat kasar (p <0,05). Selain itu, perendaman gabah selama 60 menit meningkatkan lemak kasar. Semakin lama periode perendaman menyebabkan berkurangnya total gas, produksi gas potensial, laju produksi gas dan jumlah kecernaan bahan organik (P<0,05).
Keywords: Dedak padi pratanak, perendaman, kecernaan, in vitro

ABSTRACT

Parboiled rice product has been widely produced by industry in the developing countries. The increasing of parboiled rice product will consequently increased rice bran waste. Therefore, the objective of the study was to analyze the effect of parboiling processes on nutritional value of rice bran and evaluate nutritional value of parboiled rice bran in the rumen fermentation using in vitro digestibility method. The experiment was arranged in completely randomized factorial design with 2 factors and 3 replications. The first factor is types of rice bran from different rice plant variety consisted of LIPI GO1, LIPI GO2, LIPI GO4, and Sintanur. The second factor is soaking time consist of 0 minute (control), 30 minutes and 60 minutes. Data were analyzed by using ANOVA and significant effects of each treatment were further analyzed by using the least significant difference by Duncan multiple range test (P<0.05). Kinetic parameters of Ørskov's equation were obtained by non-linear regression procedure. Results showed that the quality of parboiled rice bran was significantly affected by the parboiling process such as soaking periods and rice variety. Chemical composition, rumen gas production, and digestibility of rice bran differ depends on rice variety. Soaking for 30 minutes and 60 minutes decreased organic matter, crude protein, and increased crude fiber (P<0.05). Moreover, soaking rice grain for 60 minutes

increased crude fat. The longer of soaking periods decreased of total gas, potential gas production, gas production rate and total organic matter digestibility (P<0.05).
Keywords: parboiled rice bran, soaking periods, rice variety, digestibility, in vitro

INTRODUCTION

Rice bran, the major cereal by-product of rice industry approximately for 5–8% of milled rice (Sereewatthanawut et al., 2008; Sumantha et al., 2006). It contains 14–16% protein, 12–23% fat, 8–10% crude fiber (Kahlon, 2009; Malekian et al., 2000). The majority of rice bran produced in the world is utilized as raw material of concentrate feed for energy resources (Hagl et al., 2013). Many reports indicate that feeding cattle with rice bran could increase average body weight (Forster *et al.*, 1993; Till *et al.*, 1991; Choi et al, 2016). However, the quality of rice bran is influence by varieties and paddy treatment before grinding.

Parboiled rice product has been widely produced by industry in the developing countries. Currently, the use of Parboiling technology provides promising opportunities to improve rice nutrition. The parboiling process is done before the hulls are removed and consist of three-stage process namely soaking, steaming and drying (Fernandes, 2016). The increasing of parboiled rice product consequently increased rice bran waste. All processes on parboiling method have impacts on the quality of parboiled rice. Parboiling process could increase nutrition of rice by transfer nutrition content from bran into the inner layers of rice caryopsis and decreased percentage of broken grain (Levien *et al*., 2015). This Transfer nutrition will be effected on nutritional quality of rice bran as feedstuff. However, little is known about the influence of parboiling processes on the nutritional value of rice bran. Therefore, the objective of the study was to analyze the effect of parboiling processes on nutritional value of rice bran and evaluate nutritional value of parboiled rice bran in the rumen fermentation using in vitro digestibility method.

MATERIALS AND METHODS

Materials

Rice grains were consist of 4 different varieties i.e. LIPI GO1, LIPI GO 2, LIPI GO 4 and Sintanur. Rice grains of LIPI GO 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.

MATERIALS AND METHODS

Parboiled Rice Bran.

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

Experiment

The experiment was arranged in completely randomized factorial design with 2 factors and 3 replications. The first factor is types of rice bran from different rice plant variety consisted of LIPI GO1, LIPI GO2, LIPI GO4, and Sintanur. The second factor is soaking time consist of 0 minute (control), 30 minute and 60 minute. The dried sample of parboiled rice bran was analyzed for pH, Proximate analysis (dry mater, crude protein, ash content, crude fiber and crude fat content (AOAC 2005). *In vitro* fermentation was conducted according to the method of Theodorou *et al.*, (1994) with modification. Rumen fluid were collected from two rumen-fistulated Ongole crossbred cattle before morning feeding. The rumen-fistulated Ongole crossbred cattle were managed according to the protocols approved by the Ethic Clearance Committee of Indonesian Institute of Sciences (Number 9879/WK/HK/XI/2015). Rumen fluid that used for in vitro studies was filtered using double layer of cheesecloth, pooled in thermos, sealed and immediately transported to the laboratory. Into each fermentation bottle, 0.5 g substrate and 50 mL mixture solution of McDougall buffer and rumen fluid (3:1) were added. The tubes were flushed by CO_2 for 60 s (pH 6.5-6.9) and incubated in water bath at temperature of 39°C. Gas production was measured at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, and 48 h of incubation to obtain kinetics gas production. After 48 h of incubation, pH value of rumen-buffer mixture were analyzed. Thereafter, rumen-buffer mixture was separate using Whatman™ papers no 41 (CAT No.1441-125). Dry matter and organic matter disappearance (DMRD and OMRD) were measured after 48 h of fermentation, while dry matter and organic matter digestibility (DMD and OMD) were measured after 2×48 h of fermentation, with the addition of pepsin-HCl after 48 h of fermentation.

Statistical Analysis

Data of proximate, gas production, rumen feed disappearance, and Volatile fatty acid production were analyzed by using ANOVA with SPSS 23 for windows. Significant effects of each treatment were further analyzed by using the least significant difference by Duncan 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 gas produced by the soluble fraction, '*b*' is gas produced by the insoluble but slowly fermenting fraction, '*c*' is constant gas production rate, '*t*' is time of fermentation. Kinetic parameters of Ørskov's equation were obtained by non-linear regression procedure in SPSS 23.

RESULTS AND DISCUSSIONS

Rice bran, a valuable by product contains of essential nutrients such as protein, minerals, vitamins, fiber, amino acids and antioxidants (Younas *et al.*, 2011). Compositional data from parboiled rice bran samples are presented in Table 1. Crude protein content of the different rice brans was low, ranging between 4,2% and 4,77% and this result were lower than in other studies. Rice bran contains about 10–15% of high quality protein (Lean, 2014). The highest crude protein content in rice grain is on the outer surface of rice kernel and in bran (Champagne *et al*., 2004). There was no significant different on 4 varieties of rice bran on dry matter, crude fiber and crude fat but organic matter and crude protein content of LIPI GO4 was significantly higher ($p<0.05$). Significant differences ($P<0.05$) existed between the samples and these variations may be attributed to varietal differences. The nutritional composition of rice bran is depends on rice variety (Amissah *et al*., 2003).

Soaking rice grain for 30 minutes and 60 minutes followed by steaming decreased organic mater 81.90% and 82.43%, crude protein 3.68% and 3.96% and increased crude fiber 34.94% and 37.79%, respectively ($p<0.05$). Milled parboiled rice has been found to have greater amounts of nutrition compound and certain minerals as compared with milled raw rice (Bhattacharya *et al*., 2013) The changes of nutrition of parboiled rice was caused by internal migration of water-soluble small molecules from the bran layer into the endosperm during soaking process. The migration of water-soluble small molecules will decreased nutrition compound on rice bran. Soaking rice grain for 60 minute increased crude fat 4.29% ($p<0.05$). Rice bran is mainly composed of the aleurone layer and embryo, and contains 60% of non-starch lipids that are the major lipids present in bran layer as spherosomes (Godber and Juliano, 2004). Lipid are broken and released from the surface of kernel during parboiling process. Lipids are diffused into bran layer of parboiled rice and make bran become oily (Mahadevappa and Desikachar, 1968).

Several studies have evaluated the effects of soaking temperature and steaming duration in parboiled rice. Soaking had significant effect on chemical compositions of rice grains mainly on starch content. Decrease in starch content after soaking might be due to leaching and formation of amyloselipid complexes during heating in water (Singh *et al*., 2006; Derycke *et al*., 2005). Soaking and steaming might also have same effect on rice bran. The formation of amyloselipid made rumen microbes harder to degrade and lead to decreased of gas production.

The organic matter was decreased during the increasing of soaking periods (Table 1). Decreased of mineral in rice bran might be due to leaching of minerals from the husk and bran into the starchy endosperm during soaking process. Minerals leached into the soaking water and also diffused into the endosperm of rice (Derycke *et al,* 2005).

In vitro method was used to estimate digestibility of parboiled rice bran on rumen and post rumen digestibility. Digestibility of parboiled rice bran in the rumen was indicated by DMDR and OMDR parameters. There was no significant different on DMDR and OMDR. Ruminal pH and OMD was significantly affected ($P<0.05$) by soaking time (Table 2) but not affected ($P<0.05$) by variety of rice bran. Despite of an increase in pH value due to the soaking treatment, the pH

Table 1. Nutritional Value from Different Soaking Periods and Types of Rice Bran

Variable	DM (%)	OM (%)	CP (%)	EE (%)	CF (%)
	% DM Basis				
Types of Rice Bran mean (T)					
LIPI GO1	95.19	84.78^{c}	4.20^{a}	3.78	34.72
LIPI GO2	94.87	84.04^{b}	4.18^{a}	3.52	33.85
LIPI GO4	95.67	85.06^{c}	4.77^{b}	3.52	33.25
SINTANUR	95.15	78.61^{a}	4.18^{a}	3.56	32.44
P-Value					
Types of Rice (T)	>0.05	<0.05	<0.05	>0.05	<0.05
Soaking periods mean (S) (Minute)					
0	94.58	85.03^{c}	5.36^{a}	3.13^{a}	27.95^{a}
30	95.90	81.90^{a}	3.68^{b}	3.36^{a}	34.94^{b}
60	95.18	82.43^{b}	3.96^{b}	4.29^{b}	37.79^{c}
P-Value					
Soaking periods	>0.05	<0.05	<0.05	<0.05	<0.05
P-Value					
Interaction (T x S)	>0.05	>0.05	>0.05	>0.05	<0.05

DM: dry matter; OM: organic matter; CP: crude protein; EE: Ether Extract; CF:Crude Fiber

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

value was still within the normal pH range of the rumen. The normal rumen pH range is between 5.8 to 6.9 (Morgante *et al*., 2009). The increased of pH could be explained by higher fat content, lower fiber content, lower OM digestibility and lower potential gas production of parboiled rice bran. Soaking treatment of rice bran for 30 minutes decreased OMD (P<0.05) by 25, 68%. The decreased of OMD was caused by the increased of crude fiber and crude fat content and decreased of protein content. Higher fat content has negative effects on digestibility and fermentation of feeds including animal performance (Kumar, 2013). High fiber in the feed will cause the process of fermentation in the rumen run slowly, thus avoiding the rapid increase of gas production and acid too quickly in the rumen, which could depress pH and fiber fermentation and lead to digestive upset (Lean 2014)

Different types of rice bran affected potential gas production, gas production rate, and total gas production (P<0.05) as presented in Table 3. Chemical composition of rice bran differ depends on rice variety, climatic conditions, and rice processing methods (Taylor *et al*. 2011). Differences in chemical composition of feed ingredients will affected rumen gas production. Production of gas in the rumen related to rumen microbial activities. The kinetic gas production are presented in Figure 1. LIPI GO 4 rice bran without soaking treatment was shown the highest gas production and gas production rate. The increasing of soaking periods was decreased gas production and gas production rate.

The increasing of the soaking period will decrease the total gas production, gas production potential, and gas production rate (P<0.05). Decreased of gas production was supported by a decrease in protein levels and organic matter. Rice bran surface protein during parboiled rice making process play role in water diffusion. Because the glass transition of surface protein is slightly lower than starch, surface protein may acts as a regulator of water diffusion into the starch granules and controls granular swelling during the

Table 2. Digestibility from Different Soaking Periods and Types of Rice Bran

Variable	pH	DMDR	OMDR	DMD	OMD
		%DM Basis			
Types of Rice Bran mean (T)					
LIPI GO1	6.39	46.70[a]	51.63	46.77	52.39
LIPI GO2	6.35	37.46[a]	51.45	34.04	48.8
LIPI GO4	6.32	22.52[b]	42.03	32.04	47.37
SINTANUR	6.37	20.29[b]	37.59	35.60	46.97
P-Value					
Types of Rice (T)	>0.05	<0.05	>0.05	>0.05	>0.05
Soaking periods mean (S) (Minute)					
0	6.30[a]	31.33	53.96	41.28	57.51[b]
30	6.39[b]	29.98	42.72	32.28	42.74[a]
60	6.39[b]	33.92	40.27	37.77	46.41[ab]
P-Value					
Soaking periods	<0.05	>0.05	>0.05	>0.05	<0.05
P-Value					
Interaction (T x S)	>0.05	>0.05	>0.05	>0.05	>0.05

DMRD: dry matter disappearance, OMRD: organic matter disappearance, DMD dry matter digestibility, OMD: organic matter digestibility

[a-c] Means with different superscripts within columns significantly differed (P<0.05)

gelatinization process and make water absorption capacity become higher (Matveev *et al*., 2000). During gelatinization process, the starch and protein expand and fill in the internal spaces between granules and created strong cohesion between them (Islam, 2004; Sareepuang *et al*., 2008). Strong cohesion between starch granule in rice bran make protein and starch become harder to degrade by rumen microbes and decreased organic matter and dry matter digestibility and total gas production. Decreased of organic matter in rice bran means decreased of nutrients content in feed which can used by rumen microbes for growth. Nutrient such as protein and starch partly converted by rumen microbe into volatile fatty acid.

Matrix correlation between nutritional compound, variable, and parameter of in vitro digestibility of parboiled rice bran are presented in Table 4. The results showed, crude fat had negative correlation with rumen pH value (P<0.05). Fat content in feed become inhibitor for rumen microbial growth. Crude protein had negative correlation with pH value and positive correlation with OMD, potential gas production, and total gas production (P<0.05). Protein is easy to be digested in rumen by rumen microbe except protein which protected with certain compound. Protein will be hydrolyzed by rumen microbe and turn into ammonia and amino acids (Neeta Agarwal and Chaudhary 2015). As the decrease of protein compound in feedstuff, there is generally a corresponding increasing rumen pH values.

Crude fiber showed positive correlation with pH value and negative correlation with OMD, potential gas production, and total gas production (P<0.05). Fiber consist of acid detergent fiber (ADF) and neutral detergent fiber (NDF). Fiber is required to maintain normal ruminal pH, stimulating rumination, promoting dilution of more fermentable components of the feed, and favors ruminal buffering, but large amounts

Table 3. Gas Production Kinetic from Different Soaking Periods and Types Rf Bice bran

Variable	PGP (mL)	GPR (mL/hr)	Total Gas (mL)
Types of Rice Bran mean (T)			
LIPI GO1	35.96^{a}	0.037^{a}	29.089^{a}
LIPI GO2	37.20^{a}	0.039^{a}	31.44^{a}
LIPI GO4	48.56^{b}	0.052^{b}	44.78^{b}
SINTANUR	47.02^{b}	0.055^{b}	42.72^{b}
P-Value			
Types of Rice (T)	<0.05	<0.05	<0.05
Soaking periods mean (S) (Minute)			
0	63.18^{b}	0.047	56.72^{a}
30	31.37^{a}	0.044	26.86^{b}
60	31.99^{a}	0.042	27.44^{b}
P-Value			
Soaking periods	<0.05	>0.05	<0.05
P-Value			
Interaction (T x S)	>0.05	>0.05	>0.05

PGP: potential gas production, GPR: gas production rate
$^{a\text{-}c}$ Means with different superscripts within columns significantly differed (P<0.05).

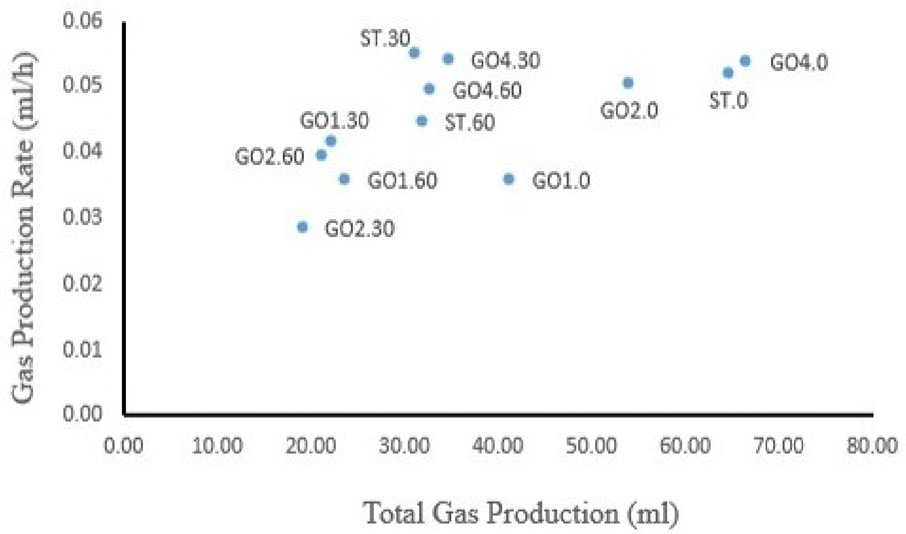

Figure 1. *In* v*itro G*as Production Kinetics of Parboiled Rice Bran

Table 4. Matrix Correlation (r) between Nutritional Value and Rumen Fermentation Variable

Variable	DMDR	OMDR	DMD	OMD	pH	PGP (ml)	GPR (ml/jam)	Total Gas (ml)	AA (% mM)	PA (% mM)	BA (% mM)	Total VFA (ml mol/lt)	AP Ratio
Nutrition value													
EE	0.15	0.09	0.01	0.07	-0.62*	-0.32	-0.23	-0.32	-0.50*	-0.44*	-0.31	-0.17	-0.36*
CF	0.11	-0.29	-0.17	-0.42*	0.55*	-0.81*	-0.20	-0.77*	-0.81*	-0.65*	-0.48*	-0.21	-0.61*
CP	-0.12	0.31	0.17	0.46*	-0.62*	0.82*	0.23	0.78*	0.56*	0.52*	0.27	0.01	0.37*
Variable													
Soaking periods	0.06	-0.32	-0.1	-0.32	0.49*	-0.77*	0.2	-0.71*	-0.84*	-0.64*	-0.64*	-0.50*	0.36*
Types of Rice Bran	-0.65*	-0.33*	-0.27	-0.14	-0.14	0.29	0.50*	0.36*	0.43*	0.33*	0.15	-0.35*	0.67*

CP: crude protein; EE: Ether Extract; CF: Crude Fiber;DMRD: dry matter disappearance; OMRD: organic matter disappearance; DMD: dry matter digestibility; OMD: organic matter digestibility; PGP: potential gas production; AA: Acetic Acid, PA: Propionic Acid , BA: Butyric Acid, acetate-propionate ratio
* Means with different superscripts within columns significantly different ($P<0.05$).

consumption of NDF will decreased feed intake (Rodrigues, 2016; Zebeli *et al*. 2012).

Main VFA in the rumen are propionate, butyrate, acetate; and small portion of isobutyrate, valerate, isovalerate, and caproate (McDonald *et al.*, 2011). The result showed (Table. 5) the longer the soaking periods will cause the decline concentration of total VFA, acetic acid, propionic acid, butyric acid and valeric acid ($P<0.05$). The decreased concentrations of volatile acids could be the response of the lower fermentation activity due to the decreasing of rice bran nutrient during the soaking process. The decreasing of VFA concentration was followed by decreased of digestibility and increased of pH. There is a positive correlation between DM digestibilities with ruminal total VFA production. Feed with low DM digestibility can contribute to low ruminal total VFA production (Cao *et al,* 2011).

The increasing of soaking periods followed by decreased A/P ratio ($P<0.05$) cause of lower acetate and higher propionate. The decrase of A/P ratio means an increase in propionate proportion. The proportion of VFA in the rumen is affected by diets, microbial growth rates, levels of feeding, and ruminal pH (López *et al.*, 2000). Probably, the decreased of A/P ratio cause by steaming treatment and the increasing of starch content of rice bran from 5,96%(0 minute) to 6.95% (30 minutes) and 15.20% (60 minutes) respectively (data unpublished). Steaming treatment on grain increased proportion of propionic acid and decreased ratio of acetic acid to propionic acid (Lee *et al.,* 1982; Zinn, 1987; Barajas and Zinn, 1998; Quiao *et al.*, 2015) The high portion of fibrous diet will result in the production of large amounts of acetate and butyrate, whereas the high starch diet results in a greater proportion of propionate, although acetate is still the dominant VFA (Beever and Mould, 2000; Gonzalez *et al.*, 2012; Li *et al.*, 2014).

CONCLUSION

The quality of rice bran from parboiled rice depends on rice variety and parboiling process. Parboiled rice bran had lower organic matter and crude protein content but higher in fat content and crude fiber content compared with non-parboiled rice. The increasing of rice grain soaking periods, decreased total gas production, gas production rate, potential gas production rate, organic matter digestibility and total VFA. Rice bran from parboiled rice waste had lower nutrition than rice

Table 5. Volatile Fatty Acid on *In vitro R*umen Feed Digestibility from Different Soaking Periods and Types of Rice Bran

Variable	AA (mL mol/lt)	PA (mL mol/lt)	BA (mL mol/lt)	VA (mL mol/lt)	Total VFA (mL mol/lt)	A/P Ratio
Types of Rice Bran mean (T)						
LIPI GO1	38.95^{a}	16.91^{a}	8.15^{b}	2.22^{c}	66.26^{a}	2.29^{b}
LIPI GO2	38.87^{a}	17.78^{b}	8.18^{b}	1.67^{ab}	66.52^{a}	2.17^{a}
LIPI GO4	43.11^{b}	19.60^{c}	7.65^{a}	1.54^{a}	71.92^{b}	2.19^{a}
SINTANUR	45.59^{c}	17.83^{b}	8.78^{c}	1.79^{b}	73.99^{c}	2.55^{c}
P-Value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Soaking periods mean (S) (Minute)						
0	48.39^{a}	19.23^{c}	8.97^{c}	2.01^{a}	78.61^{c}	2.53^{a}
30	40.86^{b}	18.07^{b}	8.16^{b}	1.95^{b}	69.05^{b}	2.26^{b}
60	35.65^{c}	16.79^{a}	7.44^{a}	1.45^{b}	61.35^{c}	2.21^{c}
P Value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Interaction (T x S)	>0.05	<0.05	<0.05	<0.05	>0.05	<0.05

AA: acetic acid, PA: propionic acid , BA: butyric acid, VA: valeric acid, A/P Ratio : acetate-propionate ratio

[a-c] Means with different superscripts within columns significantly differed (P<0.05)

bran from common rice.

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