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Supplementation of Biopeptide from Chicken Feet to the Immune System and Growth of Broiler Chicken

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ABSTRACT

The safety of poultry products, especially broiler chickens, can be stated to be relatively low. Antibiotic growth promoter (AGP) is used to improve chicken performance and maintain immunity. The ban on the use of AGP triggers the innovation of other materials to replace the function of AGP. Biopeptide is a solution that can be used. Biopeptides can be made from materials with a high protein content, for example, waste from chicken slaughterhouses. The waste that can be used is chicken feet, biopeptides produced from hydrolytic proteins have antioxidant and antimicrobial properties. These properties can improve the performance and immune system of broiler chickens. The aim of the study was to examine the supplementation of biopeptides from chicken feet on the immune system (total *Escherichia coli* and internal organ) and the growth of broiler chickens. The research material was 200 day old chickens of broiler strain cobb unsex, which were reared for 42 days of the rearing period. The treatment feeds were P0: basal feed, P1 basal feed + 2% biopeptide, P2: basal feed + 4% biopeptide, P3: basal feed + 6% biopeptide. The collected data were analyzed using analysis of variance with the Tukey test (BNJ). The results showed that biopeptide supplementation from chicken feet had a significant ($P<0.05$) effect on total *Escherichia coli*, spleen weight, bursa of Fabricius weight, body weight gain, and feed efficiency for broiler chickens. However, the results of the study had no significant effect ($P>0.05$) on liver weight and Avian Influenza antibody titers for broiler chickens. Biopeptide supplementation from chicken feet can reduce total *Escherichia coli*, and spleen weight, and increase the bursa of Fabricius weight, body weight gain, and feed efficiency for broiler chickens. Biopeptide supplementation from chicken feet of 6% gave the best results in body weight gain and feed efficiency.

Keywords: Biopeptide, Broiler, Feed, Growth, Immunity

Introduction

The safety of poultry meat products still has a low consideration, which relates to livestock drug residues in the form of antibiotics. Residues of antibiotics left behind in products can make consumers contaminated with these residues. The formation of antibiotic residues in products of animal origin is caused by the uncontrolled use of antibiotics, especially the dosage and accuracy in determining the withdrawal time before cattle are slaughtered (Lailogo *et al.*, 2015). So far, antibiotics have been added to broiler feed in small quantities as growth promoters, but since the issuance of Indonesian Minister of Agriculture Regulation No. 14 of 2017, which refers to Law no. 41 of 2014 that prohibits the use of antibiotics in medicine, other compounds are needed that can replace the function of antibiotics as growth promoters. One of the solutions offered is the use of bioactive peptides (biopeptides), which have the function of maintaining the livestock immune system. Bioactive peptides are peptides or protein fragments composed of several amino acids and have positive physiological activities for the health of the body.

Bioactive peptides are produced in several ways, consisting of enzymatic hydrolysis with digestive enzymes or microbial enzymes, fermentation by utilizing microbial activity, and chemical synthesis (Bhat *et al.*, 2015). Advances in technology and existing analytical methods facilitate the discovery and identification of new peptides that have the potential to improve animal health. Peptides are also used to improve animal performance, such as increasing body weight and increasing the work of digestive enzymes. Aside from being a drug, bioactive peptides have another function, namely stimulating the immune system (Fosgerau, 2015). An easy and cheap source of biopeptides is collagen, which comes from chicken slaughterhouse waste (chicken feet). According to data from the Central Statistics Agency, in 2021 there will be around 3,426,042.00 tons of chicken meat produced. If we assume that the slaughtered chickens weigh 1.5 kg per head, there are 2,284,028,000 chickens. This indicates that the waste of chicken feet in Indonesia is 4,568,056,000 claws (Badan Pusat Statistik, 2021). Chicken feet are mainly composed of skin and bone tissue containing large amounts of collagen. In addition, total collagen on chicken feet is 12.08% (Santosa *et al.*, 2018). Collagen hydrolysis produces gelatin, and then hydrolysis of gelatin results in small peptides (3-6 kDa). These small peptides are soluble, which have antioxidant and antimicrobial activity (Ketnawa *et al.*, 2017; Hong *et al.*, 2017).

An increased immune system is expected to prevent the disease. In addition, an increased immune system has an impact on weight of spleen, liver, bursa Fabricius, and avian influenza (AI) antibody titers broiler. Also, growth efficiency can be seen from the efficiency of the animal feed, the more efficient the feed consumed, the more efficiently the livestock is growing. On the other hand, bioactive peptides, which have antibacterial properties will suppress the population of pathogenic bacteria so that livestock become healthier. Giving biopeptide can increase bursa Fabricius weight, body weight gain, feed efficiency, and can reduce internal organ weight, total *Escherichia coli* bacteria and can support AI antibody titers to be better.

Materials and Methods

The material used in the research was 200 broiler Day Old Chicks (DOC) strain cobb unsex, basal feed, and bioactive peptides from chicken feet. The DOC used has been vaccinated against newcastle diseases, infectious bronchitis, and infectious bursal diseases, feeding was given 3 times and drinking was provided as ad libitum. The compositions and nutrient contents of basal feed and treated feed can be seen in Table 1. Biopeptides from chicken feet contain 14.10% protein, 13.81% crude fat, and 18.19% crude fiber. Biopeptides from chicken claws are made by hydrolyzing collagen in chicken claws using the papain enzyme, the results of the hydrolysis are mixed with rice bran. The experimental study used a completely randomized design (Steel and Torrie, 1995) with treatment consisting of four levels of chicken claw hydrolyzate supplementation, consisting of 0% (P0), 2% (P1), 4% (P2), and 6% (P3). Each treatment was repeated 5 times, so that there were 20 experimental units containing 10 chickens/unit and if the treatment had an effect, a further honest significant difference test was carried out.

Total *Escherichia coli* was counted using the TPC (total plate count) method according to Barrow and Feltham (1993) with a dilution of 10^{-1}–10^{-4}, then 1 mL of each dilution was pipetted and put into a petri dish containing Eosin Methylene Blue Agar (EMBA) media, which was made in duplicate. The data was expressed as log colony forming unit (cfu)/mL. Antibody titers used the method used by Syukron *et al.* (2013) that 5 mL of blood samples were taken using a disposable syringe via the brachial vein after the broiler was 31 days old. The serum was analyzed for the amount of AI antibody titers in the agglutination inhibition test. Data collection on spleen weight, liver weight and bursa of Fabricius weight was carried out after maintenance was completed at 42 days of age, 3 chickens were taken from each unit. Growth is assumed to be the difference in body weight of the chickens from the start of the feeding experiment (initial weight) until it reaches the final weight (Darwati *et al.*, 2015). Feed efficiency is obtained from body weight gain divided by the total feed consumed for 42 days multiplied by one hundred percent (Kamran *et al.*, 2008).

Results and Discussion

Immune system

The results of the study of biopeptide supplementation from chicken feed improve the immune system are presented in Table 2. Supplementation of biopeptides from chicken feet had a significant effect ($P<0.05$) on total *E.coli*, bursa of Fabricius weight, and spleen weight in broiler chickens. Treatment had no significant effect ($P>0.05$) on AI antibody titers and broiler liver weight. The results showed that P3 and P2 was significantly different from P0 for total *E.coli*. For spleen weight, P3 was significantly different from P0. And for bursa Fabricius weight, P3 was significantly different from P0 and P1. Broiler chickens have a poor immune system, prone to stress, and high potential to sick. Diseased broiler chickens can affect the growth of immune organs (bursa Fabricius, spleen, liver), thus the immune system will decrease (Wiranto *et al.*, 2020).

The mean total *E. coli* for P0 was 10.91±61 log cfu/mL, P1 was 3.64±4.98 log cfu/mL, P2 was 1.82±2.50 log cfu/mL, and P3 was 0.91±2.03 log cfu/mL. These results were lower than a previous study conducted by Krismaputri *et al.* (2016) that broiler chickens fed with soybean meal had a total *E. coli* of 4.31 log cfu/mL. In this study, the total *E.coli* decreased as the concentration of biopeptide supplementation was increased. The decrease in the population of *E.coli* in the intestine of chickens proves that biopeptide supplementation can suppress total *E.coli*. This is due to the antimicrobial content in biopeptides from chicken feet. Antimicrobials given to broiler chickens will inhibit pathogenic bacteria in the digestive tract (Yulianti *et al.*, 2019). The antimicrobial activity of biopeptides from chicken feet will break down the bacterial cell membrane thereby inhibiting cell wall synthesis, weak cell walls will rupture/lyse so the bacteria will die (Arum *et al.*, 2014; Reygaert, 2017).

Increasing biopeptide supplementation to a level of 6% could reduce spleen weight. The average weight obtained for P0 was 2.01±0.29 g, P1 was 1.82±0.18 g, P2 was 1.67±0.13 g, and P3 was 1.72±0.19 g. The results of this study are in

Table 1. Composition and nutrient content of biopeptide supplementation treatment from chicken feet

Feed ingredients	P0 (%)	P1 (%)	P2 (%)	P3 (%)
Milled corn	42	42	42	42
Rice bran	21	21	21	21
Soybean meal	23	23	23	23
Fish meal	10	10	10	10
Oil	3	3	3	3
Mineral mix	0.8	0.8	0.8	0.8
Lysine	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Biopeptide	0	2	4	6
Total	100	102	104	106
Nutrient composition				
Crude protein (%)	22.76	23.04	23.32	23.60
Metabolis energi (kcal)	2969	2963	2963	2963
Crude fat (%)	6.87	7.15	7.42	7.70
Crude fiber (%)	5.84	6.20	6.57	6.93
Calcium (%)	0.72	0.88	0.88	0.88
Phospor (%)	0.56	0.64	0.64	0.64
Lysine (%)	1.18	1.32	1.32	1.32
Methionine (%)	0.39	0.59	0.59	0.59

P0: Basal feed + 0% biopeptide; P1: Basal feed + 2% biopeptide; P2: Basal feed + 4% biopeptide; P3: Basal feed + 6% biopeptide.

accordance with the research of Landy *et al.* (2020) that the administration of a bioactive peptide from a cotton meal of 5 g/kg could reduce the weight of the spleen. In this study, the higher the level of biopeptide supplementation resulted in the better the immune system. This causes the performance of the spleen to become lighter and its weight tends to shrink. This is in accordance with the opinion of Osho *et al.* (2019) indicating that the administration of biopeptides from soybeans can withstand the effect of immunosuppression on the development of immune organs. According to Merryana *et al.* (2007) that enlargement of the spleen is due to a bacterial infection, the spleen produces lymphocytes which function as the body's resistance.

Bursa Fabricius is one of the primary defense glands in chicks that is easy to find, because it is located adjacent to the cloaca and is larger in size compared to the defense glands in other animals (Bramardipa *et al.*, 2019). Abdelaziz *et al.* (2018) stated that bursa Fabricius has an important role, namely the differentiation of B lymphocytes, besides that it is also capable of producing local antibodies. The results showed the weight of bursa of Fabricius for P0 was 14.30±0.26 g, P1 was 14.34±1.39 g, P2 was 15.26±1.33 g, and P3 was 16.54±0.69 g. This is in accordance with research conducted by Landy *et al.* (2020) that the administration of biopeptides from cottonseed had a significant effect on the weight of the bursa of Fabricius in broiler chickens. The results of the same study were reported by Abdollahi *et al.* (2017) that the weight of the bursa of Fabricius in broiler chickens increases by providing feed containing biopeptides from soybeans.

Supplementation of biopeptide from chicken feet tended to decrease antibody titers but was not statistically significant. The results of antibody titers can be seen in Table 2. AI antibody titers will be protective at an amount of approximately log 32 (Widowati *et al.*, 2022). According to the Director General of Animal Husbandry (Dirjen, 2005), antibody titers can be classified into several levels, including; negative for log 1 numbers, low for log 2 to log 16 values, moderate for log 32 to log 256, then high for log 512 to log 2048 values. Based on the results of the study, it was shown that antibody titers for all treatments were classified as moderate. The high level of challenge in the cage can occur because the cage used in this study is an open-house cage that cannot be controlled properly. According to Nurkholis *et al.* (2014), the level of challenge in question is in the form of exposure or challenge of pathogenic microorganisms (antigens) such as viruses, bacteria, protozoa, and fungi that are in the broiler-rearing environment. This greatly affects the production of broiler antibodies. In control chickens, it was suspected that high levels of antigen exposure in the broiler-rearing environment could trigger the activation of the broiler's immune system. Antigens that enter the body are immediately countered by non-specific defense systems in the form of physical, mechanical, and chemical defenses. The higher level of field challenge received by the broiler results in the more antibodies must interact with the antigen. Chickens that were given biopeptide supplementation, already have a good immune system even without a AI vaccination. A good immune system is proven by chickens treated with less titers formed because the field challenge of the

Table 2. Biopeptide supplementation for the immune system of broiler chickens

Treatment	Total *E.coli* (Log cfu/ml)	AI antibody titers	Slpeen weight (g)	Liver weight (g)	Bursa fabricius weight (g)
P0	10.91±6.09^{b}	2.34±0.54	2.03±0.18^{b}	29.02±4.93	14.30±0.26^{a}
P1	3.64±4.98^{ab}	2.28±0.45	1.93±0.15^{ab}	28.46±3.31	14.34±1.39^{a}
P2	1.82±2.50^{a}	1.86±0.33	1.79±0.10^{ab}	27.55±1.50	15.26±1.33^{ab}
P3	0.91±2.03^{a}	1.74±0.39	1.72±0.19^{a}	28.23±3.16	16.54±0.69^{b}

P0: Basal feed + 0% biopeptide; P1: Basal feed + 2% biopeptide; P2: Basal feed + 4% biopeptide; P3: Basal feed + 6% biopeptide.
^{a,b} Different superscripts in the same column indicate a significant difference (P<0.05).

Table 3. Results of biopeptide supplementation on broiler chicken growth

Treatment	Body weight gain (g)	Feed efficiency (%)	Feed consumption (g)
P0	1018,97±90.63[a]	42.74±3.41[a]	2383,86±75.42
P1	1045,47±67.27[a]	43.99±2.64[a]	2376,08±29.97
P2	1048,83±20.25[a]	43.74±1.22[a]	2398,12±21.76
P3	1182,07±59.50[b]	49.96±2.45[b]	2379,83±16.32

P0: Basal feed + 0% biopeptide; P1: Basal feed + 2% biopeptide; P2: Basal feed + 4% biopeptide; P3: Basal feed + 6% biopeptide.
[a,b] Different superscripts in the same column indicate a significant difference (P<0.05).

AI virus cannot attack the livestock body. This makes livestock less susceptible to exposure to AI viruses, thus making antibody titers lower with control feed.

The liver weights of broiler chickens receiving biopeptide supplementation from chicken feet can be seen in Table 2. The data shows relatively the same and normal liver weights for broiler liver weights. These results are greater than the research conducted by Mistiani *et al.* (2020) showing that the average liver weight is 24 to 26.8 g. In this study, the feed used for the control had good protein content because it reached 22.76% for the control feed. Crude protein (CP) content of control diet is high because the minimum standard feed for broiler starter according to Indonesian National Standard No. 8173.2.2015 (SNI, 2015) contain 20% of CP. Treatment feed with biopeptide supplementation from chicken claws can increase the CP in the feed, so that the higher the level of biopeptide, the CP in the feed will also increase.

Performance and efficiency of broiler feed

Based on the results of the analysis of variance, it was shown that supplementation of biopeptides from chicken feet had a significant (P<0.05) effect on body weight gain and feed efficiency for broiler chickens, but had no significant effect (P>0.05) on feed consumption. The results for body weight gain and feed efficiency showed that P3 was significantly (P<0.05) different from P0, P1, and P2.

Supplementation of biopeptides from chicken feet by 6% was able to increase the weight gain of broiler chickens. The average body weight gain for each treatment can be seen in Table 3. These results are in line with the study of Karimzadeh *et al.* (2016) that the given biopeptides of as much as 0.025% had a significant effect on the growth of broiler chickens. The same results were shown in a study by Osho *et al.* (2019) that the higher the percentage of biopeptide supplementation, the higher the body weight growth of broiler chickens.

According to Nadzir *et al.* (2015), achieving an optimal growth need to do some efforts, such as providing highly nutritious feed and improving management by providing optimal environmental temperature for keeping the cage. Body weight is one of the main benchmarks to show whether or not a feed is digested by the animal's body (Hidayat *et al.*, 2018). According to Has *et al.* (2020) that body weight gain is greatly influenced by feed consumption and optimization of nutrient absorption in the digestive tract. The addition of biopeptides in feed containing antimicrobials will make the digestive tract of chickens increase the nutrient absorption process because pathogenic bacteria are suppressed and the performance of other organs is more optimal. The average feed Consumption was 2376.09 to 2379.83 g/head. Relatively the same feed Consumption between treatments is an indicator that biopeptide supplementation can improve growth and feed efficiency. Feed Consumption shown in each study showed relatively the same value in each research treatment but resulted in different body weights, meaning that feed efficiency can also be increased by administering biopeptides.

The feed efficiency average for each treatment can be seen in Table 3. Feed efficiency will be improved as long as the increasing concentration of biopeptides. This is because biopeptides have an antimicrobial function which will increase the immunity of this livestock. The antimicrobials in biopeptides also stress the population of pathogenic bacteria in the digestive tract. According to Adila *et al.* (2022), the number of non-pathogenic microbes in the digestive tract can provide benefits, such as increasing nutritional efficiency in rations. Increasing the absorption of nutrients contained in the ration, the digestibility of energy and protein increases (Silalahi and Sauland, 2013). This makes the feed efficiency of livestock-given biopeptides even better. Feed efficiency is closely related to body weight gain which is influenced by several factors including gender, feed consumption, environment, seeds, and feed quality (Qurniawan, 2016).

Conclusions

Biopeptide supplementation from chicken feet can reduce total *E.coli*, and spleen weight, and increase the bursa of Fabricius weight, body weight gain, and feed efficiency for broiler chickens. Biopeptide supplementation from chicken feet of 6% gave the best results in body weight gain and feed efficiency.

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