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The effects of *Temu Ireng (Curcuma aeruginosa), Kunyit (Curcuma longa) and Jahe Merah (Zingiber officinale*) on bursa of fabricius and Histo-morphological intestine characteristics of local Indonesian chickens

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Abstract
The objectives of this study were to study the Effects of *temu ireng (Curcuma aeruginosa), kunyit (Curcuma longa) and jahe merah (Zingiber officinale*) on bursa of fabricius and histo-morphological intestine characteristics of local Indonesian chickens. The experimental design was completely randomized design with 440 birds allocated into 11 treatments and 4 replications of each; 10 birds per experimental unit. The data were subjected to ANOVA procedure, and Duncan's multiple range tests were conducted when the difference among the treatments was found. The results showed that the ratio of body/body weight ratio of Indonesian local chickens was significantly different ($P<0.05$) in Period I, but not different in period II. Otherwise, plica of fabricius height was not different in Period I ($P>0.05$), but differed in Period II ($P<0.05$). Plica of fabricius from chickens treated with phytobiotics had higher plica of fabricius ($P<0.05$) than T01 and T02 control treatments. The histo-morphological data analysis of villus height showed no differences ($P<0.05$) in all parts of the intestine among the treatments, except the villus height of T01 which is different ($P<0.01$) from the others. The same pattern occurs in the crypt and the thickness of tunica muscularis of the duodenum, jejunum and illeum. In conclusion, giving red ginger has been able to produce normal growth best in bursa of fabricius. Furthermore, overall, morphological changes have occurred in the small intestine as the effect of phytobiotics. It can be used as an indication that there has been a change in function of mainly associated with the process of absorption of nutrients occured in the intestine.

Keywords: phytobiotics, histo-morphological, bursa of fabricius, *Curcuma aeruginosa*, *Curcuma longa*, *Zingiber officinale*

1. Introduction
Ban the use of synthetic antibiotics as growth promoters in livestock industry increasingly widespread. This condition is based on the increasing number found empirical evidence, especially with the increasing resistance of some types of pathogenic microorganisms and antibiotic residues in livestock due to inappropriate use or poorly controlled (Smith, 2011) [9]. The ban their use started in Sweden in 1986 and Denmark in 1995 that prohibits the use of synthetic antibiotics in livestock industry in total. Subsequently in 2004, the European Union banned the use of the antibiotics as growth promoters in livestock industry in total. In 2010, the ban the use of most types of synthetic antibiotics extends to Asian countries in livestock industry (Smith *et al*, 2011) [9]. Finally, the prohibition in Indonesia on the use of synthetic antibiotics as growth promoters was effective from 2018. The article explains the mixing of veterinary medicines in feed only for therapy according to instructions and under the supervision of a veterinarian. Thus, the purpose of this study was to determine the the effects of *temu ireng (Curcuma aeruginosa), turmeric (Curcuma longa)* and *red ginger (Zingiber officinale*) on the bursa of fabricius characteristics; weight and the ratio the plica against the weight, and the histo-morphology of the intestine; villus height, crypt depth and thickness tunica muscularis. The results could be used hopefully as a basis for producing growing chicken feed that contains a natural antibiotic or phytobiotics as a substitute for antibiotic synthesis in the future.

2. Materials and Methods
Phytobiotics of *temu ireng*, red ginger and turmeric used were dried by the sun, after previously being washed, made thinly sliced and ground with a flour machine. The composition of the basal diet used was structured to meet the needs of energy and protein for growing chickens (NRC, 1994) for each growing period, as shown in Table 1, and phytobiotics were given with the appropriate amount of each treatment (Table 2).
The experimental design used was a completely randomized design. Four hundred forty chickens of one day of age were randomly divided into 11 treatments with 4 replicates so that each experimental unit consisted of 10 chiks. Furthermore, all parameters were tested by analysis of variance using procedure ANOVA (Freund and Littell, 1981), and if there was difference between the parameters analyzed followed by Duncan's multiple range test.

Table 1: Basal diet formulas used in the experiment.

No	Ingredients	Period I (0 – 23 days)	Period II (24 – 34 days)
		%	
1	Corn	50.0	50.0
2	Soybean	15.5	15.5

3	Fish meal	12.5	10.0
4	Rice bran	5.0	8.5
5	Palm cernel cake	5.0	5.0
6	Coconut cake	5.0	3.0
7	Coconut oil	5.0	6.0
8	Bone meal	1.5	1.5
9	Vitamin and Mineral Premix	0.50	0.50
10	Salt	0.4	0.4
	Crude protein (CP), %	22.51	20.18
	Metabolizable Energy (ME), kcal.	3,192.04	3,196.82

Table 2: Treatment feed (basal feed plus phytobiotics).

No	Treatment	
1	$T0_1$	= Basal feed
2	$T0_2$	= 0.05 mg bacitracin/kg basal feed
3	TI_1	= 0.75% *Temu ireng*
4	TI_2	= 1.0% *Temu ireng*
5	TI_3	= 1.5% *Temu ireng*
6	KU_1	= 0.75% *Kunyit*
7	KU_2	= 1.0% *Kunyit*
8	KU_3	= 1.5% *Kunyit*
9	JM_1	= 0.75% *Jahe merah*
10	JM_2	= 1.0% *Jahe merah*
11	JM_3	= 1.5% *Jahe merah*

On day 24 (Period I) and 34 (Period II), samples of bursa of fabricius and a 2-3 cm of the duodenum, jejunum, and ileum were taken for each chicken experimental unit. Samples were washed in physiological saline solution and fixed in 10% buffered formalin solution, then made preparations on a glass slide and given hematoxylin and eosin staining for analysis of histo-morphology (high/length villus and crypt and thickness of tunica muscularis).

3. Results and Discussion

Data analysis for the ratio of bursa of fabricius weight (BF) to body weight (BW) of native Indonesian chickens in Period I and Period II is illustrated in figure 1. The results show that the ratio of BF/BW of the chicken is significantly different (P<0.05) in Period I, but not different (P>0.05) in Period II. The highest average BF/BW ratio for Period I of the chickens was phytobiotic treatment with the addition of 1.0% red ginger (JM2 = 0.366) and the lowest ratio was phytobiotic treatment with the addition of antibiotic bacitacin (T02 = 0.178), and both showed significant difference (P<0.05). The reverse pattern is shown for data on plica fabricius lengths of the chickens in Period I and Period II. The analysis showed that the length of the plica fabricius was significantly different (P<0.05) in Period II, but not different (P>0.05) in Period I. The longest mean of the plica of fabricius of Period II was the control treatment T02 (0.458 μm) and the lowest was KU2 (0.366 μm), and both showed significant difference (P<0.05).

Bursa of fabricius is an organ that plays an important role to support the immune system. Ratcliffe (2006) stated that the lumen of bursa of fabricius is connected to the digestive tract (cloacal tip). It turns out that this channel has significance in relation to the development of B cell function as producing lymphocytes. There is indication that the development of the function of the bursa ofFabricius as reflected by the morphological development depends on the feed or compounds that are present or passing through the digestive tract of animals. The role of bursa of fabricius was very important in poultry because of its function as an organ of the immune system. This organ can also be an indicator of the presence of infectious diseases, especially those caused by viruses, for example infectious bursal disease (IBD). This disease will attack the B lymphocyte producing cells as characterized in inflammation or swelling of the glands of the bursa on the first 4 days, but began atrophy in the next 4 days, and will produce higher mortality rate in the following days (Ogawa *et al*, 1998; Kim *et al*, 1999) [4, 2]. From the study of Wang *et al* (2010) [10] in broiler chickens showed that the ratio of BF/BW was in the range of 0.209 to 0.221 μm. Another study carried out by Shomali *et al* (2011) [8] showed that the histo-morphological parameter of the bursa of fabricius was as an important organ in birds. The results also showed that there was a dose dependence on the addition of the Zataria multiflora herbal plant which has been known as an immunomodulatory agent in poultry on the measured parameters, including plica of the bursa of fabricius.

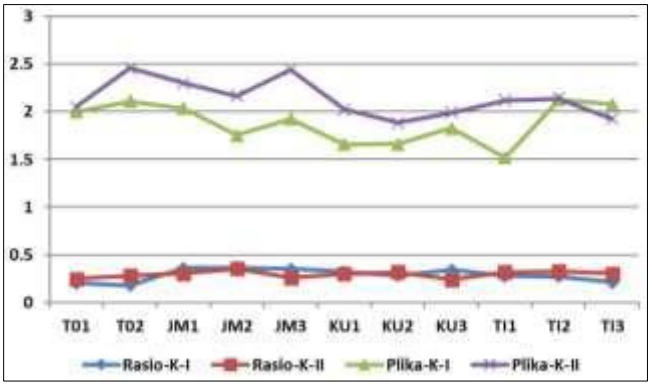

Fig 1: Weight ratio of bursa of fabricius (g x 100) against body weight (g) Period I (ratio-K-I) and Period II (ratio-K-II), and plica of bursa of fabricius (μm) Period I (plica-K-I) and Period II (plica-K-II) of chickens

In the Period I experiment (Table 3), the treatment of all types of phytobiotics (except TI1) showed that villus height in the duodenum were higher (P<0.05) compared with control treatment T01 and T02. The same pattern also occurred at measurement of jejunum and ileum villus high where phytobiotics treatments were applied. The crypts in duodenum, jejunum and ileum of intestine illustrate the thickness of the tunica muscularis, and the results showed that the thickness were different (P<0.05). The crypts in the deodenum and jejunum of the intestine showed that they were deeper (P<0.05) compared to control treatment T01 and T)2, except for the crypt in the illeum. Pattern that occurs in villus height and the crypt depth should illustrate the thickness of the tunica muscularis of the intestine, but it did not occurred in T0 control. For the record, some data could not be written because of damage to preparations which are generally due to lysis.

With the increasing growth and development of the chickens as shown in the second period of study (Table 4), histo-morphological data analysis of villus height showed no differences (P<0.05) in all parts of the intestine among the treatments, except the villus height of T01 which is different (P<0.01) from the others. The same pattern occurs in the crypt and the thickness of tunica muscularis of the duodenum, jejunum and illeum, where treatment giving phytobiotics showed no differences (P>0.05) from the T01 control treatment and T02, except for the tunica muscularis of KU2 which was different (P>0.05) from the others. These results are consistent with the research conducted by Rajput *et al* (2013) who found that adding curcumin to the dose of 200 mg/kg of feed in broiler chickens affected the morphology of the small intestine. The results showed that detailed increased villus height in the duodenum, jejunum and ileum. Thickness tunica muscularis also increased in the duodenum and jejunum. From this overall research indicated that an additional surface area of absorption occured in the intestine resulting in increased absorption of nutrients until the age of 42 days.

Morphological changes in the intestine can be used as an indication of changes in function that are mainly associated with growth, or absorption of nutrients occurring in the intestine. For example, changes in growth are related to the presence of toxins, absorption of nutrients, enzyme secretion, resistance to certain microbes / diseases, and finally changes in intestine morphology can be used as indications of overall performance changes (Xu *et al*, 2003). Research has been conducted to compare the use of natural antibiotics that have been commercialized that was AV/AGP/10 which was a mixture of the *Allium sativum, Zingiber officinale, Trigonella foenum graecum, Eruca sativa* with a natural antibiotic Bacitracin Methylene Dicyticylate and Oregostim which serves as a growth promoter (Kanduri *et al*, 2013) [1]. In this study showed that the use of AV/AGP/10 with a maximum dose of 500 g successfully increased villus height and depth of the crypt in the duodenum and ileum compared with the use of antibiotics bacitracin Methylene Dicyticylate 100 g/ton and Oregostim 250 g/ton of feed. In another study (Saki *et al*, 2012) [6] also showed that the use of medicinal plants as an antibiotic alternatives can improve height, thickness and depth of indentation villus which was also in line with the results of this study**.**

Table 3: Villus, crypt and tunica muscularis of duodenum, jejunum and ileum of the chickens in Period I (24 days).

	Treatment*										
	T01	**T02**	**JM1**	**JM2**	**JM3**	**KU1**	**KU2**	**KU3**	**TI1**	**TI2**	**TI3**
Villus, *µm*											
Duode	684.	727.	-	1733	1835.	1819	1675	1734	749.	1401	1460.
Jejunu	753.	779.	1521	-	-	1666	1609	1706	1120	1404	1578.
Illeum	796.	748.	1388	1860	1646.	1635	1569	1339	1241	1271	1338.
Crypt, *µm*											
Duode	23.4	30.2	-	26.0	35.1	39.2	35.8	39.5	38.0	29.1	24.9
Jejunu	19.1	24.8	32.6	-	-	37.6	35.8	38.9	24.7	22.9	30.3
Illeum	25.5	18.7	26.0	49.0	36.7	34.7	37.2	30.5	18.4	34.5	36.5
Tunica Muscularis, *µm*											
Duode	264.	280.	239.	291.	380.4	267.	272.	396.	384.	374.	327.3
Jejunu	264.	461.	417.	420.	302.7	278.	338.	363.	287.	300.	329.3
Illeum	165.	367.	430.	345.	345.5	351.	320.	472.	454.	442.	332.1

Note: * Lowercase different superscripts in the same raw indicate significant differences (P <0.05).

Table 4: Villus, crypt and tunica muscularis of duodenum, jejunum and ileum of the chickens in Period II (34 days)

	Treatment*										
	T01	**T02**	**JM1**	**JM2**	**JM3**	**KU1**	**KU2**	**KU3**	**TI1**	**TI2**	**TI3**
Villus, *µm*											
Duode	1470	2098	2028	2005	-	1905	1997	1833	1975	1998	1722.
Jejunu	1441	1472	2098	1815	-	1986	1895	2104	1683	1840	1717.
Illeum	1333	1352	1537	1372	-	1317	2227	2223	1378	1537	1801.
Crypt, *µm*											
Duode	28.5	29.2	38.0	34.6	28.5	31.9	33.8	29.7	36.0	32.4	29.0
Jejunu	34.4	39.1	36.9	24.8	34.4	36.5	26.1	42.4	17.4	30.5	33.0
Illeum	27.3	28.7	28.5	44.6	27.3	38.0	32.4	23.3	50.5	35.7	30.2
Tunica Muscularis, *µm*											
Duode	436.	502.	450.	382.	534.	472.	433.	356.	506.	430.	384.8
Jejunu	351.	299.	346.	422.	426.	425.	695.	301.	419.	383.	440.4
Illeum	493.	586.	485.	505.	467.	438.	413.	401.	672.	623.	411.4

Note: * Lowercase different superscripts in the same column indicate significant differences (P <0.05).

4. Conclusion

Giving *temu ireng (Curcuma aeruginosa), kunyit (Curcuma longa) and jahe merah (Zingiber officinale*) have been able to produce normal growth best in bursa of fabricius. Furthermore, overall, morphological changes have occurred in the intestine as the effect of Phytobiotics. That can be used as an indication that there has been a change in function of mainly associated the process of absorpsion of nutrients occurs in the intestine. Furthermore, the research that has been done is expected to be used as a basis for producing growing chicken feed that contains that natural antibiotic or phytobiotics as a substitute for antibiotic synthesis in the future.

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