Histomorphometrics and Small Intestinal Organ Weights in Native Chickens Fed with Maggots (*Hermetia illucens* L) at Various Levels in the Ration

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ABSTRACT

The development of the digestive tract organs, especially the intestines in utilizing nutrients correlates with body growth. This research aims to decide the effect of maggot flour on the histomorphometric and organ weight of the small intestine in eight-week-old native chickens. This study used a completely randomized design (CRD) with five treatments and four replications, namely P0 = 0% (0% maggot flour), P1 = 25% (3.74% maggot flour), P2 = 50% (7.51% maggot flour), P3 = 75% (11.26% maggot flour), P4 = 100% (15% maggot flour). On the eight weeks the birds were slaughtered, then the duodenum, jejunum, ileum were taken and weighed, then processed for making histological preparations using the paraffinization method and staining with hematoxylin-eosin (HE). The results obtained indicated that the provision of maggot flour has a significant impact ($P<0.05$) on the histomorphometric of apical width, basal width, villi height, the distance between the villi and organ weight in the three segments of the small intestine. In conclusion, the use of 11.26% maggot meal in the diet increased the apical width, basal width, villi height, the distance between the villi and the weight of the small intestine organs in native chickens.

Keywords: *native chicken, maggot meal, fish meal, histomorphometric, small intestine*

1. INTRODUCTION

The poultry industry will continue to grow to meet people's animal protein needs, along with increasing incomes, population growth, and public awareness of the nutritional value and health of chicken meat. In rural communities, free-range chickens have an important role in providing meat and eggs which are quite high. Eggs and chicken meat are sources of animal protein that are needed by the body and play a role in improving public health. However, the development of Indonesian local poultry, especially native chickens, is still very slow compared to purebred chickens. This condition is a challenge for farmers and animal feed entrepreneurs, so one alternative is to use local feed that can support the productivity of native chickens.

One of the factors that determine the quality of meat is feed, with sufficient quality and quantity. The current phenomenon in the livestock industry is the increasing price of protein source feed ingredients, including fish meal and soybean meal [1]. Prohibition of the use of antibiotic growth promoters (AGP), mainly against the background of the dangers of antibiotic residues in food products of animal origin and their potential to cause antibiotic resistance in both their livestock and consumers. This phenomenon is a challenge to find natural antibacterial sources that are safe for the health of livestock and the community as consumers, such as the use of environmentally friendly maggots (*Hermetia illucens*).

This maggot is an insect organism that plays an important role in nature, especially as a decomposer of various organic wastes [2]. Several research results reported that substrate bioconversion using organic waste by maggot as a growth medium showed that maggot body biomass contained about 42% protein, 35% fat and 8.1% minerals [3]; [4]; [5]. There is potential for maggot (*Hermetia illucens*) which is rich in protein, so maggot is an ideal raw material that can be used as animal feed. The nutritional content of maggot (*Hermetia illucens*) in the form of the crude protein has a fairly high percentage, as well as amino acids, minerals and is also *Antimicrobial peptide* (AMP) which functions to inhibit pathogenic microbial activity through membrane transport systems and intercellular activity, so that maggot is an ingredient ideal raw material that can be used as animal feed and this indicates that maggot (*Hermetia illucens*) has potential to replace fish meal and MBM.

The digestive tract of native chickens is an organ that has a function to digest and absorb feed as well as an immunological function. One indicator of the intestines can work optimally in absorbing nutrients when in good health. Intestinal health is influenced by the population of microbes that live in it. The antimicrobial properties of BSF larvae can reduce the number of pathogenic bacteria, including *Escherichia coli* found in quail intestines. *E. coli* can become pathogenic if present in the gastrointestinal tract in amounts that exceed the maximum limit and cause health problems in the digestive tract [6]. The development of the weight and length of the digestive tract, as well as the optimal growth of intestinal villi reflect a healthy digestive tract of chickens so that nutrient absorption is more optimal. Good absorption of nutrients from feed will help increase chicken growth [7]; [8]. Therefore, it is necessary to study in this study the effect of using maggot flour on histomorphometrics and organ weights of the digestive tract of native chickens.

2. MATERIALS AND METHODS

2.1. Experimental design, Native chicken, and Feeding

The study was carried out for eight weeks in the poultry production laboratory, Faculty of Animal Science, Hasanuddin University and the Maros Center for Veterinary Medicine. DOC native chickens with a

Table 1. Composition of feed and nutrient content of native chickens

Feed ingredients	Experimental diets				
	P0	P1	P2	P3	P4
Corn (Kg)	64	64	64	64	64
Fine bran (Kg)	12.5	12.5	12.5	12.5	12.5
Soybean meal (Kg)	1	1	1	1	1
Fish meal (Kg)	15	11.26	7.51	3.74	0
Maggot flour (Kg)	0	3.74	7.51	11.26	15
Coconut cake meal (Kg)	2.5	2.5	2.5	2.5	2.5
Vegetable oil (Kg)	2	2	2	2	2
CaCO3 (Kg	2	2	2	2	2
Premix (Kg)	1	1	1	1	1
Total	100	100	100	100	100
Nutrient composition *)					
Protein (%)	17.35	17.33	17.31	17.28	17.26
Fat (%)	2.32	2.68	3.16	3.64	4.13
Crude fiber (%)	6.22	6.32	6.65	7.18	7.31
Ca (%)	0.98	0.96	0.95	0.93	0.92
P (%)	0.92	0.82	0.72	0.61	0.51
Ash (%)	7.37	6.86	6.33	5.91	5.5
BETN (%)	63.74	62.81	62.65	61.89	61.80
ME** (Kkal/kg)	3,084.27	3,092.56	3,118.85	3,156.41	3,183.79

*) Calculated based on the results of the analysis and calculation of the proximate analysis table data at the Laboratory of Nutrition and Animal Feed Chemistry, Faculty of Animal Science, Hasanuddin University.

total of 140 birds were placed in colony-shaped cages. The trial design used was a completely randomized design (CRD) with five treatments and four replications, with seven birds each. Feed formulations containing maggot flour replace fish meal at grade levels of P0 = 0% (0% maggot flour), P1 = 25% (3.74% maggot flour), P2 = 50% (7.51% maggot flour), P3 = 75% (11.26% maggot flour), P4 = 100% (15% maggot flour). The feed given in this study was a starter phase in the form of commercial feed BR-1 for 14 days then continued with treatment feed until harvesting age. The rations were given for eight weeks every morning and evening, and drinking water is provided *ad libitum*. The experimental rations were formulated using feed ingredients obtained mostly from the company around Makassar except for maggot flour (*Hermetia illucens*. L), which was purchased from the maggot Cultivation, Depok, West Java, Indonesia.

2.2. Small Intestine Weight Measurement

The research parameter measured was the weight of the three segments of the small intestine by slaughtering chickens, cleaning the feathers, then processing the carcass. The small intestine organs were removed and separated from other digestive tract organs, then it was weighed using a digital scale with an accuracy of 0.01 g.

2.3. Preparation and Measurement of Small Intestine's Histomorphometric

Small intestine sample preparation was carried out on 8 weeks old chickens for histomorphometric observations and measurements. Sample preparation began with the slaughter of 1 chicken per treatment unit. After slaughtering, the feathers were plucked, followed by separating the small intestine from other organs. The contents of the intestine then were removed and cleaned. Each segment of the small intestine was cut and taken about 5 cm in the middle, then put into a sample bottle containing 10% buffered neutral formalin (BNF) for 24-48 hours, then was dehydrated. Dehydration was carried out by soaking the preparations for two consecutive

3. RESULTS AND DISCUSSION

3.1 Histomorphometric of the villi duodenum, jejenum and ileum

The results of the statistical analysis of the apical width of the third villi of the small gut segments of native chickens elderly eight weeks showed a significant effect (P<0.05) between the control and treatment P1, P2, P3 and P4. The apical width of the duodenal villi at P3 (11.26% maggot flour) was 63.24 µm wider than the treatment P0 (0% maggot meal), P1 (3.74% maggot flour, P2 (7.51% maggot flour) and P4 (15% maggot flour). The results of Duncan's test showed that the control treatment was different from P1 in the duodenum, jejunum and ileum, and P3 did not differ from P1, P2 and P4 in the duodenum, while P3 did not differ from P4 in the jejunum and ileum. The width of the apical villi of the jejunum and ileum were the largest at treatment P3 (57.33 µm and 55.73 µm) and the smallest at P0 (26.82 µm and 24.76 µm). The apical width villi of the duodenum are relatively large compared to the width of the apical villi of the jejunum and ileum. This indicates that the use level of up to 75% maggot meal in the ration has a positive effect on the apical width of the intestinal villi. The width of the apical villi in the intestine affects the level of nutrient absorption. The larger the surface area of the villi, the more efficient the absorption of nutrients that occurs [11]. The elements that affect the efficiency of nutrient absorption are hormonal work, nerves and digestive glands within the digestive tract and accent glands. Segments of the small gut have the ability to digest and soak up nutrients that change and are prompted by the surface place of the intestinal epithelium, the range of folds and the wide variety of villi and microvilli that extend the absorption vicinity [12]; [13]. In addition, the height and surface area of the villi affect the three segments of the small intestine [14].

The outcomes of statistical evaluation confirmed that the treatment had a significant effect (P<0.05) at the width of the basal villi of the three segments of the small intestine at eight weeks of age. Histomorphometrics of the three segments of the small intestine is presented in Table 2. The mean basal width of the largest villi in the duodenum was found at P4 (103.99 µm) compared to P0, P1, P2 and P4. The average width of the basal villi in the jejunum and ileum was greatest in the P3 treatment (11.26% maggot flour) which ranged from 79.17 µm to 71.48 µm, while the control treatment showed the smallest mean villi height at 26.82 µm. In this study, the width of the basal villi of the duodenum was longer than that of the jejunum and ileum. This is in line with research which states that the number of intestinal villi in the duodenum are more numerous than the villi found in the jejunum and ileum [15]. On the other hand, the shortened villi of the small intestine are associated with decreased absorption of nutrients, secretion of intestinal glands and decreased performance [16]. In addition, maggot flour has an active compound within the form of Antimicrobial peptide (AMP) which has a tremendously right defensive effect in retaining the morphometrics of chicken intestine villi. The results correspond to other studies which state that maggot flour performs a lively position in inhibiting pathogenic microbes, growing the balance of the intestines and useful microorganisms [17].

The research data are presented in table 2. Statistical test data analysis of variance shows that the treatment had a significant effect (P<0.05) on the height of the villi of the duodenum, jejunum and ileum at 8 weeks of

Table 2. Average histomorphometric and organ weight of the duodenum, jejunum and ileum in native chickens aged 8 weeks

Parameter	Experimental diets					P-Value
	P0	P1	P2	P3	P4	
The apical width of the villi (μm)						
Duodenum	40.39±4.62^{a}	57.61±8.60^{b}	57.03± 3.39^{b}	63.29± 6.25^{b}	63.22± 10.23^{b}	0.00
Jejenum	26.82 ±3.42^{a}	46.55 ±1.39 ^{b}	51.51 ± 3.63^{bc}	57.33 ± 7.96^{c}	56.48 ± 4.98^{c}	0.00
Ileum	24.76 ±3.08 ^{a}	44.05 ±1.08^{b}	45.84± 3.94^{b}	55.73± 3.99^{c}	55.33± 2.84^{c}	0.00
The basal width of the villi (μm)						
Duodenum	65.80 ± 17.20^{a}	67.99 ± 15.20^{ab}	89.43 ± 30.57^{abc}	98.74 ± 10.52^{bc}	103.99 ± 19.95^{c}	0.04
Jejenum	41.67 ±7.08^{a}	53.09 ± 3.26^{b}	70.97 ± 8.76^{c}	79.17 ± 5.68^{c}	76.63 ± 4.07^{c}	0.00
Ileum	37.48 ± 4.19^{a}	63.19 ± 3.17^{b}	66.56 ± 3.24^{bc}	71.48 ± 1.99^{c}	68.22 ± 4.09^{bc}	0.00
Villi height (μm)						
Duodenum	172.71 ± 5.18^{a}	176.42 ± 6.41^{a}	242.31 ± 15.68^{b}	269.68 ± 6.29^{c}	246.89 ± 6.02^{b}	0.00
Jejenum	145.01 ± 44.03^{a}	208.44 ± 56.85^{b}	219.84 ± 7.77^{b}	253.95 ± 8.89^{b}	236.30 ± 18.31^{b}	0.00
Ileum	139.42 ± 43.61^{a}	202.76 ± 13.18^{b}	221.17 ± 8.13^{bc}	247.65 ± 13.83^{c}	217.39 ± 34.28^{bc}	0.00
The distance between the villi (μm)						
Duodenum	7.00 ±0.82^{a}	8.25 ±1.71^{ab}	10.00 ± 1.41^{bc}	11.75 ± 1.71^{c}	10.75 ± 1.50^{c}	0.00
Jejenum	7.75 ±0.96^{a}	9.50 ±1.29^{b}	10.75 ± 0.96^{bc}	11.50 ± 0.56^{cd}	12.50 ± 1.00^{d}	0.00
Ileum	8.00 ±0.82^{a}	9.75 ±1.50^{ab}	11.00 ± 1.15^{bc}	11.75 ± 1.71^{bc}	12.75 ± 0.96^{c}	0.00
Organ weight (g)						
Duodenum	3.82 ±0.53^{a}	5.40 ±0.60^{b}	5.78 ± 0.86^{b}	5.47 ± 0.27^{b}	5.25 ± 0.76^{b}	0.01
Jejenum	6.45 ±1.55^{a}	8.10 ±1.46^{ab}	8.75 ± 1.07^{b}	8.82 ± 1.78^{b}	8.57 ± 0.91^{ab}	0,00
Ileum	4.50 ±1.01^{a}	6.25 ±0.48^{b}	5.57 ± 0.57^{ab}	7.10 ± 0.91^{c}	5.45 ± 1.75^{ab}	0,00

Different superscripts in the same line showed significant differences (P<0.05), P0 = 0% (0% maggot flour), P1 = 25% (3.74% maggot flour), P2 = 50% (7.51% maggot flour), P3 = 75% (11.26% maggot flour), P4 = 100% (15% maggot flour)

age. The mean height of the largest villi in the duodenum was found at P3 (269.68 μm) and was higher than P0, P1, P2 and P4. The highest mean villi height of the jejunum and ileum in treatment P3 (11.26% maggot flour) were 253.95 μm and 247.65 μm, while the control treatment had the smallest mean villi height at 145.01 μm. in the jejunum and ileum of 139.42 μm. The number of intestinal villi in the duodenum is longer than the villi found in the ileum [15]. The height of the villi in all three segments of the small intestine increases with the increasing use of maggot flour levels. This shows that the use of maggot flour given to chickens up to 75% (11.26% in ration) gives a positive response to the height of the small intestinal villi. The characteristic of the villi of the small intestine will grow with the growth inside the length of the villi of the jejunum and ileum [18]. Primarily based on this examine, P3 remedy with the use of 11.26% maggot flour in the ration may have a higher nutrient absorption capability than other remedies. The height of the villi within the small intestine describes a place for wider absorption of nutrients in order that the transportation all through the body is better and smoother. This is consistent with the opinion [19] that an increase in villi height is indicated by an growth in the surface area of the villi for absorption of nutrients into the bloodstream.

The research data are presented in table 2. Duncan's further test results showed significant effect (P<0.05) between P3 and P4 in the duodenum. The distance between the duodenal villi was largest in treatment P3 (11.75 μm) and the smallest at P0 (7.00 μm). The distance between the jejunal villi and the ileum was largest at P4 (1.50 μm and 12.75 μm) and the smallest at P0 (7.75 μm and 8.00 μm). The results of this study indicate that the smallest distance between the villi is in the duodenum and the largest in the ileum. Increasing the level of use of maggot flour at P3 and P4 then the distance between the villi is also getting bigger. This shows that the *Antimicrobial peptide* (AMP) contained in maggot flour work synergistically to increase the growth of beneficial bacteria such as lactic acid bacteria (LAB) and reduce pathogenic bacteria. Lactic acid bacteria (LAB) colonize the jejunum and ileum which give a positive response to the development of villi. The antibacterial substances contained in maggot flour are *Antimicrobial peptide* (AMP) and lauric acid which work actively to inhibit harmful bacteria, improve the balance of the intestine and beneficial microorganisms [20]. The density and size of the small intestinal villi are positively correlated with the rate of change in absorption on the surface area of the villi [21].

3.2 Organ Weight of the duodenum, jejenum and ileum

Statistical data in Table 2 shows that the feed treatment had a significant effect ($P<0.05$) on the weight of the three segments of the small intestine. The weight of the duodenum was the largest in treatment P3 (5.47 g) and the smallest at P0 (3.82 g). The weights of the jejunum and ileum were highest at P4 (8.82 g and 7.10 g) and the smallest at P0 (6.45 g and 4.50 g). The results showed that the weights of the three segments of the small intestine were still within normal limits and in accordance with the statement [22] stated that the weight of the duodenum is 4.03 g, jejunum 6.4 g and ileum 4.5 g. The consequences of this take look at suggesting that the use of higher concentrations of maggot flour will be followed by an increase in the weight of the small intestine. This is probably because the development of the small intestine of poultry is strongly influenced by the crude fiber content in the rations consumed by native chickens. Feed treatment is thought to cause the digesta rate to be slow because the high crude fiber in the feed requires more intensive digestion of feed. Accelerated digestibility can be caused by an increase in the capacity of the digestive organs and the presence of enzymes that hydrolysed meals materials longer in order that nutrient absorption is more effective [23].

4. CONCLUSION

Replacement of fish meal up to 75% with maggot flour in the ration has the potential to result in better development of apical width, basal width, villi height, the distance between villi and weight of the duodenum, jejunum and ileum organ compared to other treatments. Therefore maggot flour can be recommended as an alternative antibacterial in native chicken rations.

AUTHORS' CONTRIBUTIONS

The experimental study designed by Fuji Astuty Auza in consultation with Ali Bain, Yamin Yaddi, Hamdan Has, Purnaning Dhian Isnaeni and Syamsuddin supervised the experimental study, collection data, and analysis. The manuscript was written and composed by Fuji Astuty Auza. All authors read, reviewed and approved the final manuscript for submission and publication.

ACKNOWLEDGMENTS

The authors would like to convey honestly to the Laboratory of Feed Chemistry, Hasanuddin University, Poultry Production Laboratory, Hasanuddin University and the Maros Center for Veterinary Medicine. They equip the laboratory and research center during the experiment.

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