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Growth Performance, Histological Structure of Duodenum and Pectoralis Muscle of Kampung Chickens After Administration of Antibiotic Growth Promoter

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ABSTRACT

This study aimed to know the effect of AGP on growth performance, duodenal histological structure and pectoralis muscle of Kampung chicken. This research was conducted by designing 3 groups with each group consisting of 20-day old chicks (DOC) of Kampung chickens. The control group was given basal feed without AGP bacitracin, group 2 was treated with 0.125 g of bacitracin /kg of basal feed and group 3 was treated with 0.25 g bacitracin/kg of basal feed until 12 days old. The parameters observed were chicken body weight on days posthatch, 3, 5, 7, 9, and 12, morphometry, visceral organ weight, duodenal organ morphology and muscle performance of Pectoralis thoracicus at 12 days old. Data analysis used one way ANOVA test followed by Tukey test with significance of $P \leq 0,05$. The result showed that the morphology of the doudenum and the myofiber area of group 3 indicated significant differences compared to the control group. The conclusion of this study revealed that administration of AGP bacitracin with 0.25 g/ kg dose of basal feed may increase the growth performance of Kampung chicken.

Keywords : Bacitracin, Duodenum, Kampung chickens, Pectoralis thoracicus

Introduction

Kampung chicken is a native chicken originating from Indonesia which becomes most demanded source of food derived from animals (Daghir, 1995). The meat of *Kampung chicken* is favored by Indonesians as it is delicious and tasty (Suryo *et al*., 2012). Unfortunately, the population of *kampung* chickens is getting decreased compared to other chicken breeds such as broiler and layer, and its growth performance is getting slower compared to broiler chickens. Such slow growth performance of *kampung* chickens can be due to heredity and poor feeding management (Suryana and Hasbianto, 2008).

The needs for national chicken feed increase every year along with the rising demand of chicken meat. In chicken feed, other than ingredients containing protein and carbohydrates, there is also a small amount of additives added to the ration. The ingredients serve to optimize the absorption of nutrients, give color, and give distinctive smell. The examples are antibiotics, hormones, fragrances and dyes.

Antibiotic growth promoter (AGP) has been widely used to improve efficiency in feed absorption (Miles *et al*., 2006). Antibacterials are given to the chickens to control diseases such as necrotic enteritis caused by *Clostridium perfringens* and to promote faster growth as well as to increase feed conversion rate (Han *et al*., 2016). The microbial population in the gastrointestinal tract is capable of metabolic activity, and has advantages and disadvantages in host animals. The negative effect of intestinal microflora is to decrease the absorption of nutrients by competing with its host to obtain energy and protein source (Pan and Yu, 2014). One of the types of AGP is bacitracin, which is a cyclic branched-up *decapeptide* compound which serves to disrupt bacterial cell membranes, suppress cell wall formation by preventing *peptidoglycan* formation, and inhibit protein synthesis (Costa *et al*., 2017).

The use of Bacitracin as an AGP in Indonesia for animal feed such as chicken and ruminant livestock is still permitted with the applicable terms and conditions in accordance with SNI Number: 01-6366-2000 (SNI, 2000). According to Andy *et al.* (2016), the timing of stopping the AGP Zinc Bacitracin provision in broiler chickens can reduce the risk of antibiotic residue in post-harvest chicken meat so it remains safe for consumption.

A research conducted by Miles *et al*. (2006) reported that *bacitracin* or *virgianiamicyn*

AGP provision can improve the performance of broiler chickens. However, until this present time no publication has reported the effect of AGP provision on the growth and histologic profile of the small intestine and muscle in *kampung* chicken. This study aims to see a positive response of adding bacitracin to the basal feed on the growth and histology structure of the intestinal and chicken muscle.

Materials and Methods

60-day-old chicks (DOC) of *kampung* chicken are caged in the UGM's Agricultural Education, Research and Development Garden (*Kebun Pendidikan, Penelitian dan Pengembangan Pertanian/KP4)*. The chickens were given a vaccination program at the age of 4 days and given basal feed (Table 1) and water.

The chickens were divided into 3 groups and each group consisted of 20 DOCs. Group 1, as a control, was given basal feed without adding antibiotic growth promoter (AGP) type *Bacitracin methylene disalicylate* (MD) 15%. Group 2 was given basal diet plus AGP type Bacitracin 0.125 g/kg of basal feed. Group 3 was given basal feed and AGP type Bacitracin 0.25 g/kg of basal feed. *Bacitracin* powder was mixed with basal feed by *on top*.

Measurement of growth performance

The measurement including weight was conducted when the chickens were at the age of posthatch, 3, 5, 7, 9 and 12 days and the morphometry of chicken phenotype character was measured at the age of 12 days.

Measurement of duodenal morphology

A total of 5 chickens from each treatment group were euthanized at the age of 12 days for measuring the duodenal morphology, pectoralis muscle performance and visceral organ weight. Furthermore, the chickens were dissected and their duodenum were taken then fixated into *Bouin* solution for 12 hours. The organs were dehydrated by a multilevel alcohol, followed by infiltration and embedding processes. The next process was done by sectioning and then the *Hematoxylin-Eosin* staining. Duodenal specimen were observed under an optical microscope and looked for its *villus* heigth and *crypt* depth (Figure 1). The documentation of observations was made by using application and AmScope MU1400-CK 14MP USB 2.0 microscope digital camera with 10x10 magnification. Then the height of the villus and the depth of the crypt were measured by using the Miconos Image Raster 3 application (Fasina *et al*., 2010). The ratio of V/C was also obtained by dividing the value between the height of the *villus* and the depth of the *crypt*.

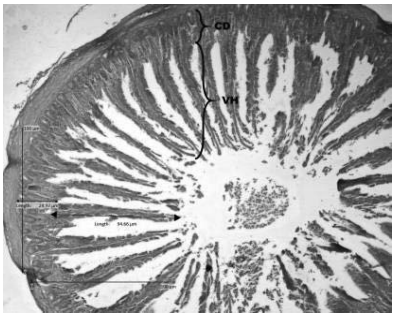

Figure 1. Dissection of duodenal tissue in chicken. VH: Height of villus, CD: The depth of the crypt.

Table 1. Nutrient content and specification in each treatment group of antibiotic growth promoter (AGP) at basal feed in *kampung* chicken of starter period (1-14 days)

Nutrient Type	Nutrient Content		
	Control	Treatment I	Treatment II
Corn (%)	63.53	63.53	63.53
Soybean meal (%)	28.38	28.38	28.38
Mbm (%)	4.70	4.70	4.70
Cpo (%)	0.95	0.95	0.95
Dcp (%)	0.67	0.67	0.67
premix mensa(%)	0.50	0.50	0.50
Steam bonemeal (%)	0.45	0.45	0.45
D,L-methionine (%)	0.23	0.23	0.23
NaCl (%)	0.22	0.22	0.22
$CaCO_3$ (%)	0.18	0.18	0.18
L-lysine HC (%)l	0.10	0.10	0.10
L-Threonine (%)	0.08	0.08	0.08
Fintox (%)	0.01	0.01	0.01
Bacitracin (Gram/Kg)	0	0,125	0,250
	Composition Calculation		
ME (kcal/kg)	3.447.176	3.447.176	3.447.176
Crude protein (%)	24.127	24.127	24.127
Crude fiber (%)	1.57	1.57	1.57
Lysin (%)	1.392	1.392	1.392
Methionine + Systein (%)	1.186	1.186	1.186
Trytophan (%)	0.289	0.289	0.289
Calsium (%)	1.034	1.034	1.034
Sodium (%)	0.154	0.154	0.154

Measurement of Pectoralis thoracicus muscle performance

Data retrieval for Pectoralis thoracicus (PT) muscle started from the left-sided PT muscle weighted to determine its weight, while the right side was used to measure the area of PT with Miconos Image Raster 3. Miofiber area measurement used the J 1.51k Java (64-bit) image application on Windows system. Pectoralis muscle specimen with Hematoxylin-Eosin staining were previously performed by cutting the PT muscle in the right side by 4x4 cm (Puspita *et al*., 2017).

Measurement of visceral organs

Weighing was undertaken to visceral organs, including intestines, heart, proventrikulus, ventriculus, liver, spleen and bursa fabricius. The visceral organs were weighed by using digital micro scales.

Data analysis

Data on growth performance, visceral organ weight, duodenal morphology, pectoralis muscle performance were obtained by 5 times repetition and were analyzed by using a variance analysis of one way ANOVA, followed by Tukey-test using SPSS version 13.0.

Result and Discussion

Performance growth

The effect of *Bacitracin* on the body weight growth and *kampung* chicken phenotype morphometry is presented in Table 2 and 3. The results showed that the growth of body weight for 12 day-old chicken did not show a significant difference but there was a tendency of increased body weight of 0.25 g/kg Bacitracin feed group compared to the control group. Meanwhile, the morphometry of phenotype between groups showed no significant difference (P≤0,05).

Duodenal morphology

The effect of AGP Bacitracin on the small intestine morphology of the chicken duodenum is presented in Table 4 and 6. Based on the research data, it is known that in chicken at the age of 12 days, the length of intestine of the 0.25 g/kg Bacitracin feed group tended to be longer than the control group and 0.125 g/kg Bacitracin feed group. The result for *villus* height (V) and *crypt* depth (C) of 0.125 g/kg Bacitracin feed group and 0.25 g/kg Bacitracin feed group showed a significant increase to the control group. Meanwhile, the V/C ratio showed that there was a significant difference between the 0.25 g/kg Bacitracin feed group and the control group. Wu *et al*. (2004) reported that the V/C ratio was associated with increased nutrient absorption and increased resistance to pain.

Performance of muscle *Pectoralis thoracicus*

The effect of giving Bacitracin on the histologic structure of *kampung* chicken pectoralis muscle is presented in Table 5. The results showed that when chickens were 12 days old, the increase in muscle weight and muscle area for the

Table 2. Average weight (gr) in each treatment group of antibiotic growth promoter (AGP) at basal feed in *kampung* chicken at the age of 0, 3, 5, 7, 9 and 12 days

Day	K	P1	P2
posthatch	24.5±2.6^{ns}	25±4.8 ^{ns}	25.3±2.7^{ns}
3	33.33±6.02^{ns}	27.16±3.19^{ns}	33.8±3.35^{ns}
5	39±7.67^{ns}	32.6±1.95^{ns}	40±6.44^{ns}
7	47.5±9.85^{ns}	39.6±2.3^{ns}	47.8±9.58^{ns}
9	5.7±9.67^{ns}	48.25±2.36^{ns}	61±10^{ns}
12	74.25±4.79^{ns}	62.5±3.87^{ns}	77±13.49^{ns}

K: control group with basal feed; P1: basal feed treatment + concentration of 1 antibiotic growth promoter (AGP); P2: basal feed treatment + concentration of 2 antibiotic growth promoter (AGP).
^{ns}Not significant.

Table 3. Average phenotype morphometry in each treatment group of antibiotic growth promoter (AGP) at basal feed in *kampung* chicken at the age of 12 days

Variabel	Kontrol	P1	P2
Total height (cm)	13.05±1^{ns}	12.2±0.45^{ns}	12.5±1.62^{ns}
Body height (cm)	10.75±1.47^{ns}	9.3±1.3^{ns}	9.8±1.52^{ns}
Beak width (cm)	1.25±0.48^{ns}	1.1±0.33^{ns}	1.32±0.29^{ns}
Beak length (cm)	2.17±0.26^{ns}	1.66±0.48^{ns}	1.76±0.18^{ns}
Head length (cm)	3.7±0.56^{ns}	3.5±0.88^{ns}	3.66±0.95^{ns}
Head width (cm)	2.2±0.3^{ns}	2.16±0.15^{ns}	2.1±0.22^{ns}
Body length (cm)	7.83±1.29^{ns}	7.32±1.35^{ns}	8.2±1.95^{ns}
Body width (cm)	5.33±0.75^{ns}	4.84±0.85^{ns}	4.86±0.79^{ns}
Chest circumference (cm)	9.92±0.74^{ns}	7.5±0.71^{ns}	8.46±1.02^{ns}
Wing length (cm)	10.42±1.07^{ns}	9.36±0.91^{ns}	10.2±1.36^{ns}
Neck length (cm)	3.4±0.86^{ns}	2.8±0.47^{ns}	2.66±0.42^{ns}
Calves length (cm)	2.58±0.35^{ns}	2.08±0.13^{ns}	2.5±0.35^{ns}
Thigh lenght (cm)	4.82±0.74^{ns}	4.3±0.83^{ns}	4.94±0.44^{ns}

K: control group with basal feed; P1: basal feed treatment + concentration of 1 antibiotic growth promoter (AGP); P2: basal feed treatment + concentration of 2 antibiotic growth promoter (AGP).
^{ns}Not signifant.

Table 4. Average *villus* heigth duodenum (µm), *crypt* depth (µm) and *villus* heigth / *crypt* depth duodenum ratios in each treatment group of antibiotic growth promoter (AGP) at basal feed in *kampung* chicken at the age of 12 days

Variables	K	P1	P2
villus height	82.95±10.12[a]	97.65±12.2[b]	96.44±6.12[b]
crypt depth	20.2±2.3[a]	28.6±3.4[b]	29.38±2.9[b]
ratio V/C	3.7±0.8[a]	2.8±0.5[a]	2.7±0.4[b]

K: control group with basal feed; P1: basal feed treatment + concentration of 1 antibiotic growth promoter (AGP); P2: basal feed treatment + concentration of 2 antibiotic growth promoter (AGP).
[a,b] Different superscripts on the same rows show a noticeable difference (P≤0,05).

Table 5. Average weight of *pectoralis thoracicus* muscle (gr), muscle area (mm2) and myofiber area (µm2) in each treatment group of antibiotic growth promoter (AGP) at basal feed in Kampung chicken at the age 12 days

Variables	K	P1	P2
Muscle weight	3.01±0.35[ns]	2.74±0.23[ns]	3.56±1.23[ns]
Muscle width	9.61±0.51[ns]	8.31±1.1[ns]	10.11±2.3[ns]
Myofiber width	1.89±0.16[a]	1.94±0.24[b]	1.99±0.12[b]

K: control group with basal feed; P1: basal feed treatment + concentration of 1 antibiotic growth promoter (AGP); P2: basal feed treatment + concentration of 2 antibiotic growth promoter (AGP).
[a,b] Different superscripts on the same rows show a noticeable difference (P≤0.05).
[ns] not significant.

0.25 g/kg Bacitracin feed group tended to increase compared to the control group and 0.125 g/kg Bacitracin feed group. The width of the myofiber *pectoralis thoracicus* muscle of 0.25 g/kg Bacitracin feed group showed significant improvement (P≤0.05) compared to the control group and 0.125 g/kg Bacitracin feed group.

Measurement of visceral organs

The effect of giving Bacitracin on the visceral weight of *kampung* chicken organs is presented in Table 6. Based on the research data, it was found when chickens were 12 days old, the weight of visceral organ for all groups showed no significant difference (P≤0.05).

Studies on the importance of antibiotic growth promoter (AGP) in chicken breeds such as broiler have been done from various aspects, from growth aspect to morphology of visceral organs. However, the study on the effect of AGP on Indonesian *kampung* chicken, in term of histologic structure of the small intestine and pectoral muscle, has not been widely performed.

The reason for the selecting MD bacitracin in this study is for its ability to inhibit the formation of bacterial cell wall by preventing peptidoglycan formation and to inhibit bacterial protein synthesis (Costa *et al*., 2017). According to Huyghebaert *et al*. (2011) antibiotic growth promoter is known for its capability in inhibiting early microbial proliferation, which is usually a nutritional competitor with intestinal cells so AGP can help the process of bowel maturation run better. The process occurs in chickens at the age of 6-9 days.

Yang *et al*. (2009) reported that adding antibiotics to broiler chicken feed increases body weight, feed intake and feed conversion ratio. Bacitracin is an antibiotic polypeptide produced by Bacillus *licheniformis* and used as a growth promoter (Awais *et al*., 2007). Adding bacitracin in broiler chicken can improve growth performance like weight gaining up to 0.8%, carcass characteristics and reduce the population of intestinal bacteria (Singh *et al*., 2008; Abdulrahim, 2010). Costa *et al*. (2017) reported that adding AGPs such as *bacitracin* MD and *virginiamicyn* creates good effects on growth and is able to improve feed conversion in broiler chicken at all age of raising. The present research using Indonesian *kampung* chickens also showed similar results to previous studies in terms of increased weight growth and intestinal growth.

This study showed that giving *Bacitracin* can improve the morphology of *villi* and *crypt* of duodenal intestines. The use of antibiotics in feed can support the growth of the gastrointestinal tract (Miles *et al*., 2006). It is likely possible due to some factors such as reduced nutrient competition

Table 6. Average weight of the small intestine (gr) and Length of the small intestine (cm), weight of the heart, proventricular, ventricular, liver, spleen and bursa fabricius (gr) in each group of antibiotic growth promoter (AGP) at basal diet in chicken at the age of 12 days

Variables	K	P1	P2
Small intestine weight	4.8±0.95[ns]	4.26±0.9[ns]	4.54±0.47[ns]
Small intestine lenght	54.1±7.4[ns]	52.1±5.63[ns]	59.6±5.3[ns]
Heart	0.78±0.12[ns]	0.66±0.06[ns]	0.83±0.17[ns]
Proventriculus	0.83±0.19[ns]	0.69±0.03[ns]	0.82±0.08[ns]
Ventriculus	6.13±0.86[ns]	4.64±0.27[ns]	6.21±1.09[ns]
Liver	2.64±0.33[ns]	2.22±0.12[ns]	2.68±0.2[ns]
Spleen	0.09±0.009[ns]	0.075±0.0[ns]	0.09±0.03[ns]
Bursa fabricius	0.25±0.02[ns]	0.135±0.05[ns]	0.17±0.05[ns]

K: control group with basal feed; P1: basal feed treatment + concentration of 1 antibiotic growth promoter (AGP); P2: basal feed treatment + concentration of 2 antibiotic growth promoter (AGP).
[ns] not significant.

in the small intestine, reduced local inflammation since it can control pathogens, diminish intestinal thickness and longer bowel size as a result of increased digestion and decreased pathogens (Apajalahti and Vienola, 2016). A study by Markovic *et al*. (2009) indicated that by adding AGP, the *villi* size of broiler chicken's duodenum is longer than the control group with an average of 80 µm, thus increasing the surface area for food absorption. In general, AGP can work well to suppress bacterial populations such as *Campylobacter jejuni* and *Clostridium perfringens* so as to alter the physical form of the gastrointestinal tract. There are several benefits of adding AGP to chicken feed, including among others, increasing the absorption of various nutrients and causing some anticipated changes in gastrointestinal morphology which include decreased cell proliferation, thinner mucosa, reduced lamina propria, and increased absorptive surface area (Gaucher *et al*., 2015). Smirnov *et al*. (2005) reported that giving AGP significantly also affects goblet cell density, but does not affect the thickness of chicken's mucous layer in small intestines.

The AGP mechanism in increasing growth is by suppressing the bacterial population in the gut which can lead to gastrointestinal infections. Reduced levels of gastrointestinal infections may increase muscle mass (Contreras-Castillo *et al*., 2008). This study also gives the same idea that giving Bacitracin as AGP can increase muscle growth with the increase in myofiber area of pectoralis muscle. Visceral organ growth in this study showed that adding AGP is not different from basal feeding.

Conclusion

This study showed that giving Bacitracin antibiotic growth promoter (AGP) in 0.25 g/kg dose of basal diet can improve the growth performance, duodenal morphology and muscle growth of *kampung* chicken.

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