Oral Presentation (MP-12)

Effect of AKBISprob Supplementation on Antibacterial-Producing Lactic Acid Bacteria (LAB) Isolated from Laying Hens Intestine

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INTRODUCTION

Generally, Bacteria in the intestine consist of beneficial bacteria and bacteria that have the potential to disrupt animal health such as pathogenic bacteria. The presence of pathogenic bacteria is one of the factors that cause gastrointestinal dysfunction, but can be suppressed by maintaining the presence of beneficial bacteria for the digestive tract, especially the type of Lactic Acid Bacteria (LAB) (Astuti, 2016). LAB has antagonistic activity because it is able to inhibit pathogenic bacteria so it can compete to maintain normal flora balance in the digestive tract. The main inhibitory activity of LAB is caused by the accumulation of primary metabolites such as lactic acid, acetic acid, ethanol, and carbon dioxide. In addition, LAB is also capable of producing antibacterial compounds such as bacteriocins (Furtado *et al*., 2014)**.** Naturally LAB has existed in the digestive tract of chickens, but it is easy to experience changes in the amount due to the influence of given the feed (Widodo *et al*., 2015). Feed and the environment can affect the composition of microbes in the digestive tract in chickens (Ghadban, 2002; Apajalahti *et al*., 2004). Provision of fermented feed can improve the composition of intestinal microflora and increase the number of LAB. Fermented feed is generally easily biodegradable and has a higher nutritional value than the original ingredient and it can reduce the pH of the digestive tract.

AKBISprob is an alternative product to increase poultry production in the form of supplement made from a mixture of soybean wate, and palm kernel meal which is fermented with Aspergillus niger. The fermentation process using *Aspergillus niger* can reduce the crude fiber contained in AKBIS, because the mold can produce amylase, pectinase, amyloglucosidase and cellulase enzymes which can degrade cellulose so that it is easier to digest. In addition, Aspergillus niger also produces metabolites in the form of citric acid which is a component that can reduce intestinal pH so that it is suitable for LAB growth. Based on research conducted by Nurliana *et al*. (2016; 2017), supplementation of 4% AKBISprob can maintain the chickens production and health as well increase the number of LAB and reduce the number of pathogenic bacteria such as *Escherichia coli* and *Salmonella* sp. in chickens intestine. Thus creating a balance of microflora in the digestive tract of laying hens. Based on the above reasons, it is necessary to conduct research on antibacterial detection of BAL in laying hens that have been given AKBISprob.

MATERIALS AND METHODS

Maintenance of chicken, administration of AKBISprob and preparation of intestinal

All chickens were adapted for one month, fed commercial (324-2R) of 110 g / head. After adaptation of layer were grouped into four treatments as follows no supplemented by 4% AKBISprob (P0), 4% AKBISprob daily (P1), 4% AKBISprob once every three days (P2) and 4% AKBISprob once every five days (P3). Intestinal preparation was performed on day 31. All the organs of the gastrointestinal tract are removed to get the intestine. Chicken intestine were cut and removed from the body cavity and then weighed. Furthermore the contents of each intestinal section are inserted into the plastic separated between the contents of the cecum and small intestine respectively by 3.5 grams, accommodated on sterile plastic, stored in a freezer -5^{0}C.

Detection and measure the number of antibacterial-producing LAB

One ml of intestinal contents was diluted in Buffered Peptone Water (BPW) and and NaCl 0.85% from 10^{-2}-10^{-7} dilution. 1000 µl from 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were taken and put onto the petri disc, then it were poured with solid MRSA (which is still liquid with a temperature of

about 50°C) and homogenized, incubated for 24 hours at a temperature of 30°C. LAB colonies grow on the MRSA were calculated. To screen the antibacterial-producing BAL, it were overlaid with indicator bacteria. Six µl of *Bacillus subtilis, E.coli*, and *Salmonella* inoculated into Mueller Hinton Agar (MHA), Eosin Methilen Blue Agar (EMBA), Salmonella Shigella Agar (SSA). it were respectively poured onto the surface LAB colonies on MRSA, incubated for 48 hours at 37°C. Bacterial colonies which addressed the inhibition of indicator bacteria would show clear zones around it. Overlay agar methods was according to modification of Nurliana *et al*., (2009).

Characterization of LAB based on colony morphology, Gram staining and catalase

LAB colonies that grow separately, observed of form, elevation, edge shape, colony surface, color and size of colonies. Furthermore, LAB were observed microscopically based on Gram staining. The catalase test was carried out using 2-3 drops of H2O2 reagent on the preparation to cover the surface of the preparation. Changes that occur are to be positive if a gas bubble is formed, and vice versa.

Data analysis

The datas of total Antibacetrial-producing LAB, *E. coli*, and *Salmonella* sp were analyzed by variance analysis (ANAVA) supported by SPSS for Windows 16.0 program, then different treatments were continued analysis by Duncan test. The data of characterization of antibacterial-producing BAL were descriptive analyzed.

RESULTS AND DISCUSSIONS

Total of LAB and Antibacterials-Producing LAB in Small Intestine and Cecum of Layer.

Based on the results of the study The average ± SD total LAB in the small intestine and cecum of laying hens that have been given 4% AKBISprob are shown in Table 1. Based on statistical analysis, the supplementation of 4% AKBISprob had a very significantly effect (P <0,05) on the total LAB and antibacterial-producing LAB compared with those only given commercial feed in the intestine of laying hens (Table 1). The highest number of LAB and antibacterials-producing LAB occurred at 4% AKBISprob once in five days, both in the small intestine and caecum (Table 1).

Tabel 1. Average±SD of LAB and antibacterials-producing LAB (log10 CFU/g) in small intestine and caecum of laying hens after supplementation of 4% AKBISprob.

Organs	Bacteria	Treatments			
		P0	P1	P2	P3
Small intestine	LAB	7,60±0,22^{a}	8,10±0,15^{b}	8,39±0,09^{c}	8,39±0,08^{c}
	APLAB	5,26±0,24^{a}	6,85±0,14^{b}	7,05±0,08^{b}	7,11±0,09^{b}
caecum	LAB	7,82±0,14^{a}	8,18±0,13^{b}	8,35±0,08^{b}	8,41±0,13^{b}
	APLAB	5,49±0,19^{a}	6,83±0,16^{b}	7,02±0,06^{bc}	7,14±0,12^{c}

Lactic acid bacteria (LAB); Antibacterials-Producing Lactic Acid Bacteria (APLAB); different superscripts on the same line show very different (P <0.05).

The treatment of P2 and P3 had a significant effect (P <0.05) to the increase total of LAB in the small intestine compared to P1 treatment, but the total of LAB of P1 was not significantly different (P> 0.05) with P2 and P3 treatment in the cecum (Table 1). The treatment of P1, P2, and P3 had no significant effect (P> 0.05) on the number of BAL producing antibacterial in the small intestine, as well as the cecum between P1 and P2 was not significantly different, whereas P1 with P3 was significantly different (P <0,05).

Giving 4% AKBISprob with a time interval of once every five days was better in increasing the number of antibacterial-producing LABs and LABs. This is presumably because the nutrients given are not excessive and in accordance with the needs of the LAB and its host so that no nutrients are wasted and utilized by pathogenic bacteria. In addition, the symbiotic relationship between *A. niger* and LAB is more optimal at the time interval of five days given compared to other time intervals (Nurliana et al., *2016*). According to Widodo *et al*. (2015), feeding fermentation could increase the number of LAB and improve the composition of microflora in the digestive tract of chicken. Fermentation feed can reduce intestinal pH (Supartini and Fitasasi, 2011) so that it was suitable for LAB growth. LAB growth required acidic pH and could live up to pH 3. AKBISprob contained *A. niger* could crush crude fiber from soybean waste and palm kernel meal and produced citric acid. Citric acid can create an acidic atmosphere with a pH of 4.0 to 3.5 in the intestinal tract and made suitable condition for the development of LAB (Chowdhury *et al*., 2009).

The ability of Antibacterial produced by LAB against *E. coli* and *Salmonella*.

Based on the results of 24 isolates antibacterial-producing LAB in which 13 colonies originated from the small intestine and 11 colonies came from the cecum which still consistently showed an inhibitory zone (clear zone formed around the colony). Eigth LAB isolates of the 24 LAB isolates that produced antibacterial which could inhibit *Bacillus subtilis* (narrow spectrum

LAB), while 16 isolates are antibacterial against Gram positive and negative bacteria (broad spectrum LAB). The LAB colonies which previously showed inhibitory zones and after the confirmation test did not show the inhibitory zone was suspected because the inhibition zone was formed not because of the antibacterial (bacteriocins) produced by LAB. There were differences in inhibition zones of antibacterial (bacteriocin) LAB inhibited *E. coli* and *Salmonella* sp. (Table 2). AKB 11 is one of isolates antibacterial-producing LAB which is a very active antibacterial category because it had a large inhibition zone of >11.3 mm to E.coli, while other isolates had moderate antibacterial inhibition zone activity. Antibacterials such as bacteriocins from 16 LAB isolates were able to inhibit *Salmonella* sp., a barrier zone between 6-11 mm, it was categorized as a moderate active barrier zone.

Table 2. Antibacterial (bakteriosin) produce of LAB isolated laying hens intestine after supplement of AKBISpron to inhibit *E.coli* dan *Salmonella*

Source of isolat	Tetrasiklin		*E.coli*		*Salmonella* sp.	
	Inhibit zones (mm)	Activity	Inhibit zones (mm)	Activity	Inhibit zones (mm)	Activity
P12H52	6,5	+	8	+	7.3	+
P12S72	6,5	+	9.0	+	7.3	+
P21S71	6,9	+	8.2	+	8.8	+
P23H21	6,5	+	7.6	+	7.1	+
P23H31	6,5	+	7.6	+	8.9	+
P23H33	7,1	+	8.1	+	7.0	+
P31H32	7,1	+	9.0	+	7.4	+
P31H54	6,9	+	8.2	+	7.7	+
P31H61	7,1	+	8.3	+	7.4	+
P31H62	6,5	+	7.2	+	7.3	+
P31S71	7,1	+	11.3	+	8.5	+
P31S72	7,1	+	7.8	++	8.6	+
P32H62	6,9	+	9.4	+	8.6	+
P33S51	6,9	+	7,7	+	8,4	+
P33S52	6,5	+	9.3	+	10	+
P33S62	6,9	+	7.6	+	8.9	+

Remarks: - : no active (inhibit zones<6 mm);
+: moderate active (inhibit zones: 6-11 mm)
++: very active (inhibit zones >11mm)

According to Rai (2009), antibacterial such as bacteriocin activity produced clear, round and wide zones. The diameter of the barrier zone formed can be bactericidal (killing bacteria) and pseudo zone diameter which showed bacteriostatic properties (inhibits bacterial growth). Factors that influence bacteriocin activity include environmental conditions such as temperature, pH, ypes of bacteria, bacteriocin concentration and nutrient content in the media (Romadhon *et al*., 2012).

CONCLUSION

Supplementation of 4% AKBISprob once every five days increased the total of antibacterial-producing LABin the layer intestine. It were founded 8 LAB isolates that produce antibacterials are narrow spectrum activity and 16 isolates are broad spectrumactivity.

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