Simple fermentation using shrimp paste on the nutritional and functional properties of rice bran as a broiler chicken feed ingredient

by Sugiharto Sugiharto

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Sugiharto Sugiharto, Endang Widiastuti, Ratno Murwani, Turrini Yudiarti, Hanny Indrat Wahyuni and Tri Agus Sartono

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia (50275)
sgh_undip@yahoo.co.id

Abstract

The study aimed to investigate the effect of simple fermentation using shrimp paste as a starter on the nutritional and functional characteristics of rice bran as an alternative feed ingredient for broiler chickens. To produce the fermented rice bran, the autoclaved rice bran (200 g) was placed in an anaerobic jar and added with sterile water (1: 3, g: mL). Shrimp paste (2 g) was added to the mixture and then mixed thoroughly. Solid-state fermentation was conducted anaerobically for 0, 2, 4 and 6 days. The fermented rice bran was sun-dried and then analyzed. Fermentation using shrimp paste increased crude protein content of rice bran. Crude fiber linearly increased with incubation period. Gross energy was lower in fermented than that in unfermented rice bran. Fermentation resulted in higher total acidity. Six days of fermentation increased total phenolics content, but antioxidant activity was higher in unfermented than in fermented rice bran. The longer fermentation period linearly increased essential amino acid L-methionine, L-phenylalanine, L-histidine, L-threonine and L-valine, but decreased L-lysine HCL. Fermentation also increased non-essential amino acid L-cysteine, L-serine and L-tyrosine, while decreased L-arginine, L-aspartate acid and L-glutamic acids. In conclusion, simple fermentation using shrimp paste improved the nutritional values of rice bran as a broiler feed ingredient.

Keywords: *antioxidants, fermentation starter, lactic acid bacteria, nutritional properties, rice bran*

Introduction

In the commercial broiler industry, feed is the most dominant factor in total production costs. As a response to the increase in the price of conventional feed ingredients (e.g., corn and soybean meal), there is currently high interest in reusing by-products from agro-industry as feed constituents in broiler rations. This not only has a positive impact on environment, but can also reduce feed costs in broiler production (Abbasi et al 2015, Kasapidou et al 2015). Rice bran is an example of agro-industrial waste that has been used as a feed ingredient for broiler chickens (Supriyati et al 2015, Sugiharto et al 2018). However, there are some limitations to use this by-product in broiler rations. The high crude fiber and low protein contents as well as the presence of anti-nutritional factors can limit the use of rice bran as a feed ingredient for broiler chickens (Sugiharto et al 2018).

Fermentation using lactic acid bacteria (LAB) has widely been conducted to improve the nutritional and functional values of feed ingredients for broiler chickens (Sugiharto and Ranjitkar 2019). The LAB are generally isolated from the digestive tract of livestock, but recent knowledge shows that LAB can also be isolated from various fermentation products. One of the fermentation products that contain LAB is shrimp paste (Amalia et al 2018, Romadhon et al 2018), which is a traditional fermented fish or shrimp product in the form of a solid paste. There are several LAB species isolated from shrimp paste, including *Lactobacillus plantarum*, *Lactococcus lactis*, *Vagococcus fluvialis* and*Lactococcus garvieae* (Maeda et al 2014), among which *L. plantarum* is the most dominant LAB species (Amalia et al 2018). Altogether, due to its high LAB content, shrimp paste may therefore function as a fermentation starter. Yet, there is no study documenting the application of shrimp paste as a fermentation starter so far.

The purpose of this study was to investigate the effect of a simple fermentation using shrimp paste as a starter on the nutritional and functional characteristics of rice bran as an alternative feed ingredient for broiler chickens.

Materials and methods

Production of fermented rice bran

Rice bran was purchased from a local poultry shop in Semarang, Central Java, while shrimp paste from a traditional market in Rembang city, Central Java, Indonesia. To make fermented rice bran, 200 g of rice bran was sterilized using an autoclave and then placed in an anaerobic jar. Sterile water was added to rice bran (1: 3, g: mL) and then 2 g of shrimp paste was added to the mixture. It was then mixed thoroughly. Fermentation was carried out based on the solid-state fermentation method and conducted anaerobically for 0, 2, 4 and 6 days. After incubation the fermented rice bran was sun-dried and then stored at room temperature before analysis. The study was conducted in triplicates.

Laboratory analysis

Total plate count analysis was conducted to count LAB population in shrimp paste. The enumeration was performed on de Man, Rogosa and Sharpe agar (Merck KGaA, Darmstadt, Germany) following anaerobic incubation at 38°C for 2 days. The analysis showed that shrimp paste used in this study contained 16.4 log cfu/g. The chemical compositions of the fermented rice bran were measured based on the standard proximate analysis (AOAC 1995). The gross energy content were determined using oxygen bomb calorimeter (Parr Instruments Co., Moline, IL, USA). Benzoic acid was used as a standard of calibration. Total acidity of the fermented rice bran was quantified according to the titration method (Apriyantono et al 1989). The total acidity was measured by neutralizing the acid in the sample of fermented rice bran with 0.1 M NaOH standardized using Potassium Hydrogen Phthalate. The change in colour of phenolphthalein indicated the titration endpoint. To assess the antioxidant potential of fermented rice bran, the sample was subjected to the 2,2-diphenylpicrylhydrazyl (DPPH) free radical neutralizing assay (Wu et al 2009). The absorbance was measured at 515 nm. Ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference. The phenolic compounds of fermented rice bran was measured on the basis of Folin-Ciocalteu procedure (Orak 2006). The fermented rice bran (0.5 mg), distilled water (8 mL), Folin-Ciocalteu reagent (0.5 mL, Merck KGaA, Darmstadt, Germany) and sodium carbonate (1 mL, Merck KGaA) were mixed thoroughly. It was then incubated at room temperature for 30 min. The absorbance was measured at 765 nm with a spectrophotometer. Gallic acid was employed to prepare standard curve. The content of amino acids in the fermented rice bran was measured according to a standard ultra-performance liquid chromatography procedure (Szkudzińska et al 2017).

Statistical analysis

Data were treated with analysis of variance (ANOVA, SPSS 16.0 version). Duncan multi-range test was performed when the impact ($p<0.05$) of fermentation was found. Also, data were subjected to linear regression to determine the effect of incubation period on the parameters measured.

Results and discussion

Data on the proximate compositions, total acidity and antioxidant capacity of fermented rice bran are listed in Table 1. Fermentation using shrimp paste as starter increased ($p<0.05$) crude protein content of rice bran. This finding confirmed that the LAB contained in shrimp paste exerted a fermentation process resulting in elevated protein content of rice bran. This result was in agreement with Suphalucksana and Soytong (2018) showing an increased protein content in feedstuff with LAB (*Pediococcus acidilactici*) fermentation, but differed from Day and Morawicki (2018) who did not find any effect of fermentation using amylolytic LAB on the protein content of grain sorghum. The increased protein in rice bran after fermentation seemed due to the increased accumulation of bacterial biomass, which is rich in protein (Day and Morawicki 2018). Regression analysis showed that the content of crude fiber linearly increased (p<0.05) with the longer incubation period. This result was in accordance with Sugiharto et al (2018) showing the increased crude fiber content of the by-product of herbal medicine with *Bacillus*-fermentation. In such case, fermentation was most likely to break down polysaccharides (complex carbohydrates) into oligosaccharides (simpler carbohydrates) (Sugiharto et al 2018: Yudiarti et al 2019). The latter condition may result in the increased crude fiber and decreased carbohydrates concentrations in the fermented rice bran.

Table 1. Nutritional compositions, total acidity and antioxidant capacity of fermented rice bran

Items	Incubation period				SEM	*p* value-ANOVA[1]	*p* value-regression[2]
	Day 0	Day 2	Day 4	Day 6			
DM (%)	89.8[a]	78.1[c]	81.7[bc]	85.2[b]	1.42	<0.01	0.44
Crude protein (% DM)	8.26[b]	8.89[a]	8.80[a]	8.93[a]	0.10	0.02	0.02
Crude fat (% DM)	14.1	15.9	20.8	15.2	1.33	0.33	0.52
Crude fiber (% DM)	29.3	31.8	35.8	34.9	1.08	0.10	0.02
Crude ash (% DM)	10.5	11.0	10.9	11.0	0.09	0.10	0.05
Gross energy (kcal/kg DM)	4,365[a]	4,096[c]	4,153[bc]	4,232[b]	32.9	<0.01	0.26
Total acidity (%)	4.94[c]	46.7[b]	47.2[a]	47.2[a]	5.50	<0.01	<0.01
Total phenolics (%)	0.19[b]	0.20[ab]	0.20[ab]	0.21[a]	<0.01	0.04	0.01
Antioxidant activity (IC_{50}, ppm)[3]	1,881[d]	2,514[a]	2,392[b]	2,197[c]	73.6	<0.01	0.23

[a,b,c,d] Means in the same row with various letters indicate notable differences (p<0.05);[1] Refers to analysis of variance (ANOVA);[2] Refers to regression analysis; [3] IC_{50} is acknowledged as the effective concentration at which the 2,2-diphenylpicrylhydrazyl (DPPH) radicals were scavenged by about 50%. A higher DPPH radical scavenging activity is associated with a lower IC_{50} value DM: dry matter

Gross energy was lower (p<0.05) in the fermented rice bran than that in unfermented one. This result was contrary to Supriyati et al (2015) showing an increased gross energy content of rice bran with *Bacillus amyloliquefaciens*-fermentation. As inferred previously, fermentation using shrimp paste was associated with the decreased carbohydrate content, and hence decreased gross energy in the fermented rice bran. Owing to this, fermentation is economically justified as a means of improving the protein quality of the rice bran but probably the gross energy value of the rice bran has been reduced as the ash content was higher after fermentation. In this study, fermentation with shrimp paste resulted in higher (p<0.05) total acidity in rice bran. This was in agreement with Liang et al (2020) reporting that LAB-fermentation increased total acid in Hong Qu glutinous rice wine. In this case, LAB in shrimp paste exerted the fermentation process and thus converted carbohydrates into lactic acid leading to the increased total acidity in the rice bran.

Fermentation with LAB has been associated with the increased phenolic compounds in rice bran (Le et al 2019). In agreement, our finding showed that 6 days fermentation using shrimp paste increased (p<0.05) the content of total phenolics in rice bran. Le et al (2019) suggested that LAB may produce several enzymes that can hydrolyze the insoluble bound phenolic acids to the soluble/free phenolic acids. The latter condition may thereby increase the content of phenolic components in rice bran. In general, the increased total phenolics was attributed to the increased antioxidant activity of rice bran (Nisa et al 2019). This was, however, different from our present study, at which the increased total phenolics was not accompanied by the increased antioxidant activity of rice bran. In the previous study, Sugiharto et al (2018) also reported that fermentation using *Bacillus subtilis* lowered antioxidant activity of the by-product of herbal medicine. The latter investigators suggested that fermentation may destruct the phenolic compounds resulting in poor scavenging activity of phenolics against free radicals. It should also be noted that other than phenolics, there are other compounds that may contribute for the antioxidant activity of the substrates, including flavonoids, tannins, β-carotene, tocopherols, ascorbic acids, etc. (Sugiharto et al 2016).

Table 2. Amino acid contents of fermented rice bran

Items (mg/kg)	Incubation period				SEM	*p* value-ANOVA[1]	*p* value-regression[2]
	Day 0	Day 2	Day 4	Day 6			
L-Methionine	385	386	393	392	1.37	0.15	0.04
L-Cysteine	468^{b}	577^{b}	1,237^{a}	1,211^{a}	79.0	<0.01	<0.01
Glycine	5,190	5,504	5,251	5,486	67.5	0.24	0.30
L-Alanine	4,932	4,856	5,222	5,005	61.9	0.18	0.30
L-Arginine	7,505	7,569	6,807	7,009	123	0.06	0.04
L-Aspartate acid	6,525^{a}	4,680^{c}	5,326^{b}	4,890^{bc}	178	<0.01	<0.01
L-glutamic acids	10,997^{a}	8,240^{c}	9,351^{b}	8,622^{bc}	274	<0.01	0.01
L-Phenylalanine	4,169^{b}	5,350^{a}	4,872^{a}	5,227^{a}	121	<0.01	<0.01
L-Histidine	2,554	2,873	2,540	2,666	50.8	0.06	0.99
L-Isoleucine	2,937	2,990	3,067	2,984	29.1	0.49	0.41
L-Leucine	5,861	5,925	6,135	6,059	66.5	0.48	0.18
L-Lysine HCL	4,740^{a}	3,391^{b}	3,644^{b}	3,644^{b}	116	<0.01	0.02
L-Proline	3,883	3,838	4,109	3,987	45.3	0.15	0.16
L-Serine	3,827^{b}	4,419^{a}	3,652^{b}	4,054^{ab}	98.0	0.02	0.93
L-Threonine	3,613	3,942	3,671	3,845	52.1	0.08	0.38
L-Tyrosine	2,310^{b}	2,838^{a}	2,564^{ab}	2,674^{a}	64.5	0.02	0.16
Valine	5,005	5,347	5,332	5,345	58,9	0.09	0.05

^{a,b} *Means in the same row with various letters indicate notable differences (p<0.05)*

[1]*Refers to analysis of variance (ANOVA);* [2]*Refers to regression analysis*

Table 2 presents the amino acid contents of fermented rice bran. The longer period of fermentation using shrimp paste was attributed to the linear increase (p<0.05) in essential amino acid L-methionine of rice bran. Another essential amino acids (i.e., L-phenylalanine, L-histidine, L-threonine, L-valine) were also increased by shrimp paste-fermentation. Our present finding was in accordance with Lim et al (2019) showing the proteolytic activity (decomposing of protein into amino acids) of LAB, and thus capable of producing a number of amino acids. In this study, there was no source of additional nitrogen hence the improvement in amino acids represented some of the crude protein in the rice bran being converted to "true" protein of superior amino acid values. In contrast to the above-mentioned essential amino acids, one essential amino acid, L-lysine HCL, decreased with shrimp paste-fermentation. In this case, LAB may use particular amino acid (such as lysine) to support their growth (Lee et al 2014), and therefore reduced the content of the respective amino acid in the substrate. With regard to non-essential amino acids, fermentation using shrimp paste increased the concentrations of L-cysteine, L-serine and L-tyrosine, while decreased L-arginine, L-aspartate acid and L-glutamic acids. Again, LAB may produce (Lim et al 2019) as well as utilized (Lee et al 2014) amino acids and thereby affecting the concentrations of particular amino acids in rice bran.

Conclusions

- It could be concluded that simple fermentation using shrimp paste improved the nutritional values of rice bran as a broiler feed ingredient. Hence, shrimp paste may be employed as a cheap and convenient fermentation starter for feedstuffs.

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