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Research Article

Effect of Mixed Acidifier Supplementation in Feed on Small Intestinal Morphology of Laying Duck

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Abstract: The purpose of this study was to examine effect of mixed acidifier supplementation as feed additive on small intestinal morphology in laying duck. The acidifier used was in 2 forms, namely formic and fumaric acids and mixed at ratio of 1:1. This research method used *in vivo* experiment by using Completely Randomized Design (CRD). One hundred and fifty female ducks aged 50 weeks were randomly distributed into 5 treatments and 5 replications. The treatments consisted of T0-= basal feed/control. T0+= basal feed with addition of Bacitracin antibiotic 0.1%. T1= basal feed + 0.1% mixture of formic and fumaric acids at ratio of 1:1, T2= basal feed + 0.2% mixture of formic and fumaric acid at rasio 1:1, and T3= basal feed + 0.3% mixture of formic and fumaric acids at ratio of 1:1. The variables observed were intestinal morphology of ileum consisting of villus number, villus height, crypt depth, basal width, apical width, and villus surface area. The results showed that there were significant effects (P>0.05) in the villus height and apical width and highly significant effects (P>0.01) in the total villus number. On the other hand, treatments showed no difference effect (P<0.05) on crypt depth, basal width and surface area. In conclusion, the acidifier treatment improves intestinal morphology, especially when formic and fumaric acid mixture were supplemented at 0.1%.

Keywords: antibiotic replacer, formic acid. fumaric acid, intestinal morphology, laying duck

INTRODUCTION

Antibiotic Growth Promoter (AGP) was commonly used to increase the production of poultry, but recently, the Indonesian government prohibited the use of AGP added into feed due to health concern. The concern is when the products, like egg and meat, are consumed by human. Since AGP was banned by the government, the use of natural growth promoter (NGP) one way of avoiding production loss. Excluding AGP has made many farmers suffered because of mortality increased and productivity dropped. Therefore, current researchers are mostly focused on finding the alternative replacer for AGP. Beside findings which have been reported with success, some others could not perform as expected.

Acidifier is commonly known as is one of the AGP replacer that can be added into feed mixture with the purpose of improving performance. The examples of acidifier are like benzoic acid, fumaric acid, formic acid, etc. There are two kind of acidifiers based on its form, which are solid and liquid. Solid form has advantage because of its easiness in application and protected to be able to pass through the intestine. Acidifiers are able to escalate the intestinal mucosa and its morphology of birds, beside organic acid can also improve immunity, reduce pathogen activity and balance the population of bacteria in the intestine. These acids could improve intestinal environmental pH due to production of lactic acid by bacteria and help to inhibit pathogenic microflora to develop. It was reported that *Salmonella* growth can be minimized by lowering pH in gastrointestinal tract due to the use of acidifier (Sterzo, *et al.*, 2007). Acidifier or commonly called as organic acid supplementation have been reported in decreasing the colonization of pathogenic bacteria that produce toxic metabolites, and also improve digestibility of protein and minerals like Ca, P, Mg, and Zn (Adil, *et al*., 2011). In the previous *in vitro* experiment, we found that a mixture of formic and fumaric acids showed a significantly better antimicrobial effect than if either formic or fumaric acid given individually (Ramadhan *et al.*, (2020). This research focused to examine *in vivo* evaluation of intenstinal morphology change in laying duck fed diet supplemented with a mixture of formic and fumaric acid at the same ratio.

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MATERIAL AND METHOD

Time and Location Research

The research started from September 29th, 2019 until November 20th, 2019. *In vivo* experiment was carried out in David Kurniawan Farm, at Doko Sub-District , Kesamben District, Blitar, East Java, Indonesia.

Birds and Dietary Treatments

One hundred and fifty Laying Mojosari duck of 50 weeks old were used. They were allocated to 5 treatments and 5 replications. Each experimental unit used 6 ducks and had an area of 0.4 m2. Each unit was equipped with feeder and drinker and lighting used was 2 ten watts of lamp provided daily between 17.00 p.m. to 5.00 a.m. Feed was given twice a day with daily total feed allowance was restricted as much as 160 g/bird. Water was provided *ad libitum*. The length of experiment was 7 weeks. The respective formula and nutrient content of basal diet were described in the following Table:

TABLE I. Formula of basal feed

Ingredients	Proportion (%)
Yellow corn	48.00
Soy bean meal	21.00
Rice bran	12.20
Meat bone meal	8.00
Soybean oil	2.00
Grit	5.00
DL-methionine	0.20
Mineral premix	2.00
Vitamin premix	0.50
Salt	0.10
Total	100.00

TABLE 2. Calculated Nutrient content of basal feed

Nutrient	Unit
Crude protein (%)	19.34
Metabolizable Energy (kcal/kg)	2,800
Crude Fibre (%)	4.5
Crude Fat (%)	4.0
Calcium	3.0
Phosphor	0.5
Lysine	1.05
Methionine	0.5

The experiment was designed by using Completely Randomized Design (CRD) consisted of 5 treatments with 6 ducks for each replication and repeated 5 times. The treatments given were:

T0-: basal feed

T0+: basal feed with addition of Bacitracin antibiotic 0.1%

T1: basal feed + 0.1% mixture of formic and fumaric acids (1:1)

T2: basal feed + 0.2% mixture of formic and fumaric acids (1:1)

T3: basal feed + 0.3% mixture of formic and fumaric acids (1:1)

The variables observed in this experiment were villus number, villus height, crypt depth, basal width, apical width, and villus surface area.

Procedure of collecting data

One duck for each experimental unit was randomly chosen and slaughtered. After slaughtering, the ileum of 4-5 cm long were cut, the ileal content was gentle removed by employing syringe filled with 0.01% physiological NaCl solution. The empty ileum then soaked in 10% formalin solution, before the ileum samples were then taken to laboratory.

The preparation of ileal samples were made by following the laboratory procedure of Laudadio, *et al.*, (2012). The histomorphometric study were observed with the Olympus BX51 DIC microscope. Ten villi were measured per sample by using Image Raster software. Variables observed included:

1. **Number of villus**
   The number of villus was measured by counting the villus observed per transversal cut under the microscope

2. **Villus height**
   The height of the villus was measured from the basal part of the villus to the apical villus (μm).

3. **Apical width**
   Apical width was measured from the upper side width of villus

4. **Basal width**
   Basal width was measured from the lower side width of villus

5. **Crypt depth**
   Measurement of crypt depth starts from the base (part of the lamina propria) to the base of villus (μm).

6. **Villus surface area**
   Villus surface area was estimated from the trigonometric relationships of villus basal width, villus apical width and villus height

   Villus surface area= $\frac{(b+c)}{cxa}$, where:

   a = villus height
   b = basal width
   c = apical width (Iji, *et al.*, 2001).

Statistical Analysis

Data obtained from this research were analyzed by using one way analysis of variance (ANOVA) then continued with Duncan's Multiple Range Test (DMRT) if significant effect appeared.

RESULT AND DISCUSSIONS

The summarized results on the effect of acidifier as feed additive on intestinal morphology in laying duck are shown in Table 3.

TABLE 3. Intestinal Morphology of laying duck fed with treatments

	T0-	T0+	T1	T2	T3
Villus number**	62.6±4.61^{b}	48.2±2.17^{a}	48.8±5.63^{a}	46.2±3.90^{a}	48.4±3.91^{a}
Villus height (μm)*	492.80±59.68^{a}	601.41±71.59^{b}	675.67±141.14^{c}	605.95±49.27^{b}	608.81±75.80^{b}
Crypt depth (μm)	120.95±15.96	146.26±20.55	143.81±29.81	130.22±25.42	139.03±14.24
Basal width (μm)	97.10±13.36	127.49±21.15	126.71±29.65	106.54 ±23.11	112.17±1.72
Apical width (μm)*	76.51±5.94^{a}	104.55±26.49^{b}	118.98 ±34.13^{c}	99.01±7.96^{b}	105.92±5.97^{b}
Villus surface area (μm^2)	1123.49±173.61	1342.27±67.64	1429.18±399.53	1247.91±41.25	1256.97±175.10

Notes:
*The different superscripts in the same row showed significant differences (p>0.05).
**The different superscripts in the same row showed highly significant differences (p>0.01).

A. Total Villus Number

Based on the results presented in the Table 3 indicated that dietary treatments gave a significant different (p>0.05) effect on villus number of laying duck's ileum. The result showed that the villus number decreased with addition of both antibiotics (T0+) and any levels of mixture of formic and fumaric acids. It seems that the number of villi was badly affected by the use of antibiotic and acidifiers in duck. On the other hand, no changes in the number of villi was reported in ileum of broiler when virginiamycin or bacitracin was added into the feed (Miles, et al., 2006). Different results might partly indicate different sensitivity or response toward application of antibiotic and acidifiers for chicken and duck used in both experiment. Austic, *et al.*, (1990) stated that small intestine ability to digest and absorb feed nutrients were affected by surface area of epithelium, the number of mucosal folds and the number of villi and microvilli. The number of the villi was directly related to the shape, height and surface area of the villus (Maneewan, *et al.*, 2005).

B. Villus Height

Based on the results as presented in the Table 3, it was found that the dietary treatment gave a significant different (p>0.05) effect to the villus height of laying duck's ileum. The results showed that inclusion of antibiotic and any levels of acidifier to the diets increased the villus height, but the highest villus height was obtained for T1. It may indicate that at an ideal level of 0.1% the use of a mixture of acidifier in this experiment could stimulate a better response on villus growth. Garcia, *et al.*, (2007), Hernandez *et al.*, (2006) and Miles *et al.*, (2006) reported that the use of both 5,000 and 10,000 ppm of formic acid in broiler feed also found a significant increase in villus height. Short-chain fatty acids as a kind of organic acid usually used as acidifiers have been demonstrated to stimulate the proliferation of normal crypt cells, enhancing healthy tissue turnover and maintenance (Awad, *et al.*, 2008).

C. Crypt Depth

Based on the result that shown in the Table 3, laying duck fed different dietary treatments did not show significant (p<0.05) effect on crypt depth. The results might suggest that similar response of effect of acidifier on crypt depth between broiler chicken and duck, because similar effect has been reported by Miles *et al.*, (2006) and Gracia, *et al.* (2007). However, Adeniji *et al.* (2015) reported that feeding broiler chicken supplemented with single dose of formic acid improved the crypt depth by 22%. The crypt depth is considered important, because the deeper crypt means faster tissue turn over to permit the renewal of the villus needed in sloughing or inflammation from pathogens or their toxins (Yason, *et al.*, 1987). The main role of crypt is to reserve nutrients, then they will be absorbed by the crypt itself. Crypt can also be called as the base of villi. The thicker the crypt, the quicker villi regeneration mechanism (Laudadio, *et al.*, 2012).

D. Basal and Apical width

Based on the result that shown in the Table 3, laying duck fed experimental diet showed no difference ($p<0.05$) on basal width. On the other hand, the observed apical width showed different results, of which apical width of villi was significantly ($p>0.05$) affected by experimental diet. The tendency showed that dietary treatment groups showed significantly wider apical width of villi, with the widest was reported for duck fed 0.1% acidifier. This result indicated that the experimental diet could improve the villus apical width. Cheng, *et al.*, (1974) stated that the apical surface in gut, is responsible to secrete antimicrobial peptides, mucus and hormones, respectively. Apical surface has a part called cell protuberances on the villus, have been demonstrated to show an activated absorptive function of the villi (Yamauchi, *et al.*, 2005). Maneewan, *et al.*, (2005) also stated that the condition of intestinal villi and epithelial cells on the apical surface of the villi are known as good indicators of the enteral nutrient absorption of feed ingredients in chicken.

E. Villus surface area

Based on the result that shown in the Table 3, laying duck fed experimental diet showed no different ($p>0.05$) effect on the villus surface area. The widest surface area showed by T1 with an average of 1429.18±399.53 μm^2 while the narrowest showed by T0- with an average of 1123.49±173.61 μm^2. Despite it showed no statistical difference, it signaled that acidifier fed to laying duck gives a substantial improvement in villus surface area compared to control group. Hernandez, *et al.*, (2006) stated that response of villus surface area of broiler fed diet supplemented with did not differ from control and avilamycin. Villus surface area greatly depends on villus height, basal width, and apical width respectively. Awad, *et al.*, (2008) stated that the increase of villus height would indicate of an increasing of surface area for greater absorption of available nutrients while deeper crypt depth is implicated in a greater production of enterokinase which is the precursor for the production of trypsin. Trypsin is needed for the digestion of protein which culminates in increased availability of amino acids which is also vital for improvement of bird performance.

CONCLUSION

Based on the results of this study, it can be concluded that the mixture of 2 acidifiers which are formic and fumaric acid at the same proportion can be used as feed additive alternative to antibiotic in laying duck. The optimum level of mixture of acidifier was 0.1% to improve the villus characteristics of the ileum of laying duck.

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