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The improvement of meat nutrient composition in broiler chickens fed diets containing rice bran tempeh supplemented with cellulose

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ABSTRACT

This study was aimed to evaluate the effect of cellulase supplementation in diets containing rice bran tempeh on body conformation, fat deposition, nutritional composition of broiler meat and blood biochemical concentrations in broiler chickens. This research was carried out for 3 weeks from 15 days to 35 days of age. Two hundred broilers aged 15 days were divided into 4 treatment groups with 5 replications (8 broilers for each replication) for each treatment, as follows: T1 = 0% cellulase; T2 = 0.05% cellulase; T3 = 0.1% cellulase, and T4 = 0.15% cellulase. Body conformation, chemical meat composition, fat deposition, blood pH, glucose and uric acid were measured. The addition of 0.05% or 0.15% cellulase significantly reduced leggedness ($P<0.05$), whereas 0,1% or 0,15% cellulase addtion significantly reduced the content of meat fat and ash plus carbohydrate. In addition, the inclusion of 0.05% cellulase increased the content of meat protein ($P<0.01$). The inclusion of cellulase from 0.05-0.15% did not decrease fat deposition, blood concentration of uric acid, cholesterol, glucose and pH. In conclusion, the addition of 0.1% or 0.15% cellulase improved meat nutrient composition without improving body conformation.

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

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 (Osaka *et al.*, 2021), coronary heart disease, obesity and stroke (Grisotto *et al.*, 2021). In addition, high levels of fat deposition in the abdomen and other parts reduce the profit level of broiler carcass producers because of lower prices of fat depots.

One way to reduce fat depots is to use low-energy feed ingredients (Fouad and El-Senousey, 2014). One of the potential feed ingredients is fermented rice bran, because these feedstuffs contains low energy, and has antilipid properties (Park *et al.*, 2021). Feeding rice bran tempeh as much as 20% reduced meat fat content (cholesterol), but this reduction was still below 25% (Santoso e*t al*., 2022). Furthermore, this product tended to reduce body weight.

To overcome the weaknesses of that research, rice bran tempeh can be enriched with cellulase. Cellulase reduces levels of crude fiber, such as cellulose in feed ingredients (Rohman *et al.*, 2015). Cellulases are able to hydrolyze cell walls (Holland *et al.*, 2020; Jamet and Dunand, 2020; Camacho- Fernández *et al.*, 2021).), so that cell walls 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 diet containing rice bran tempeh could reduce fat deposition and fat content in meat. So far, the reduction of meat fat with 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. Amerah (2015) reported that there was an interaction between wheat characteristics and xylanase in increasing body weight gain, feed intake, feed conversion ratio in broiler chickens. 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.

The addition of cellulase has the potential to hydrolyze cellulose in the feed into simpler compounds and more easily digested, and thus would improve broiler performances (Zulkarnain *et al.*, 2017). In addition, cellulase also has the potential to reduce fat deposition. However, 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 diets containing rice bran tempeh on body conformation, fat deposition, blood chemistry profile, and nutrient composition of meat in broiler chickens. It is suspected that the addition of cellulase reduced fat deposition and improved broiler meat composition without decreasing body conformation and blood biochemistry profiles.

MATERIALS AND METHODS

Animals and Diets

Broiler chickens were maintained in litter cage with 100 x 100 x 65 cm (width x length x height), with humidity ranged from 60% to 70% and temperatures ranged from 26^{o} to 32^{o}C. Rice bran tempeh was made using the method of Santoso *et al.* (2022). There were four treatment groups consisted of 160 fifteen-days-old broilers (strain Lohmann) with a ratio of male to female 1:1. In this study there was no 0% rice bran tempeh treatment as a control. The four treatment groups, with five replications for each group, were treated as follows: T1 = 0% cellulase; T2 = 0.05% cellulase; T3 = 0.1% cellulase, and T4 =

Table 1. The Composition of Experimental Diets

Feedstuffs (g/kg)	T1	T2	T3	T4
Yellow corn	420	420	420	420
Rice Bran Tempeh	200	200	200	200
Broiler Concentrate	342	342	342	342
Plam oil	15	15	15	15
Mineral mixture	17	17	17	17
Garam dapur	1	1	1	1
Top mix	5.0	4.5	4.0	3.5
Cellulase	0	0.5	1.0	1.5
Total	1000	1000	1000	1000
Calculated analysis				
Protein, %	20.01	20.01	20.01	20.01
ME, kcal/kg	2944.22	2944.22	2944.22	2944.22
Crude fiber, %	7.54	7.54	7.54	7.54
Crude fat, %	4.76	4.76	4.76	4.76

0.15% cellulase. 20% rice bran tempeh was used in this research because the results of previous research showed that although the effect was not significant, there was a tendency for weight loss (body conformation). The cellulase used in the study was liquid and was a commercial product, but there was no information on cellulase concentration. Cellulase level referred to the research results of Zulkarnain *et al*. (2017). Broilers were reared up to 35 days old. The composition of the experimental feed ingredients is shown in Table 1. As shown in Table 1, all treatment group contained 20% rice bran tempeh. Diets and drinking water were given *ad libitum*. Body conformation was measured at 35 days old. Leggedness, massiveness, compactness was measured by the calculation method according to Kokoszynski *et al.* (2017). Body shape index was measured using the method of Guillaume (1976).

Sampling and Laboratory Analysis

At 35 days of age, 5 broilers for each treatment group were slaughtered, and fat depots were separated and weighed. Before cutting, blood samples were taken through the *Vena brachialis* 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 were measured using the AOAC method (2016). Ash content + carbohydrates was 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.

RESULTS AND DISCUSSION

Body Conformation

Table 2 shows the impact of adding cellulase to a diet that includes rice bran tempeh on the body conformation of broiler chickens. According to the findings, the treatment mentioned had no significant effect (P>0.05) on massiveness, compactness and body shape index. However, it had a significant impact on leggedness (P<0.01). The leggedness of T2 and T4 was lower than that of T1 and T3. In overall, body conformation did not change by cellulase inclusion. Body conformation is a reflection of body weight, which means that adding cellulase did not improve body weight. This study agree with Sharmila *et al.* (2014) study. They reported that supplementation of 0.2% cellulase tended to reduce body weight in broiler chickens.

Fat Deposition

Table 3 demonstrates the effect of adding cellulase to a diet that includes rice bran tempeh 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. Pectin, cellulose, and ferulic acid, which make up cell wall, are released through hydrolysis (Holland *et al.*, 2020; Jamet and Dunand, 2020; Camacho-Fernández *et al.*, 2021). The potential of cellulose (Cui *et al.*, 2022) and pectin (Sagedhy *et al.* (2015) to reduce abdominal fat is widely known. It is believed that the cellulose and pectin that result from cell wall degradation are not sufficient to decrease abdominal fat. Zulkarnain *et al.* (2017) reported that the addition of cellulase enzymes reduced abdominal fat deposition in broilers, but the research results do not match their findings. The cellulase level used in Zulkarnain *et al*. (2017) is 1 g/kg sago waste or as much as 0.1%. It is believed that the differences in feed ingredients used are one of the reason for the difference in results between these two studies. This assumption is supported by Ramaiyulis *et al.* (2018) who reported that the differences in the combination of feed ingredients used would influence the livestock's response to the addition of cellulase.

The abdominal fat depot average of Loughmann strain female broilers aged 35 days in the present study was 0.63%. The average female

Table 2. Effect of Adding Cellulase to the Diet containing Rice Bran Tempeh on Body Conformaion of Broiler Chickens

Variables	T1	T2	T3	T4	P
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 shape index	4.85±0.55	4.38±0.44	4.81±0.57	4.58±0.45	0.238

T1 = 0% cellulase; T2 = 20% 0.05% cellulase; T3 = 0.1% cellulase, and P4 = 0.15% cellulase. [a,b] Different superscripts in the same row are significantly different (P<0.05).

Table 3. Effect of Adding Cellulase to the Diet containing Rice Bran Tempeh on Fat Deposition in Broiler Chickens

Variables	T1	T2	T3	T4	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

T1 = 0% cellulase; T2 = 20% 0.05% cellulase; T3 = 0.1% cellulase, and P4 = 0.15% cellulase.

broiler abdominal fat in the same strain in Santoso *et al.* (2018), Santoso *et al.* (2019), and Santoso *et al.* (2020) were 1.28%, 0.62%, and 0.80%, respectively. So, abdominal fat in the Lohmann strain at 35 days of age ranges from 0.62% to 1.28%. These researchers (Santoso *et al.,* 2018; Santoso *et al.,* 2019; and Santoso *et al.,* 2020) used diet with relatively the same energy and protein levels. Thus, other factors might involve influencing the abdominal fat deposition. Other factors that influence abdominal fat deposition include environmental factors (Luo *et al.*, 2022) and broiler rearing management. The amount of fat deposited varied among years (Presstrud and Nilssen, 1992; He *et al.*, 2020).

Blood Biochemistry Analysis

Table 4 presents the effect of adding cellulase to a diet containing rice bran tempeh 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 hydrolyzes the cell walls of feed ingredients thereby increasing the availability of glucose. The results of cellulose degradation by cellulase include glucose, oligosaccharides, shorter polysaccharides (Lu *et al.*, 2019). It was predicted that the glucose resulting from cellulose degradation in the present study is not sufficient to increase blood glucose concentration.

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 reduces blood cholesterol concentration. Cellulase releases pectin, hemicellulose, cellulose, lignin, ferulic acid, minerals, and proteins that make up the cell wall through hydrolysis (Holland *et al.*, 2020; Jamet and Dunand, 2020; Camacho-Fernández *et al.*, 2021). Ferulic acid (de Melo *et al.*, 2017), cellulose (Menge *et al.,*1974) and pectin (Terpstra *et al.*, 1998) reduce the concentration of total cholesterol in the blood.

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,

Table 4. Effect of Adding Cellulase to the Diet containing Rice Bran Tempeh on Blood Biochemistry Profiles

Variables	T1	T2	T3	T4	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

T1 = 0% cellulase; T2 = 20% 0.05% cellulase; T3 = 0.1% cellulase, and P4 = 0.15% cellulase.

Table 5. Effect of Adding Cellulase to the Diet containing Rice Bran Tempeh on the Nutrient Composition of Broiler Meat

Variables	T1	T2	T3	T4	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

T1 = 0% cellulase; T2 = 20% 0.05% cellulase; T3 = 0.1% cellulase, and P4 = 0.15% cellulase. ^{a,b} Different superscripts in the same row are significantly different (P<0.05).

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.

Uric acid concentration is in part regulated by blood glucose (Ding *et al.*, 2021). Therefore, not decreasing blood glucose is one of the causes of not decreasing blood uric acid. 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 are 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 Nutrient Composition

Table 5 presents the effect of adding cellulase to a diet containing rice bran tempeh 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 cell wall polysaccharides encapsulate starch and protein, making them less accessible for digestion. The non starch polysaccharide (NSP) can be partly depolymerized by supplemental cellulase, which can improve protein digestibility. Cellulase has the ability to hydrolyze certain types of carbohydrate protein complexes that have a protein component that resists proteolysis by substituting bulky carbohydrate groups. This could have contributed to some improvements in protein digestibility (Ranjan *et al.*, 2018). The digestibility of dry matter, organic matter and protein in broiler chickens is enhanced by the addition of cellulase (Zulkarnain *et al.*, 2017). According to Ranjan *et al.* (2018), adding cellulase led to an increase protein digestibility in *Labeo rohita*. The Increase in meat protein content in T2 is thought to be a result of an increase in protein digestibility. The reason for the decrease in meat protein levels in line with further addition of cellulase (T3 and T4) is still unkown.

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. Shahzad *et al.* (2021) reported that the addition of phytase increased the digestibility of minerals. 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.*, 2017). 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.

An interesting phenomenon in this research is that although cellulase reduced meat fat content, it did not reduce abdominal fat. There are different mechanism which regulate the deposition of abdominal fat and intramuscular fat in chickens (Zhang *et al.*, 2019). The genes Glyceraldehyde-3-phosphate dehydrogenase, Lactate Dehydrogenase A, Glutathione Peroxidase 1, and 1,4-Alpha-Glucan Branching Enzyme 1 were involved in regulating intramuscular fat deposition, and the genes Fatty Acid Binding Protein 1, ELOVL Fatty Acid Elongase 6, Stearoyl-CoA Desaturase, and Adiponectin, C1Q And Collagen Domain Containing determined abdominal fat deposition (Luo e*t al*., 2022). In addition, intramuscular fat is regulated by glycolysis/gluconeogenesis and other signaling pathways, whereas abdominal fat is regulated by peroxisome proliferatot-activated receptor (PPAR) metabolism controls abdominal fat deposition (Luo *et al.*, 2022).

The mechanism for reducing fat content of meat by cellulase is unkown. 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 hydrolyzes 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 walls 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). It is believed that the decrease in lipase leads to a decrease in the availability of substrates for fatty acid synthesis, leading to a decrease in fat levels. Pectin supplementation result in a decrease in fat deposition in mice (Sefcikova and Racek, 2016). 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 acid 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

In conclusion, the addition of 0.1% or 0.15% cellulase to diet containing rice bran tempeh improved meat nutrient composition without improving body conformation.

CONFLICT OF INTEREST

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

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