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PERFORMANCE, CARCASS WEIGHT, TOTAL INTESTINAL BACTERIA, AND FEED DIGESTIBILITY OF BROILERS FED CHICKEN FOOT-DERIVED BIOACTIVE PEPTIDES

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ABSTRACT: The aim of this study was to evaluate the supplementation of chicken claw-derived bioactive peptides on performance, carcass weight, total intestinal bacteria, and feed dry matter (DMD) and organic matter (OMD) digestibility of broilers. A completely randomized design and five repetitions were applied in this experiment. The research material consisted of 200 DOC strain CP-707 grown up to 35 days of age, and the biopeptide was produced by hydrolyzing chicken claws protein with a commercial papain enzyme. Bioactive peptides were added to feed treatments in amounts of 0, 2, 4, and 6%. The differences between treatments were tested using the honestly significant difference test. The addition of chicken claws biopeptides had a significant influence (P<0.01) on OMD and carcass weight, as well as a significant effect (P<0.05) on body weight gain, feed efficiency, DMD and total bacteria. Addition of chicken claws-derived peptides in rations up to 6% enhanced body weight gain, feed efficiency, carcass weight, DMD, OMD, and total intestinal bacteria in broiler chickens.

Keywords: Biopeptides, Chicken, Claw, Digestibility, Carcass quality.

INTRODUCTION

One of the feed additives which is currently prohibited in chicken ration is antibiotic growth promoters (AGPs). It causes resistance effect of pathogenic bacteria that lives inside the digestive tract and their abandoned residues in animal products (Bahar and Ren, 2013) as laws in many nations forbid using antibiotics (Prihambodo et al., 2021). Therefore, efforts are required to substitute the role of AGP in feed through the use of various natural ingredients that are secure for consumers and environmentally friendly. Biopeptide are peptides that have a measurement of 2-20 kDa and have various biological functions, they could act as antioxidants, antimicrobials, antihypertensives drugs and immunomodulators (Hartmann and Meisel, 2007).

Biopeptides are produced by hydrolysis of proteins using enzymes or microorganisms. Livestock product such as chicken claw has been studied to generate biopeptides. Chicken claw contains 60% water, 12.87% fat, 17.17% protein, 9.94% ash (Santana et al., 2020), 9.07%-12.8% collagen (Santosa et al, 2018), 1.70 mg phosphorus, 2.87 mg calcium, 5.5 g unsaturated fat, 2.571 mg omega-6 and 187 mg omega-3 (Muyonga et al., 2004), and cholesterol 108 mg/100 g ceker (USDA, 2018). Glycine (33%), proline and hydroxyproline (22%) are the main amino acids that form collagen. The primary structure of collagen is a triple alpha-helix, each helical chain has a molecular weight of around 100 kDa and is made up of 1014 amino acids, so the overall collagen molecular weight is 300 kDa respectively (Leon-Lopez et al., 2019). Chicken water-soluble collagen has the role of increasing immunity (Tong et al., 2010), omega-3 as anti-inflammatory and beneficial for brain function (Soeparno, 2011). Enzymatic hydrolysis of collagen produces smaller gelatin proteins, and if gelatin is hydrolyzed further, it will produce smaller-sized peptides which have the ability as bioactive peptides.

Papain (EC.3.4.22.2) is a kind of cysteine protease enzyme that consists of a polypeptide chain with three disulphide bridges and one sulfhydryl group that is essential for catalysis (Bakar, 2010). Papain's molecular weight is 23 kDa (Ming et al., 2002). Pure papain has a specific activity of 0.119-0.347 mg/ml in distilled water. Papain was found in papaya sap, leaves, seeds and fruit skin (*Carica papaya*) it has maximum activity at 60^{0}C and pH 8.0 and is stable at 60^{0}C for 30 minutes (Khatun et al., 2023). Papain was reported to have a selective advantage for hydrolyzing fishbone collagen (Hidayat et al., 2016). The use of the enzyme papain for the hydrolysis of chicken claws potentially produces bioactive peptides as antioxidants and antimicrobials. The antibacterial activity of papain was reported to inhibit the growth of acne-causing bacteria (Ardina, 2012). The degree of papain hydrolysis in the amount of 20% was reported to be able to produce bioactive peptides of 2-5 kDa respectively (Hema et al., 2017).

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An immune system that was maintained by biopeptide intake was indicated by increased growth and carcass weight of broiler chickens. Good immunity causes the use of feed protein more efficient so it improves meat protein deposition (Jamilah and Mahfudz, 2013). On the other hand, the biological function of peptides as antioxidants and antibacterial improve intestinal function through the development and integrity of intestinal cells as well as the diversity of intestinal microorganisms so that the digestibility of feed nutrients is increasing. Supplementation of soybean meal bioactive peptides up to 6 g/kg feed markedly increased FCR and tended to escalate dry matter digestibility and intestinal villi length (Abdollahi et al., 2017). The addition of small commercial peptides as much as 4.5 g/kg feed significantly increased the diversity of microorganisms in the intestines of laying hens (Zhao et al., 2022). The aim of this study was to examine the effect of chicken claw biopeptides supplementation on carcass weight, total intestinal bacteria, dry matter (DMD) and organic matter digestibility (OMD) in broiler feed.

MATERIALS AND METHODS

This experiment was performed in accordance with regulations of University of Jenderal Soedirman number 1310/UN23/HK.03/2021. The authors complied with the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines 2.0.

Experimental design and treatments

In this study, 200 unsexed birds CP 707 strains of day-old chicks were raised for 35 days. The birds were grown in an open house arrangement divided into 20 pens, each with ten birds. Prepare a feeder and a drinker tube for ad libitum supply. The basal feed consists of 42% milled corn, 21% rice bran, 23% soybean meal, 10% fish meal, 3% vegetable oil, 0.8% mineral mix, 0.1% methionine and 0.1% lysine. The levels of chicken claws biopeptides were 0% (T0), 2% (T1), 4% (T2) and 6% (T3).

Table 1 - Nutritional content of feed treatments

Nutrients	T0 (%)	T1 (%)	T2 (%)	T3 (%)
Crude protein (%)	22.76	23.04	23.32	23.60
Metabolizable energy (Kcal/kg)	2969	2990	3015	3024
Crude fat (%)	6.870	7.150	7.420	7.700
Crude fibre (%)	5.840	6.200	6.570	6.930
Calcium (%)	0.720	0.880	0.880	0.880
Phosphor (%)	0.560	0.640	0.640	0.640
Methionine (%)	1.180	1.320	1.320	1.320
Lysine (%)	0.390	0.590	0.590	0.590

Production of biopeptides

A total of 100 g chicken claws were added to 500 ml of water and then extracted by autoclave for 30 min. Filtering the chicken claws to obtain extracts liquid, the extracted fat was separated using an extract solution stored in the refrigerator overnight, so that the fat was easily separated from the collagen. The protein content of the chicken claws extracts was 0.662 g/ml. Papain commercial (0.602 U/mg) was dissolved in 250 ml 0.02 M Tris-Cl pH 7.00 then added to chicken claws extracts and homogenized. The reaction mixture was incubated at 50^{0}C for 24 h. Chicken claws hydrolyzate was mixed with sterilized rice bran (4:1) as a carrier and dried in an oven at 60^{0}C for 48 h. The chicken claws biopeptides were ground and stored for in vivo treatments, it contains 14.10% protein, 13.81% fat and 18.19% crude fibre.

Data collection

Carcass traits

To assess the carcass at the end of the trial, two birds from each pen were chosen and slaughtered, the corpses were de-feathered and the giblet was hand eviscerated. The process of slaughtering followed the rules of halal according to Islamic religion that is UU No. 33/2014 and regulation of University of Jenderal Soedirman. The carcass was then divided into several components, including the breast, thigh, wings, and back weight, and the proportion of each yield weight over the carcass weight was computed.

Total bacteria

Samples for total bacteria calculation were obtained when the chickens were 35 days old. A total of 20 chickens were slaughtered and digesta samples were taken from the duodenum, jejunum and ileum and homogenized. For slaughtering chickens were fasted in a comfortable cage for 12-24 h, then each bird were taken and slaughtered according to halal rules (cutting oesophagus, trachea and vena jugularis using a sharp knife). Digesta samples were stored in sterile vials and tightly closed and then put in ice bags and moved to the laboratory for total bacteria analysis. Total bacteria were calculated by total plate count method according to Barrow and Feltham (1993). One-gram of the digested sample was diluted in stages from 10^{-1} to 10^{-9} using sterile distilled water. A total of 100 µl of the sample was placed in a petri dish, and then sterile nutrient agar media was poured and left to solidify. All sample were incubating at 40°C for 24 h. The number of intestinal bacterial colonies was counted using the Quebec colony counter at a 10^{-5} dilution.

Determination of feed digestibility

Residual feed and fecal samples were collected for five days when chickens reached 30 days of age. The total residual feed and fecal weighing data were obtained by collecting and weighing the samples every morning before being fed. As much as 10% samples were taken, samples were dried at 105°C for 8 h. The dry matter content (%) is calculated based on the wet weight minus the moisture content of the samples. Then the dry samples were composited and analyzed for ash content. The organic matter content (%) was obtained after the dry matter content was reduced by the ash content (AOAC, 2012). The coefficient digestibility of dry matter and organic matter were measured as:

$$\text{Nutrients digestibility (\%)} = \frac{[(\text{\% nutrient in feed x Feed Intake}) - (\text{\% nutrient in faeces x Total Faeces})]\text{x } 100}{(\text{\% nutrient in feed x FI})}$$

Statistical analysis

A completely randomized design (CRD) was applied with four levels of chicken claw bio-peptide supplementation viz. 0% (T0), 2% (T1), 4% (T2) and 6% (T3). Each treatment was repeated five times, so there were 20 trial units fraught with 10 birds/unit. To find out the differences between treatments, an honest significant difference test was carried out.

RESULTS AND DISCUSSION

Chicken claws that were extracted using an autoclave were solved with a fairly high protein content is 0.662 g/ml and the protein content increased to 1.005 g/ml after being precipitated by 40% ammonium sulphate. Chicken claws hydrolysate produced by commercial papain resulted in a 2.90% degree of hydrolysis and it has antioxidant activity in the amount of 92.29% and antibacterial (Table 2). Supplementation of 4% and 6% chicken claws bioactive peptides significantly affected organic matter digestibility (OMD) and carcass weight (P<0.01), dry matter digestibility (DMD) and total intestine bacterial (P<0.05). Increasing bioactive peptides level in feed significantly improved carcass weight, digestibility and total bacteria (Table 3), body weight gain and feed efficiency but it has no effect on feed intake (Table 4). I t also led to an increase in the levels of feed protein, fat and crude fibre (Table 1). The crude fibre content of feed increased due to the use of rice bran as a carrier for chicken claw peptides.

Table 2 - Biological activity of hydrolysates chicken claws

Biological activity	Type of assay	Process	Hydrolyzate	References
Antioxidant	In vitro	Hydrolysis with papain	Protein content= 0.662 g/ml	Hartoyo et al. (2022)
		Precipitated by 40% ammonium sulphate	Protein content= 1.005 g/ml, antioxidant activity= 92.29%	
Antibacterial	Animal study	Hydrolysis with papain	Protein content= 0.662 g/ml	Hakim et al. (2023)
		Precipitated by 40% ammonium sulphate	Protein content= 1.005 g/ml, intestinal *E. Coli* decreased by 67% at 1.6% supplementation	

Table 3 - Effect of chicken claw biopeptides on carcass weight, total intestine bacteria, dry matter and organic matter digestibility in broiler

Variables	T0	T1	T2	T3
Carcass weight (kg/b)	0.67 ± 0.02^{a}	0.69 ± 0.02^{ab}	0.71 ± 0.01^{bc}	0.72 ± 0.01^{c}
Total bacteria of digesta (cfu/mL)	7.20 ± 3.09^{a}	6.40 ± 2.98^{b}	6.80 ± 1.50^{c}	7.50 ± 2.03^{ac}
Dry matter digestibility (%)	76.9 ± 1.99^{a}	77.8 ± 2.74^{b}	79.2 ± 2.76^{bc}	82.0 ± 1.73^{c}
Organic matter digestibility (%)	77.3 ± 2.03^{a}	78.6 ± 1.69^{b}	80.2 ± 2.08^{bc}	81.6 ± 1.45^{c}

^{abc} superscript with different letters in the same column indicates a difference in P<0.05 or P<0.01. T0 = basal feed; T1 = basal feed + 2% biopeptides; T2 = basal feed + 4% biopeptides; T3 = basal feed + 6% biopeptides

Table 4 - Effect of chicken claw biopeptides on performance of broiler*

Variables	T0	T1	T2	T3
Body weight gain (g)	1018,97±90.63^{a}	1045,47±67.27^{a}	1048,83±20.25^{a}	1182,07±59.50^{b}
Feed efficiency (%)	42.74±3.41^{a}	43.99±2.64^{a}	43.74±1.22^{a}	49.96±2.45^{b}
Feed intake (g)	2383,86±75.42	2376,08±29.97	2398,12±21.76	2379,83±16.32
Total *E. coli* (log cfu/ml)	10.91±6.09^{b}	3.64±4.98^{ab}	1.82±2.50^{a}	0.91±2.03^{a}

^{abc} superscript with different letters in the same column indicates a difference in P<0,05. T0 = basal feed; T1 = basal feed + 2% biopeptides; T2 = basal feed + 4% biopeptides; T3 = basal feed + 6% biopeptides. * Hakim et al. (2023)

Hydrolysis of chicken claw protein (collagen) by papain will produce gelatin, and further, gelatin hydrolysis generates small peptides (3-6 kDa) that are soluble and have antioxidant and antibacterial activity (Ketnawa et al., 2017). Bioactive peptides are short amino acids chain possessing biological activity, such as antibacterial, antioxidant, antihypertensive, and immunomodulatory effects (Hou et al., 2017). These bioactive peptides are typically 2-20 AA residues long; however, some may have more than 20 AA residues (Bah et al., 2016). The length of time hydrolysis by enzymes affects the amount and type of producing peptides. In this study, the hydrolysis time of chicken claws by papain was 24 hours. It was suspected that this time has resulted in many small peptides with different lengths and amino acid compositions that have various biological functions. According to Hou et al. (2017) generally, biopeptides production by enzyme hydrolysis takes 4-48 hours. Production of antioxidant peptides from casein cow's milk using pepsin and trypsin for 24-28 hours produces small-sized peptides with several amino acid residues of 2-8 kDa (Power et al., 2013).

Increasing carcass weight associated with chicken claws peptides supplementation improved the chicken's immune system, this condition directly resulted in growth rate and meat protein deposition, which got higher. Meat protein deposition is influenced by feed protein content and it will determine carcass weight (Widiyawati et al., 2020). T3 (6% chicken claws peptides) resulted in the highest carcass weight and total intestinal bacteria, which were 0.721 kg/bird and 7.2 x 10-5 cfu/ml compared to T0 (control). The addition of chicken claws peptides of as much as 6% (T3) afforded to produce meat tissue of as a carcass component. Carcass weight was directly proportional to body and linearly to weight gain (BWG). These results are consistent with the research of Hakim et al. (2023) that BWG of broiler chickens fed chicken claws peptides up to 6% improved bursa fabricius weight which has a positive impact to immunomodulators and antioxidants agent that affect the health of chickens. According to Abdelaziz et al. (2018), bursa fabricius plays an important role in cell differentiation that maintains the immune system, that is B lymphocytes and it has the ability to produce local antibodies. The addition of antimicrobial peptides (AMP) has a linear pattern, it increased the immune response and antioxidant activity of broiler chickens (Sholikin et al., 2021). Antioxidant compounds are capable of capturing and neutralizing free radicals so that oxidative stress stops and cell damage can be avoided. In this study, the effects of immunomodulatory and antioxidant compounds in chicken claws peptides were reflected in carcass weight increase. Besides that, there is a strong correlation between biopeptide with an increment of carcass weight by nitrogen metabolism pathway (Seifi et al., 2018). As we know, nitrogen is a part of protein which is needed due to its metabolism to produce amino acids.

Antibacterial peptides in chicken claws significantly (P<0.01) reduced *E. coli* population in chicken intestines (Table 4) from 10.91 x 10^{-5} cfu/ml to 0.91 x 10^{-5} cfu/ml when it supplemented up to 6% in broiler rations (Hakim et al., 2023). In this study, the addition of 2-4% chicken claws peptides was able to reduce the total intestinal bacteria (Table 2). This result agrees with Sholikin et al. (2021) who stated that increasing the level of antimicrobial peptides (AMP) linearly reduced the number of intestinal bacteria and broiler feces (coliform, *E. coli*, *Clostridium* spp., lactic acids bacteria and total aerobic bacteria), however, several other bacterial species were not affected by the addition of AMP. Batt and Lou Tortorello (2014) reported that *E. coli* and total aerobic bacteria are a group of coliforms, which are gram-negative and lactose-utilization bacteria. Coliform bacteria are reported can produce various toxins such as indole, scatole and thionine which stimulate the development of cancer and cause diarrhea (Girard and Bee, 2020). Gram-negative bacteria viz. *E. coli* was reported to be inhibited by AMP cecropin from maggot *H. illucens* and lysozyme (Park and Yoe, 2017). Papain hydrolysis on chicken claws protein presumably generates small peptides which can reduce total bacteria in the chicken intestines.

A decrease in total intestinal bacteria increased the DMD and OMD of feed (Table 2). The enhancement level of chicken claws peptides induced the value of DMD from 76.9 ± 1.99 (T0) to 82 ± 1.73 (T3) and OMD 77.3 ± 2.03 (T0) to 81.6 ± 1.45 (T3) (OMD). Karimzadeh et al. (2016) informed that the addition of Canola peptides as much as 200 and 250 mg/kg broiler feed improved villi length the duodenal and ileum and the activity of hydrolytic enzymes i.e., amylase, protease and lipase as well as DMD and OMD. The DMD value increased from 70.64% (control) to 74.60% and the OMD from 71.5% (control) to 74.0%. According to Zhao et al. (2022), small peptides supplementation increases intestinal function through stimulation of intestinal development, integrity, barrier function and the diversity of intestinal microbiota in growing hens. In this study, chicken claw peptides presumably caused an increase in digestive enzyme activity, its boost the DMD and OMD values of feed and nutrient absorption. Improving the feed digestibility was proven by the higher carcass weight.

CONCLUSION

Performance, carcass weight, dry matter and organic matter digestibility and total intestinal bacteria of broiler chickens improved by biopeptides from chicken claw up to the level of 6%. The next research is to study the optimal level of bioactive peptides supplementation derived from chicken feet in broiler chicken or laying hen feed.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

SR came up with the idea, created the basic outline, and wrote the manuscript. SR, BH, and TW assisted with experimental coordination and data collecting, while EAR performed statistical analyses. TRP and MB wrote and proofread the manuscript, while FMS evaluated, critiqued, and edited it based on its intellectual content. The final manuscript was read and approved by all authors.

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Competing interests

The authors declare no competing interests in this research and publication.

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