The effect of chitosan and bran fermentation on the weight of abdominal fat, blood cholesterol and local duck eggs

Eli Sahara, Sofia Sandi and Fitra Yossi

Faculty of Agriculture, University Sriwijaya, Palembang, Indonesia. Corresponding author email: elisahara.unsri@gmail.com

Abstract. Duck eggs are one of the sources of animal protein from poultry. Duck animal products are known to contain fat and cholesterol higher than chicken eggs. This egg cholesterol can be suppressed by manipulating ration. Chitosan and bran have cholesterol binding properties. The purpose of this study was to get low cholesterol meat and eggs. The study used a completely randomized design (CRD) with 4 treatments and 5 replications. Each replication consisted of 2 ducks as the experimental unit. The treatments were R0 = 45% corn + 35% concentrate + 20% fermented bran, R1 = 45% corn + 35% concentrate + 20% fermented bran, R2 = 45% corn + 35% concentrate + 19.5% fermented bran + 0.5% chitosan and R3 = 45% corn + 35% concentrate + 17.5% fermented bran + 2.5% chitosan. The variables measured were abdominal fat, blood cholesterol and duck egg cholesterol. The data were processed using SAS Windows 16 program. The results showed that giving chitosan gave an average percentage of abdominal fat weight was not significantly different between treatments (P> 0.05) and R1 treatment significantly lowered the lowest duck blood cholesterol level (P <0.05) which was 2.9 (ml/dl). While the lowest egg cholesterol was shown by the treatment of R3 (2.78 mg/dg) compared to other treatments.

1. Introduction

Increasing human civilization, causing people to better understand the importance of body health. Foods that contain cholesterol are more likely to be avoided by consumers because they are predicted to be the cause of coronary heart disease. Products from poultry such as meat and eggs contain certain amounts of fat and cholesterol. In fact, the yolk portion is dominated by fat content, as in the form of conjugate fat and cholesterol triglycerides. Poultry carcasses that contain lots of fat are less desirable, as is cholesterol contained in egg yolks. There are parts of consumers who avoid consuming foods that contain fat and high cholesterol because of high blood pressure or arteriosclerosis. As a result, low-fat food is becoming more popular. This is a market opportunity for suppliers of foodstuffs from livestock to provide low fat and cholesterol products. Therefore, the solution to reduce the fat and cholesterol content of animal-derived products is by manipulating the ration or by mixing substances that are binding cholesterol into the livestock ration.

Chitosan is the result of waste from crustaceans which are eco-friendly because they are not toxic and are strongly binding to cholesterol. Hasri [1] states that 5 grams of chitosan in 50 ml of fat affect the percentage of cholesterol absorption by 45.46%. While bran oil also produces unsaturated fatty acids which give a hypocholesterolemic effect by reducing levels of bad cholesterol (Low-Density Lipoprotein, LDL) in the blood and increasing levels of good cholesterol (High-Density Lipoprotein, HDL) [2]. This was reinforced by Sujono [3] that fermented bran used as animal feed can reduce cholesterol levels in meat and eggs. Chitosan is known to be polar and nonpolar which is able to bind oil and water. In addition, the structure of chitosan contains hydroxyl groups and amines which are positive polycation, able to bind strongly with negative elements from other ingredients in the digestive tract. Chitosan is a long polymer chain because it is built by a combination of monomers. Chitosan

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consists of poly (2-deoxy-2-acetylamine-2-glucose) and poly (2-deoxy-2-aminoglucose) which bind (1-4) \(\beta\)-glycosidic.

The polymer chain of chitosan will roll up fat and cholesterol so that it is trapped in chitosan and discharged out through feces. As a result, fat and cholesterol in the ration will be eliminated and not absorbed by the body. The bond that is formed is the van der walls bond which is a very strong bond like the opposite two magnetic poles. There is another opinion that says that the form of chitosan is porous, so it can absorb (sorbs) fat and cholesterol, some organic matter and water. Chitosan dissolves in weak acids, so that if chitosan passes through segments of the gastrointestinal tract with different pH, it is predicted that its structure will expand and deflate, especially in the gastrointestinal segment that has a low pH. As a result, certain parts of fat and cholesterol are trapped and eventually discharged out with feces. Based on the method of work and the nature of chitosan and the physiology of the digestive tract, this study focused on looking at its effects if it was collaborated with fermented bran to bind cholesterol to the body's livestock.

2. Materials And Methods

2.1. Materials

2.1.1. Ration
Experiments used ration raw materials, namely corn, concentrates of 32% protein laying, fermented bran, chitosan and premix. The chitosan used was pure chitosan from the IPB Fisheries Processing Technology Laboratory.

2.1.2. Duck
The ducks used are in production, as many as 40 animals are placed in cages that are equipped with lighting, where to eat and drink. Ducks were kept for 7 weeks and eggs in the last two weeks of the study were collected for cholesterol analysis. At the end of the study, 20 ducks were cut (5 tails / treatment) to take meat samples and abdominal fat weight. Before the blood is cut, the lower part of the wing is taken and then taken to the laboratory for analysis.

2.2. Research Methods
The study used Completely Randomized Design (CRD) with 4 treatments and 5 replications, each replication consisting of 2 ducks. The treatments used were: 
- \(R_0 = 45\% \text{ corn} + 35\% \text{ concentrate} + 20\% \text{ fermented bran}\),
- \(R_1 = 45\% \text{ corn} + 35\% \text{ concentrate} + 20\% \text{ fermented bran}\),
- \(R_2 = 45\% \text{ corn} + 35\% \text{ concentrate} + 19.5\% \text{ fermented bran} + 0.5\% \text{ chitosan}\),
- \(R_3 = 45\% \text{ corn} + 35\% \text{ concentrate} + 17.5\% \text{ fermented bran} + 2.5\% \text{ chitosan}\).

The treatment ration was made by mixing homogeneously all the ingredients for each treatment based on their composition. The parameters measured are 3, namely: weight of abdominal fat, blood cholesterol and egg cholesterol. During maintenance, the ration is given twice a day, in the morning and evening. Maintenance is carried out for 7 weeks, and drinking water is given ad libitum.

2.3. Data Analysis
The data obtained were processed by variance analysis (ANOVA) using SAS Windows 16. If there are further tested the effect of treatment of multiple Duncan [4]. Especially for cholesterol eggs are presented descriptively.
3. Results and Discussion

3.1. Results

3.1.1. The effect of giving chitosan and bran fermentation to the weight of Abdominal fat of Tegal Ducks

Abdomen fat is a layer of fat found around the gizzard and the lining between the abdominal and intestinal muscles. The average abdominal fat weight of Tegal ducks was almost the same (P > 0.05), which ranged from 1.00 to 1.59 grams (Table 1).

Table 1. Average weight of abdominal fat by giving chitosan and fermented bran

| Parameters                          | Treatments |
|-------------------------------------|------------|
| Abdominal fat weight (g)            | R0         | R1         | R2         | R3         |
|                                     | 1.38       | 1.00       | 1.15       | 1.59       |

R0 = 45% corn + 35% concentrate + 20% bran without fermentation.
R1 = 45% corn + 35% concentrate + 20% fermented bran.
R2 = 45% corn + 35% concentrate + 19.5% fermented bran + 0.5% chitosan.
R3 = 45% corn + 35% concentrate + 17.5% fermented bran + 2.5% chitosan.

The age of ducks used for research is almost the same so that the body weight of each duck is also almost the same. According to Cherry et al., (1978) cited by Subekti et al. [5] that abdominal fat is influenced by body weight. Except for that, the nutrients consumed by ducks will be used for the first time for life, after that for production. This is thought to cause a limitation of the fat depot and suppress excess body fat. As a result, each individual research duck in each treatment had balanced abdominal fat. Abdominal fat in this study was lower than broiler abdominal fat applied feed additives of curcuma zanthorriza 1-3% in ration were 1.43 - 1.53. This is understandable because energy consumption is prioritized for basic life and production.

The main ingredient of eggs is protein and fat. So the nutrient fat consumed is to fill the material content of an egg. The condition of ducks that are currently being produced is thought to greatly affect the body's fat deposits because the utilization of energy and existing fat reserves are more focused on meeting basic life needs and egg production. Abdominal fat has a high correlation with total body fat and fat in various depots. In ducks that are actively producing energy and fat reserves are used for eggs, so that body weight will be relatively uniform and stable. Ducks that lay eggs on abdominal fat will be small while ducks that do not lay eggs, usually more abdominal fat. The weight of abdominal fat tends to increase with weight gain [6].

3.1.2. Effect of giving chitosan and bran fermentation on blood cholesterol Tegal ducks

The average blood cholesterol of Tegal ducks ranges from 2.91 to 7.02 ml/dl. Giving 20% fermented bran in ration (R1), a mixture of 19.5% fermented bran and 0.5% chitosan in ration (R2), and a mixture of 17.5% fermented bran and 2.5% chitosan in ration (R3) did not differ from control (fermented bran in ration / R0) (P > 0.05). However, the administration of 20% fermented bran (R1) was real (P < 0.05) showed lower blood cholesterol levels than R3 treatment (17.5% mixture of fermented bran and 2.5% chitosan in the diet) (Table 2).

Fermented bran causes an increase in the content of unsaturated fatty acids that contain high amounts of sterols. The sterols contained in the form of β sitosterol are 5% of the total sterols (Ardiansyah, 2008 cited by [2]. Sterols play a role in inhibiting the absorption of blood plasma cholesterol and increasing excretion so as to reduce the absorption of total cholesterol. Because the level of bran oil produced by R1 treatment is greater than other treatments, this is thought to be the cause of lower blood cholesterol levels than other treatments. Unsaturated fatty acids bran provide a dual effect with sterols in reducing cholesterol levels. Unsaturated fatty acids make blood cholesterol levels at normal levels, especially linoleic and oleic acids. The higher the composition of fermented bran in the
ration, the higher the unsaturated fatty acids produced, which are accompanied by an increase in the content of linoleic and oleic acids as happened in the R1 treatment. This R1 cholesterol level is almost the same as the control treatment (R0), but it is significantly lower than R3 treatment. The cause is suspected because the sterol produced by R3 treatment is lower, while chitosan which is mixed into the ration shows its function in the digestive tract to bind cholesterol. Bran fermentation will increase nutrient digestibility and ration quality. The treatment of a higher fermentation dose is expected to increase the breakdown of unsaturated fatty acids that enter the blood.

### Table 2. Average blood cholesterol levels by giving chitosan and fermented bran

| Parameters       | Treatments |
|------------------|------------|
| Blood cholesterol (ml/dl) | R0  | R1  | R2  | R3  |
|                  | 4.82a     | 2.91b | 5.08ab | 7.02b |

R0 = 45% corn + 35% concentrate + 20% bran without fermentation.
R1 = 45% corn + 35% concentrate + 20% fermented bran.
R2 = 45% corn + 35% concentrate + 19.5% fermented bran + 0.5% chitosan.
R3 = 45% corn + 35% concentrate + 17.5% fermented bran + 2.5% chitosan.

The effect of giving chitosan and fermented bran to the cholesterol of Tegal duck eggs

The average cholesterol level of Tegal duck eggs research ranged from 2.78 to 9.44 mg/dg. Giving 17.5% fermented bran and 2.5% chitosan showed the lowest egg cholesterol level (Table 3).

### Table 3. Average cholesterol level of eggs by giving chitosan and fermented bran

| Parameters       | Treatments |
|------------------|------------|
| Eggs cholesterol (mg/dg) | R0  | R1  | R2  | R3  |
|                  | 9.44      | 6.60 | 4.39 | 2.78 |

R0 = 45% corn + 35% concentrate + 20% bran without fermentation.
R1 = 45% corn + 35% concentrate + 20% fermented bran.
R2 = 45% corn + 35% concentrate + 19.5% fermented bran + 0.5% chitosan.
R3 = 45% corn + 35% concentrate + 17.5% fermented bran + 2.5% chitosan.

Based on Table 3, the decrease in cholesterol levels were 30.08% (R1), 53.50% (R2) and 70.55% (R3) respectively. The lowest egg cholesterol level was found in R3 treatment in addition to 17.5% fermented bran and 2.5% chitosan into the ration which was 2.78 mg/dg. The symbiotic effectiveness of the role of fermented bran and chitosan in binding cholesterol can be seen from the treatment of R3. Unsaturated fatty acids from fermented bran in producing sterols are able to bind cholesterol in the blood and amine and hydroxyl chitosan groups in the gastrointestinal tract are able to bind cholesterol that passes through the digestive tract and are discharged with faeces. This has proven that chitosan is able to bind fat and cholesterol in the body so that it affects the improvement of production quality. Deuchi et al., [7] states that chitosan has a high potential as a hypocholesterolemic. The assumption is that chitosan in the ration that enters the digestive tract is able to bind fat and cholesterol to be excreted with faeces. The form of porous chitosan and has polar and non-polar properties, making chitosan able to bind water and oil [8]. This will determine the journey of chitosan in the digestive tract of ducks, namely as a mechanism for the work of chitosan in the process of binding fat and cholesterol. In addition chitosan with a fiber-like structure will trigger an improvement in the growth of the main microflora in the digestive tract, thus helping in the provision of digestive enzymes. This is in accordance with Ref. [9] statement about the role of chitosan, which is the process of making enzyme immobility, the manufacture of complex compounds with proteins, anti-cholesterol, can eliminate harmful metals and chemicals. Guided by the role of chitosan, it is very possible that the treatment of R3 with the highest chitosan dose (2.5%) will accelerate fat catabolism to produce unsaturated fatty acids with the help of
enzymes produced by the main microflora, so that it is more absorbed. The accumulation of excess unsaturated fatty acids will produce excess sterols also in inhibiting cholesterol deposits to the eggs.

In addition, chitosan has amine and hydroxyl groups that are positively charged or cationic, and at pH <6.5 chitosan in the solution will be positively charged [10]. The role of these clusters is known to be highly reactive and has a high binding capacity to fat and cholesterol. Hasri [1] reported that the mass of 5 grams of chitosan in 50 ml of fat affected the percentage of cholesterol absorption by 45.46%. The positively charged chitosan amine group attracts the negative side of fatty acids and cholesterol like pulling bonds pulling the magnetic poles and forming bonds that cannot be digested [13] This means that with this perspective, fat and cholesterol that are strongly bound to chitosan, are excreted with faeces, so that the fat absorbed is low. As a result, eggs that become animal products of ducks contain low levels of fat and cholesterol. This can be understood according to the form of chitosan-like fiber which cannot be absorbed, so that if the fat is strongly bound to it, it will not be absorbed.

4. Conclusion
The application of 19.75% fermented bran and 2.5% chitosan in the ration can reduce the cholesterol level of Tegal duck eggs.

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