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The reduction of fat deposition in broiler chickens fed diet containing modified rice bran tempeh

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ABSTRACT

The aim of this study was to assess the impact of modified rice bran tempeh on performance, carcass quality, organoleptic properties, fat deposition, and nutritional meat composition in broiler. This research used a completely randomized design. Two hundred broilers (a male to female ratio of 1:1) were divided into 4 treatment groups with 5 replications for each treatment, as follows: P1 = Feed without modified rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; and; P4 = Feed with 25% modified rice bran tempeh. The experimental results showed that all treatment groups had similar performance. The treatments significantly reduced ($P<0.05$) abdominal fat and total fat deposition. In addition, the treatments also significantly reduced meat fat and ash content ($P<0.01$), but it significantly increased ($P<0.01$) moisture contents. It was concluded that feeding 20% or 25% modified rice bran tempeh reduced meat fat content and fat deposition without reducing broiler performance.

Keywords: Broiler chickens, Fat deposition, Meat fat, Modified rice bran tempeh

INTRODUCTION

Feed costs account for 50%-80% of production costs in broiler chicken farming, even though feed prices are very high. High feed price is caused by the high price of main feedstuffs such as corn, soybean meal and fish meal. Corn accounts for about 60% in broiler chicken feed formulations. Therefore, replacing corn with cheaper feedstuffs will reduce feed costs. One potential feedstuff is the rice bran. However, the rice bran has several weaknesses, including higher crude fiber and phytic acid, and lower energy compared to corn. To increase the use of rice bran, this feedstuff could be fermented, among others, with tempeh mold (rice bran tempeh).

The previous results showed that feeding 20% rice bran tempeh did not reduce broiler performance (Rahmat *et al.*, 2021; Santoso *et al.*, 2022, 2023b). However, this product still has a weakness, namely the tempeh mold is unable to penetrate the rice bran layer. This causes the tempeh mold did not develop to the deepest layer (Santoso *et al.*, 2022, 2023).

To increase the use of rice bran tempeh, the manufacture of rice bran tempeh needs to be modified by adding a starter that is able to penetrate the inner layer of rice bran. One of the potential starters is cassava yeast (*Saccharomyces sp.*) (Fatimah *et al.*, 2020). The use of two starters for fermenting feedstuffs could result in synergistic interactions so that the fermentation results will be better than a single starter. Khasanah *et al.* (2022) reported that there is a synergistic interaction when two types of starters are combined to ferment feedstuffs. The synergistic interaction resulted in improving nutreint digestibility. Adesulu-Dohunsi *et al.* (2020) reported that a synergistic interaction results in an increase in nutrient availability, feed quality, feed palatability, organoleptic qualities and digestibility, and improved immune system. The use of these two starters are also aimed to reduce fat deposition in various organs. Reducing the fat depot in broilers has an important meaning for the broiler carcass processing industry, because this will increase the industry's income. In addition, the lower fat content of broiler meat is highly preferred by consumers. It has been documented that consuming a high fat meats could cause several non-infectious diseases. (Grisotto *et al.*, 2021; Osaka *et al.*, 2021).

Saccharomyces cerevisiae (cassava yeast) could reduce the percentage of abdominal fat in broilers (Afsharmanesh *et al.*, 2010). This is reinforced by research of Manday *et al.* (2018) who found that the deposition of abdominal fat was decreased by feeding cassava yeast fermented pineapple waste flour. Furthermore, the products fermented with *Rhizopus* also have the potential to reduce fat deposition. Sukma *et al.* (2022) reported that fermenting banana peel with *Rhyzopus oryzae* for 24 hours resulted in lower fat content. The higher the administration of this product, the lower the abdominal fat deposition. The results of the study showed that the addition of 4 g of tempeh mold to rice bran resulted in higher mycelial growth. Thus, this dose was used to produce rice bran tempeh (Fatimah *et al.*, 2020, Santoso *et al.*, 2022). Meanwhile, the use of 0.1% cassava yeast was based on the results of preliminary research in the present study which showed that the combination of 4 g of tempeh mold and 0.1% cassava yeast produced better mycelial growth and produced a distinctive cassava yeast aroma.

Based on this description, the addition of cassava yeast in rice bran tempeh manufacture has the potential to reduce fat deposition and meat fat content in broiler chickens. Therefore, the aim of this study was to analyze the effect of feeding the modified rice bran tempeh on performance, fat deposition in various fat depot locations, and nutritional composition of meat in broiler chickens. It was hypothesized that feeding modified rice bran tempeh reduced fat deposition and meat fat content without reducing broiler performance.

MATERIALS AND METHODS

Manufacture of Modified Rice Bran Tempeh

Manufacture of modified rice bran tempeh was done by mixing rice bran and water in a ratio of 1: 1. The rice bran was then steamed for 30 minutes so that it became softer and had less contaminants. The rice bran was then cooled and given 4 g/kg of tempeh mold (Santoso *et al.*, 2022) and 0.1% cassava yeast. Rice bran was wrapped in plastic, and a hole is made in the surface of the plastic, and fermented for 48 hours. The modified rice bran tempeh was then dried, ground and stored in a plastic bag.

Broiler Chickens and Treatment

Three hundred broiler chickens (strain Loghmann) aged one day were placed in brooding pens. Newly arrived broiler chickens were given sugar water to reduce stress caused by travel. The brooder temperature was maintained at the ranging of 32°-33°C. At the ages of 4 and 21 days, broiler chickens were vaccinated with ND. At 15 days of age, two hundred broilers (a male to female ratio at 1:1) were divided into 4 treatment groups with 5 replication for each treatment. The 4 treatments were as follows: P1 = Feed without rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; P4 = Feed with 25% modified rice bran tempeh.

The compositions of experimental diets are shown in Table 1. The protein content of feed at P1, P2, P3 and P4 was 19.75%, 20.11%, 20.25% and 20.38% respectively, while the energy content of the feed at P1, P2, P3 and P4 was 3,110.84 kcal/kg, 2,954.3 kcal/kg, 2,956.61 kcal/kg, and 2,904.86 kcal/kg. The crude fiber content in P1, P2, P3 and P4 was 2.87%, 4.52%, 0.45% and 5.04%, respectively. Inclusion of rice bran tempeh to the feed increased fiber content of feed. Broilers were given freedom to consume feed and drinking water. Parameters measured were performance, fat deposition (abdominal fat, sartorial fat, gizzard fat), and meat composition.

Table 1. The Composition of Experimental Diets

Feedstuffs (%)	P1	P2	P3	P4
Yellow corn	57.0	42.0	42.0	37.0
Rice bran	5.0	0	0	0
Rice bran tempeh	0	20.0	0	0
Modified rice bran tempeh	0	0	20.0	25.0
Broiler concentrate	34.2	34.2	34.2	34.2
Palm oil	1.5	1.5	1.5	1.5
Mineral mixture	1.7	1.7	1.7	17
Salt	0.1	0.1	0.1	0.1
Top mix	0.5	0.5	0.5	0.5
Nutrient composition				
Protein, %	19.75	20.11	20.25	20.38
ME, kcal/kg	3110.84	2954.3	2956.61	2904.86
Crude fiber, %	2.87	4.52	4.45	5.04
Fat, %	5.05	5.95	5.93	6.29
Calsium, %	1.06	1.12	1.12	1.13
Phosphor	0.67	0.75	0.75	0.78

Laboratory Sampling and Analysis

At 35 days of age, 5 female broilers for each treatment group were slaughtered, and fat depots, and leg meats (thigh and drumstick) were removed and weighed. The leg meats were then measured for moisture, protein, fat and ash contents using AOAC methods (2016).

Data Analysis

The research results were analyzed by ANOVA and if they are significantly different they were tested further with Duncan's multiple range test. Correlation-regression analysis was used to predict meat fat content using abdominal fat, or fatty liver score (FLS).

RESULTS AND DISCUSSION

Broiler Performance

Table 2 shows the effect of modified rice bran tempeh on broiler performance. Experimental results showed that feeding modified rice bran tempeh did not significantly reduce ($P>0.05$) body weight, body weight gain, feed intake, and feed conversion ratio. Table 3 shows the effect of treatments on nutrient intake. It was shown that the treatments had no effect on energy and protein intake ($P>0.05$), but significantly influenced fat intake and crude fiber intake ($P<0.01$). It was shown that fat intake of P1 was lower than that of P2, P3 and P4, whereas crude fiber intake of P1 was lower than that of P2, P3 and P4. In addition, crude fiber intake of P2 and P3 was lower than that of P4.

Rice bran tempeh had a lower energy content than corn. Therefore, the inclusion of higher rice bran tempeh to the feed would cause a decrease in feed energy. However, the results showed that giving rice bran tempeh did not reduce body weight. The lack of weight loss in broilers fed feed with rice bran tempeh was caused, among other things, by not decreasing energy intake and protein intake (Table 3). The higher fat intake in P2, P3 and P4 might contribute to the similar body weight among the treatments. In addition, rice bran which was fermented with tempeh mold or cassava yeast contained lower phytic acid and crude fiber, and higher protein content (Fatimah *et al.*, 2020). Reducing phytic acid levels in rice bran tempeh would increase the minerals and protein availabilities, which has a positive impact on increasing the use of rice bran tempeh (Fatimah *et al.*, 2020; Santoso *et al.*, 2022; Santoso *et al.*, 2023b). Furthermore, the increase in the protein content of rice bran tempeh caused the increasing availability of protein for the growth of broiler chickens.

The results of the correlation analysis

Table 2. The Effect of Modified Rice Bran Tempeh on Broiler Performance

Variables	P1	P2	P3	P4	P
Body weight, g/bird	1894.6±76.04	1921.41±89.6	1873.4±95.8	1894.8±86.3	0.858
Weight gain, g/bird	1308.7±78.2	1331.9±86.8	1294.1±97.1	1319.8±78.2	0.910
Feed intake, g/bird	2462.5±215.8	2608.2±239.1	2666.7±230.4	2754.2±285.0	0.320
Feed conversion ratio	1.88±0.16	1.96±0.14	2.06±0.15	2.08±0.12	0.148

P1 = Feed without rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; P4 = Feed with 25% modified rice bran tempeh.

Table 3. The Effect of Modified Rice Bran Tempeh on Nutrient Intake

Variables	P1	P2	P3	P4	P
Energy Intake, kcal.kg	7660.4±671.2	7705.5±706.4	7884.4±681.1	8000.6±828.0	0.868
Protein Intake, g/bird	486.3±42.6	524.5±48.1	540.0±46.6	561.3±58.1	0.145
Crude Fat Intake, g/bird	124.4±10.9[a]	155.2±14.2[b]	158.1±13.7[b]	173.2±17.9[b]	0.001
Crude Fiber Intake, g/bird	70.67±6.19[a]	117.9±10.8[b]	120.0±10.4[b]	7138.8±14.4[c]	0.000

P1 = Feed without rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; P4 = Feed with 25% modified rice bran tempeh.

showed that there was a moderate negative correlation between dietary energy level and feed intake (r=-0.419, p=0.066) although it was not significant. This correlation shows that if dietary energy is low then feed intake will increase to meet energy needs. Dietary energy content strongly regulates feed intake (Ahiwe *et al.,* 2018*).*

The results of previous research (Santoso *et al.*, 2022, 2023a, b) showed that rice bran tempeh could only be given as much as 20%. However, the results of current research showed that modified rice bran tempeh could be given as much as 25%. Cassava yeast was able to penetrate the inner layers of the rice bran so that it could ferment not only the surface but also the inner layers of the rice bran. Thus, the combination of tempeh mold and cassava yeast was able to ferment all the rice bran. Higher fermentation quality is thought to increase digestibility and availability of nutrients for broilers. Apart from that, it is suspected that the combination of the cassava yeast and tempeh mold can reduce phytic acid to a greater extent compared to single yeast or mold. This of course would increase the availability of minerals and protein for broilers.

The higher crude fiber intake in rice bran tempeh groups did not reduce broiler body weight. This fact indicated that crude fiber of feed ranges from 2.87% to 5.04% is still in normal range. Mandey *et al.* (2017) reported that feeding crude fiber up to 11% did not reduce body weight. Thus, feeding modified rice bran tempeh up to 25% to substitute yellow corn did not reduce the performance of broilers.

Fat Deposition

Table 4 shows the effect of modified rice bran tempeh on fat deposition of broiler chickens. Feeding modified rice bran tempeh or rice bran tempeh reduced abdominal fat (P<0.05), and total fat deposition (P<0.05), but it did not significantly reduce fat deposition in the proventriculus, gizzard, heart, neck, sartorial, and fatty liver score (P>0.05). Thus, the present study showed that rice bran tempeh or modified rice bran tempeh feeding reduced abdominal fat and total fat deposition. The present results were in contrast with the observation of Santoso *et al.* (2023[a]) who reported that rice bran tempeh did not reduce abdominal fat and total fat deposition.

Rice bran is rich in compounds that have antioxidant properties (Hefnawy and El-Shoourbagy, 2014; Tan *et al.*, 2020). Fermenting

Table 4. The Effect of Modified Rice Bran Tempeh on Fat Deposition

Variables	P1	P2	P3	P4	P
Abdomen, %	1.28±0.43[b]	0.60±0.31[a]	0.64±0.36[a]	0.54±0.16[a]	0.027
Proventriculus, %	0.076±0.31	0.051±0.46	0.035±0.67	0.038±0.53	0.126
Gizzard, %	0.42±0.13	0.59±0.39	0.22±0.16	0.37±0.09	0.223
Heart, %	0.04±0.02	0.03±0.01	0.02±0.00	0.03±0.01	0.27
Neck, %	0.04±0.01	0.03±0.01	0.03±0.01	0.02±0.01	0.248
Sartorial, %	0.64±0.27	0.45±0.20	0.38±0.20	0.31±0.09	0.094
Total Fat, %	2.50±0.79[b]	1.16±1.04[a]	0.90±0.76[a]	1.46±0.35[a]	0.015
FLS	2.85±0.34	2.67±0.01	2.67±0.00	2.13±0.25	0.058

P1 = Feed without rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; P4 = Feed with 25% modified rice bran tempeh.

Table 5. The Effect of Modified Rice Bran Tempeh on Meat Composition

Variables	P1	P2	P3	P4	P
Fat, %	5.58±0.30[b]	4.91±0.75[b]	3.91±0.97[a]	3.25±0.53[a]	0.000
Protein, %	18.65±0.31	18.53±0.46	18.65±0.67	18.12±0.53	0.330
Moisture, %	74.34±0.37[a]	75.15±0.89[a]	76.68±1.41[b]	77.87±0.81[b]	0.000
Ash, %	1.42±0.30[b]	1.40±0.41[b]	0.76±0.15[a]	0.75±0.22[a]	0.001

P1 = Feed without rice bran tempeh; P2 = Feed with 20% rice bran tempeh; P3 = Feed with 20% modified rice bran tempeh; P4 = Feed with 25% modified rice bran tempeh.

feedstuffs would increase their antioxidant properties (Erskine *et al.*, 2023; Ikusika *et al.*, 2024; Sugiharto *et al.*, 2019). Therefore, rice bran tempeh is thought to contain higher antioxidant compounds when compared to rice bran. Natural antioxidant has hypolipidemic properties (Hannan *et al.*, 2016). The decrease in fat deposition in broilers given rice bran tempeh was thought to be caused by the higher crude fiber content of rice bran tempeh (25.28%) and high oryzanol content. The addition of oryzanol decreased blood triglyceride concentrations (Kobayashi *et al.*, 2019), liver triglyceride contents and intracellular triglyceride deposition (Wang *et al.*, 2015).

The crude fiber intake in broilers given rice bran tempeh was higher than control (P1). Crude fiber also has the potential to reduce fat deposition. Mandey *et al.* (2017) reported that higher crude fiber content decreased abdominal fat deposition. Crude fiber inclusion decreased hepatic lipid deposition via inhibiting hepatic fatty acid synthase, and acetyl-CoA carboxylase. (Qin *et al.*, 2017). Tempeh is rich in *Lactobacillus sp* bacteria (Barus *et al.*, 2021). *Lactobacillus sp.* plays a role in reducing fat deposition. *Lactobacillus sp.* decreased the mRNA expression of sterol regulatory element-binding protein) and its target gene fatty acid synthase in the liver and decreased free fatty acid in the blood. Thus, *Lactobacillus sp.* reduced fat synthesis (Yonejima *et al.*, 2013).

There was a correlation between fatty liver score (FLS) and abdominal fat (r=0.629, p=0.012), FLS and total fat (r=0.445, p=0.096); abdominal fat and proventriculus fat (r=0.581, p=0.007), abdominal and sartorial fat (r=0.844, p=0.000). These results are in accordance with the observations of Agnes *et al.* (2023) who reported that there was a positive correlation between abdominal fat and sartorial fat or proventriculus fat or total fat. Thus, abdominal fat deposition can be used as an indicator in predicting total fat, sartorial fat and proventriculus fat deposition.

Meat Composition

Table 5 shows the effect of modified rice bran tempeh on meat composition. The treatments significantly affected fat content, moisture content and ash content (P<0.01), but it had no effect on protein content. The meat fat and ash contents of P1 and P2 were significantly higher than those of P3 and P4, whereas the meat moisture contents of P1 and P2 were lower than those of P3 and P4.

The lower content of meat fat in broilers fed feed containing modified rice bran tempeh indicates that there is a possibility of interaction between the two starters in the fermentation process. Wulandari *et al.* (2020) reported that the addition of *Saccharomyces cerevisiae* (cassava yeast) reduced cholesterol content of broiler breast meat. Furthermore, the addition of *Saccharomyces* fermented product reduced lipid oxidation of broiler breast meat. The compounds that play a role in reducing meat fat in modified rice bran tempeh are thought to be crude fiber and oryzanol. Oryzanol reduces triglyceride levels in the liver and intracellular triglyceride (Wang *et al.*, 2015), whereas dietary crude fibre decreased hepatic lipid deposition (Qin *et al.*, 2017). In addition, fermentation of feedstuffs with *Rhizopus sp.* increases linoleic acid and linolenic acid (Sudaryatiningsih and Supyani, 2009). Omega-3 polyunsaturated fatty acids (linolenic acid) could inhibit adipocyte differentiation, induce fatty acid oxidation, and enhance energy expenditure (Kim and Voy, 2021).

It is unknown why modified rice bran tempeh increased meat moisture. Ogodo *et al.* (2017) reported fermentation increased moisture content of feedstuffs. Zhang *et al.* (2021) reported that the inclusion of *Saccharomyces* culture increased meat moisture of geese. The results of the correlation-regression analysis showed that there was a positive correlation between meat fat content and meat water content (r=0.918, p=0.000). Thus, decreasing the fat content of meat will increase the water content of meat. An *et al.* (2022) shows a high correlation between the meat fat content and moisture content (r= 0.90).

The decrease in phytic acid of rice bran tempeh (Fatimat *et al.*, 2020) will release a number of minerals and protein, so that the availability of minerals and protein for broilers increases. This means that there is the potential for the ash content in the meat to increase. However, in this study, broilers given modified rice bran tempeh produced lower ash content in meat than the control (P1) and broilers given rice bran tempeh (P2). It is thought that these minerals are distributed more for bone mineralization than accumulated in the meat. This assumption is supported by Nari and Ghasemi (2020) who reported that the inclusion of yeast (*Saccharomyces sp.*) increased tibia ash.

There was a correlation between abdominal fat and meat fat (r=0.611, p=0.016), total fat and meat fat (r=0.551, p=0.033), sartorial fat and meat fat (r=0.439, p=0.101, and FLS and meat fat (r=0.730, p=0.002). The results of this study show that meat fat content can be predicted by FLS, abdominal fat or total fat. FLS is the best predictor of meat fat. Chen *et al.* (2008) and Ge *et al.* (2020) also reported that there was a positive correlation between abdominal fat and intramuscular fat levels. Santoso *et al.* (1995) reported that there was a positive correlation between abdominal fat and carcass fat in broilers, which means that abdominal fat could be used as an indicator to predict carcass fat.

CONCLUSION

It was concluded that the inclusion of 20% or 25% modified rice bran tempeh reduced meat fat content, abdominal fat, and total fat depositions without reducing broiler performance.

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