# Effect of cellulase inclusion to rice bran tempeh containing diets on body conformation, fat deposition and meat nutrient composition in broiler

*by* Urip Santoso

# Effect of cellulase inclusion to rice bran tempeh containing diets on body conformation, fat deposition and meat nutrient composition in broiler

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## ABSTRACT

This study aims to evaluate the effect of cellulase supplementation to rice bran tempeh containign diets on body conformation, fat deposition, nutritional composition of broiler meat and blood biochemical concentrations in broiler chickens. Two hundred broilers aged 15 days (the ratio of male to female was 1:1) were divided into 4 treatment groups with 5 replications for each treatment, as follows. P1 = 20% rice bran tempeh; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase. The addition of cellulase significantly reduced body mass index, leggedness and fat content of meat (P<0.05), increased protein content (P<0.01) and meat ash plus carbohydrate (P<0.05), but did not decrease fat deposition, blood concentration of uric acid , cholesterol, glucose and pH. It can be concluded that the addition of 0.1-0.15% cellulase to rice bran

tempeh containing diets reduced the fat content but increases the ash plus carbohydrate content of broiler meat. In addition, the addition of 0.5% cellulase increased the protein content of the meat.

*Key words: Meat fat, Rice bran tempeh, Body conformation, Cellulase, Broiler*

## INTRODUCTION

The high fat content in broiler meat is a consideration for consumers in consuming broiler meat. Consumption of products containing high fat can increase several metabolic diseases such as atherosclerosis, coronary heart disease, obesity and stroke. In addition, high levels of fat deposition in the abdomen and other parts reduce the profit level of broiler carcass producers because of low prices of fat depots.

One way to reduce fat depots is to use low-energy feed ingredients. One of the potential feed ingredients is rice bran tempeh. The results of previous studies, giving rice bran tempeh as much as 20% reduced fat depots of broiler chickens. However, rice tempeh inclusion reduce body weight (Santoso *et al*., 2021). Lower body weight was mainly due to the high crude fiber in rice bran tempeh. In addition, even though rice bran tempeh was able to reduce the fat content of the meat, the fat content of the meat was still above 4% (Santoso *et al.*, 2021).

To overcome the weaknesses of that research, rice bran tempeh can be enriched with cellulase. Cellulase reduce levels of crude fiber, NDF, ADF and hemicellulose in feed ingredients (Jefry *et al*., 2021). Cellulases are able to hydrolyze cell walls (Holland *et al*., 2020; Jamet and Dunand, 2020; Camacho- Fernández *et al*., 2021).), so that cell wall components such as pectin and ferulic acid are released from cell wall complex compounds. Pectin and ferulic acid inclusion reduce fat depots in animals (Adam *et al*., 2016; de Melo *et al*., 2017). Therefore, it is suspected that cellulase addition to rice bran tempeh containing diet could reduce fat deposition and fat content in meat. So far, the reduction of meat fat by the addition of cellulase has not been investigated. In addition, cellulase responds differently when added to different feed ingredients, which indicates that there is an interaction between cellulase and feed ingredients. Ramaiyulis *et al*. (2018) showed the interaction between feed supplements and feed ingredients. They reported that the interaction effect of cattle feed supplement and concentrate was shown by decreased degradability of ADF and cellulose in full forage diet but an increase in forage-concentrate diet and a higher linear increase to degradability of crude fiber, NDF and hemicellulose in forage-concentrate diet than full forage diet. Therefore, it is very possible that there is an interaction between cellulase and rice bran tempeh, so that broiler responses can be different when compared to previous studies using different feed ingredients.

Based on this description, the cellulase addition to rice bran tempeh containing diet has the potential to reduce crude fiber content so that giving 20% rice bran tempeh does not reduce body conformation. In addition, cellulase also has the potential to reduce fat deposition. No information was found about the use of cellulase to reduce the fat content of meat. Therefore, the present study evaluated the effect of adding cellulase to rice bran tempeh containing diet on body conformation, fat deposition, blood chemistry profile, and nutritional composition of meat in broiler chickens. It is suspected that the addition of cellulase reduces fat deposition and improves broiler meat composition without decreasing body conformation and blood biochemistry profiles.

## MATERIALS AND METHODS

### Animals and Diets

Rice bran tempeh was made using the method of Santoso *et al*. (2022). There were four treatment groups consisted of 200 fifteen-days-old broilers (strain Loughmann) with a ratio of male to female 1:1. The four treatment groups, with five replications for each group, were treated as follows: P1 = 20% rice bran tempeh; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase. Cellulase level refers to the research results of Noferdiman *et al*. (2018) who reported that the addition of 0.1% cellulase improved performance. Broilers were reared up to 35 days old. Diets and drinking water were given *ad libitum*. The composition of the experimental feed ingredients had been published on another journal (Santoso *et al*., 2023). Body conformation was measured at 35 days old. Leggedness, masssiveness, compactness are measured by the calculation method according to Kokoszynski et al. (2017). Body mass index was measured using the method of  Mendes et al. (2007), whereas body shape index was measured using the method of Guillaume (1976).

### Sampling and laboratory analysis

At the end of the study (age 35 days), 5 broilers for each treatment group were slaughtered, and fat depots were separated and weighed. Before cutting, blood samples were taken through the wings for analysis of pH, glucose, uric acid and cholesterol.

Thigh meat samples were taken to analyze the water, fat and protein content of the meat. Moisture, fat and protein content was measured using the AOAC method (2016). Ash content + carbohydrates is calculated using the following equation.

Ash content + carbohydrates (%) = 100– (protein content + moisture content + fat content).

Data Analysis

All data were analyzed for variance and if it had a significant effect it was further tested with Duncan's Multiple Range Test.

## RASULTS AND DISCUSSION

### Body Conformation

Table 2 presents the effect of adding cellulase to rice bran tempeh containing diet on the body conformation of broiler chickens. The findings suggest that the aforementioned treatment had no significant effect ($P>0.05$) on massiveness, compactness and body shape index. However, it had a significant effect on leggedness, body mass index and body length ($P<0.01$). P2 and P4 had lower leggedness and body mass index when compared to P1 and P3. Body lengths of P2 and P4 were longer than P1 and P3. Body conformation reflects broiler body weight. Santoso et al. (2021) reported that there was a correlation between body weight and massiveness, whereas no significant correlation was found between body weight and compactness, long-leggedness , body mass index, or body shape index. Thus, the decrease in leggedness and body mass index at P2 and P4 in the current study does not reflect a reduction in broiler weight.

### Fat Deposition

Table 3 shows the effect of adding cellulase to rice bran tempeh containing diets on fat deposition in broiler chickens. The addition of cellulase did not significantly reduce fat deposition in the abdomen, neck, heart, gizzard, sartorial and total fat deposition. The research results are not in accordance with the results of Zulkarnain *et al*. (2017) who reported that the addition of cellulase enzymes reduced abdominal fat deposition in broilers. The cellulase level used in Zulkarnain et al. (2017) is 1 g/kg sago waste or as much as 0.1%. It is suspected that the difference in feed ingredients used is one of the factors causing the difference in results between these two studies. Ramaiyulis *et al*. (2018) showed the interaction between feed supplements and feed ingredients. They reported that the interaction effect of cattle feed supplement and concentrate was shown by decreased degradability of ADF and cellulose in full forage diet but an increase in forage-concentrate diet, and a higher linear increase in degradability of crude fiber, NDF and hemicellulose in forage-concentrate than in full forage diet.

The abdominal fat depot average of Loughmann strain female broilers aged 35 days in the present study was 0.63%. The average female broiler abdominal fat in the same strain in Santoso *et al*. (2018), Santoso *et al*. (2019), Santoso *et al*. (2020) and Santoso *et al*. (2021) were 1.28%, 0.62%, 0.80% and 0.52%, respectively. Based on these data it could be concluded that the abdominal fat depot in female broiler chickens of the Loughmann strain at 35 days of age varies greatly.

**Blood Biochemistry Analysis**

Table 4 presents the effect of adding cellulase to rice bran tempeh containing diet on the blood biochemistry profile of broiler chickens. The results showed that the administration of cellulase diet did not change blood glucose, uric acid, pH and cholesterol levels (P>0.05). Cellulase hydrolizes the cell walls of feed ingredients thereby increasing the availability of glucose. The results of cellulose degradation by cellulases include glucose, oligosaccharides, shorter polysaccharides. The results of the study adding cellulase increased the digestibility of glucose (Siagian, 1998). However, increasing the availability of glucose and digestibility of glucose did not significantly increase the concentration of glucose in this study.

There is a negative correlation between the level of cellulase addition and blood cholesterol concentration (r=-0.477, P=0.033) with the cholesterol equation = 175.9 – 5.74 cellulase level. This shows that the higher the addition of cellulase, the lower the blood cholesterol concentration. Mandey *et al*. (2016) reported that cellulase reduce blood cholesterol concentration. Likewise, there was a negative correlation between cellulase levels and blood pH (r=-0.492, P=0.077) with the equation 7.81 – 0.44 cellulase levels. So, the higher the addition of cellulase, the lower the blood pH. The present study showed that cellulase had a pH of 5. Thus, the addition of cellulase would lower the pH of the diets. The decrease in the pH of the diets is thought to be a factor that lowers the blood pH in the present study. Hartini and Purwaningsih (2016) reported that the addition of cellulase increased acetic acid, propionic acid and butyric acid, causing a decrease in pH in the digestive tract (doudenum, jejunum, ileum and cecum).

Albumin is the main protein contained in blood plasma which is responsible for osmotic pressure and as a transport agent for various small molecules in the blood such as fatty acids and bile pigments (Mushawwir and Latipudin, 2011). Plasma albumin can be an indicator to determine the status of tissue function (Mushawwir *et al*., 2020;

Tanuwiria and Mushawhir, 2020). This is related to the function of liver tissue as a producer of albumin protein which contribute 75% of blood plasma protein. Thus, the function of liver is not disturbed as indicated by no change in blood albumin.

The remaining products of protein metabolism excreted through the kidneys are uric acid, creatinine, urea, and albumin. Increased concentrations of uric acid, creatine and urea. is a sign of kidney damage. Because the addition of cellulase does not increase these compounds, it can be predicted that the addition of cellulase will not cause damage to the kidneys.

**Broiler Meat Nutritional Composition**

Table 5 presents the effect of adding cellulase to rice bran tempeh containing diets on the nutritional composition of broiler meat. The results showed that the addition of cellulase had a significant effect on fat, protein and ash content ($P<0.05$), but had no significant effect on meat moisture content ($P>0.05$). The starch and protein are encapsulated by the cell wall polysaccharides and thus less available for digestion. Supplemental cellulase may partially depolymerize the NSP thereby improving protein digestibility. Cellulase could hydrolize certain types of carbohydrate protein complexes in which the protein component is resistant to proteolysis because of its substitution with bulky carbohydrate groups. This could have contributed to some improvements in protein digestibility (Meng *et al*., 2005). The addition of cellulase increases the digestibility of dry matter, organic matter and protein in broiler chickens (Zulkarnain *et al*., 2016). Ranjan *et al*. (2018) also reported that the addition of cellulase increased protein digestibility in *Labeo rohita*. Increased protein digestibility is thought to be one of the factors causing the increase in meat protein content at P1. Ranjan *et al*. (2018)

reported that the addition of cellulase increased carcass protein levels in *Labeo rohita*. However, it is not yet known what causes the decrease in meat protein levels in line with the addition of cellulase at higher levels.

Mineral absorption is inhibited, among others, by crude fiber (Wang *et al*., 2008). Furthermore, it was stated that the interaction between phytase and cellulase also increased the absorption of calcium and zinc. It is known that tempeh yeast produces phytase (Surya et al., 2013). The addition of cellulase will break down the crude fiber, especially cellulose, which means that the inhibition of mineral absorption is reduced. It is suspected that the phytase found in rice bran tempeh also interacts positively with cellulase, which results in increased absorption of minerals, especially calcium and zinc. Knowlton *et al*. (2005) reported that the addition of phytase and cellulase increased the digestibility of phosphorus in lactating dairy cows. Holland *et al*. (2020) reported that plant cell walls contain a number of minerals. The hydrolysis of the cell wall is thought to increase the availability of minerals. These factors might cause an increase in the ash content in the meat as happened in this study. Another factor that is thought to be the cause of the increased mineral content of meat is the increased digestibility of dry matter by cellulase (Zulkarnain *et al*., 2016). Ranjan *et al*. (2018) reported that the addition of cellulase increased the digestibility of carbohydrates, so it was suspected that the levels of carbohydrates in broiler meat also increased. Thus, increased digestibility of carbohydrates and minerals is one of the factors causing increased ash content + carbohydrates in broiler meat.

The mechanism for reducing the fat content of meat by cellulase is unknown. The cell wall encompasses an organized network of cellulose microfibrils integrated within a hydrated gel-type matrix typically consisting of pectin, hemicelluloses, and small amounts of glycoproteins, phenolic acids, minerals and, in some specialized cell-types, lignin (Holland *et al*., 2020; Jamet and Dunand, 2020; Camacho-Fernández *et al*., 2021). Cellulase hydrolized the cell wall so that the compounds that compose it are hydrolyzed into simpler components, for example cellulose is converted to glucose, simpler polysaccharides and oligosaccharides. The hydrolysis of the cell wall releases pectin, hemicellulose, cellulose, lignin, ferulic acid, minerals and proteins that make up the cell wall. Ferulic acid reduces the concentration of glucose, insulin, amylase, lipase, triglycerides, total cholesterol in the blood (de Melo *et al*., 2017). The decrease in lipase is thought to reduce the availability of substrates for fatty acid synthesis, which results in decreased fat levels. Pectin supplementation reduces fat deposition in mice (Sefcikova and Racek, 2016). Pectin has fat binding properties (Sharefiabadi and Serdaroglu, 2020). Adam *et al*. (2016) reported that pectin supplementation reduced body fat in mice fed a high-fat diet. Thus, one of the factors causing the decrease in meat fat content is due to the increased availability of ferulic acid and pectin. Another mechanism for reducing meat fat content is thought to be a decrease in bile acid synthesis. This assumption is based on the results of this study showing a tendency to decrease blood cholesterol concentrations. It is known that cholesterol is the main substrate for the synthesis of bile acids. A reduction in the synthesis of bile acids would reduce bile acid content. Bile acids act to emulsify fat so that fat is more easily digested and absorbed in the small intestine. Therefore, a decreased bile acids could decrease digestion and absorption of fat in the small intestine. Azzaz *et al*. (2021) adding cellulase increased the digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber. There is a negative linear correlation between ash content + meat carbohydrates and meat fat content (r=-0.467, p=0.068) in the present study.

## CONCLUSION

It can be concluded that the supplementation of 0.1-0.15% cellulase to rice bran tempeh containing diets reduced the meat fat content but increased the meat ash + carbohydrate content. In addition, the supplementation of 0.5% cellulase increased the meat protein content.

## CONFLICT OF INTEREST

The author declares there is no conflict of interest in this research


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Table 1. The composition of experimental diets.

| Feedstuffs (g/kg) | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Yellow corn | 420 | 420 | 420 | 420 |
| Rice bran tempeh | 200 | 200 | 200 | 200 |
| Broiler concentrate | 342 | 342 | 342 | 342 |
| Palm oil | 15 | 15 | 15 | 15 |
| Mineral mixture | 17 | 17 | 17 | 17 |
| Salt | 1 | 1 | 1 | 1 |
| Top mix | 5 | 4,5 | 4,0 | 3,5 |
| Cellulase | 0 | 0.5 | 1.0 | 1.5 |
| Total | 1.000 | 1.000 | 1.000 | 1.000 |

Table 2. Effect of adding cellulase to feed containing bran tempeh on broiler performance

| Variables | P1 | P2 | P3 | P4 | P |
|---|---|---|---|---|---|
| Body length, cm | 26.25±1.07[a] | 28.95±1.20[b] | 26.25±1.67[a] | 28.05±1.92[b] | 0.002 |
| Massiveness | 10.07±0.96 | 9.92±0.40 | 9.85±0.49 | 10.5±1.40 | 0.723 |
| Compactness | 187.22±16.14 | 187.07±12.37 | 184.02±9.58 | 192.87±26.58 | 0.783 |
| Leggedness | 22.07±2.96[b] | 17.94±1.88[a] | 21.87±2.45[b] | 19.25±2.41[a] | 0.005 |
| Body mass index | 2.46±0.28[b] | 1.98±0.17[a] | 2.48±0.30[b] | 2.08±0.30[a] | 0.001 |
| Body shape index | 4.85±0.55 | 4.38±0.44 | 4.81±0.57 | 4.58±0.45 | 0.238 |

P1 = 20% rice bran tempeh plus 0% cellulase; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase.

**Table 3.** Effect of adding cellulase to feed containing bran tempeh on fat deposition in broiler chickens

| Variables | P1 | P2 | P3 | P4 | P |
|---|---|---|---|---|---|
| Abdominal fat, % | 0.58±0.24 | 0.66±0.43 | 0,72±0,20 | 0,54±0,15 | 0,564 |
| Neck fat, % | 0.017±0.009 | 0.022±0.01 | 0,029±0,013 | 0,015±0,007 | 0,054 |
| Heart fat, % | 0.024±0.018 | 0.032±0.015 | 0,037±0,017 | 0,029±0,008 | 0,364 |
| Gizzard fat, % | 0.30±0.12 | 0.28±0.13 | 0,36±0,13 | 0,29±0,11 | 0,654 |
| Sartorial fat, % | 0.29±0.15 | 0.36±0.20 | 0,32±0,11 | 0,30±0,10 | 0,741 |
| Total fat, % | 1.21±0.45 | 1.36±0.57 | 1,46±0,31 | 1,18±0,22 | 0,485 |
| Fatty liver score | 2.53±0.28 | 2.44±0.65 | 2,56±0,18 | 2,22±0,45 | 0,389 |

P1 = 20% rice bran tempeh plus 0% cellulase; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase.

**Table 4.** The effect of adding cellulase to feed containing bran tempeh on blood biochemistry profiles

| Variables | P1 | P2 | P3 | P4 | P |
|---|---|---|---|---|---|
| Cholesterol, mg/dL | 171.4±12.52 | 164.4±12.35 | 155±11.35 | 155.4±9.93 | 0.179 |
| Glucose, mg/dL | 205±27.66 | 192.2±23.76 | 224±29.75 | 210.2±25.59 | 0.433 |
| Uric acid, mg/dL | 10.26±3.34 | 11.16±1.64 | 9.9±1.71 | 11.28±1.35 | 0.759 |
| pH | 7.74±0.10 | 7.74±0.05 | 7.72±0.07 | 7.60±0.09 | 0.077 |

P1 = 20% rice bran tempeh plus 0% cellulase; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase.

**Table 5.** The effect of adding cellulase to feed containing bran tempeh on the nutritional composition of broiler meat

| Variables | P1 | P2 | P3 | P4 | P |
|---|---|---|---|---|---|
| Moisture, % | 76.07±2.26 | 74.57±2.07 | 75.12±1.33 | 75.87±0.87 | 0.601 |
| Protein, % | 15.40±1.44$^{a}$ | 18.98±1.80$^{b}$ | 15.25±0.86$^{a}$ | 14.47±0.84$^{a}$ | 0.002 |
| Fat, % | 5.87±1.72$^{b}$ | 4.34±1.02$^{ab}$ | 3.44±1.28$^{a}$ | 2.90±0.76$^{a}$ | 0.027 |
| Ash+carbohydrate, % | 2.66±1.74$^{a}$ | 2.12±1.48$^{a}$ | 6.20±0.89$^{b}$ | 6.77±0.99$^{b}$ | 0.017 |

P1 = 20% rice bran tempeh plus 0% cellulase; P2 = 20% rice bran tempeh plus 0.05% cellulase; P3 = 20% rice bran tempeh plus 0.1% cellulase, and P4 = 20% rice bran tempeh plus 0.15% cellulase.

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