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Evaluation on Addition of Powder And Liquid Probiotic In Poultry Feed Towards Intestinal Microflora of Layer

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Abstract: *This research aims to discover the effect of powder and liquid probiotic addition towards intestinal microflora of layer. This research involved 144 Isa Brown hens with 6 months (24 weeks) age and liquid probiotic containing Lactobacillus sp bacteria with the composition of 1,4 x 1010 cfu/ml, while the powder probiotic that was used using skim milk media was processed into powder. The method used was experimental, employing nested Completely Randomized Design (CRD) with 2 factors and 6 actions, in which every action is given 4 times repetition; each repetition consisted of 6 layers. The data was analyzed using variety analysis and examined further by the Duncan test. Research result showed that the addition of powder or liquid Lactobacillus sp probiotic gave a positive effect on the amount of TPC Lactic Acid Bacteria (LAB), Salmonella sp, and Escherecia coli. It can be concluded that the addition of powder probiotic is better than the liquid one on the amount of TPC LAB, Salmonella sp, and Escherecia coli in intestine of layer.*

Key Word: *Probiotic, Microflora, Intestinal*

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I. Introduction

Laying chicken farming business in Indonesia has many obstacles, one of which faced by farmers is the unstable price of chicken eggs while feed prices tend to increase. This resulted in the income received is not worth the cost to be sufficient to meet the cost of feed, maintenance and medicine. Feed is the most important factor that has a contribution of 60-70% of the total cost of production in the livestock business. Production costs can be reduced if feed efficiency increases. Feed efficiency can be achieved if the feeding nutrient provided provides for the livestock needs for basic living, production and reproduction. Maximum efficiency can be achieved if the digestive tract can digest and absorb nutrients optimally, so the productivity of layer can be improved properly. Technology and innovation in the field of livestock began to be developed with attention to the health of livestock so as to positively affect the livestock productivity and reduce the negative effects that arise due to some antibiotic compounds used as additives in animal feed. Probiotics is one of the innovations made to replace antibiotic compounds.

Microflora in the digestive tract plays an important role in livestock productivity and health associated with gastrointestinal morphology, nutrient absorption, pathogensity and immunity (Lu et al, 2003). Probiotics is one of the additive feeds that can give positive influence in the form of control on microflora in the digestive tract. Probiotics can generally replace the role of antibiotics, increase egg production, egg quality, balance the microflora of the gastrointestinal tract, increase the absorption of food intestines in the small intestine and increase the gain. From these problems it is necessary to do research on how the effect of giving liquid and powder probiotics in the feed against spawning layer chicken microflora. The purpose of this study was to find out how well the use of probiotics in powder or liquid form is fed into the feed mixture by looking at the identification of microbes found in the intestine of layer.

II. Material And Methods

The method used was field experiments or experimental using nested patterns in Completely Randomized Design (CRD) with 2 factors and 6 treatments. The first factor is the form (B1 = Liquid form and B2 = powder form) nested in second factor that is the level of probiotic addition in feed (L1 = 0,4%, L2 = 0,6%). Each treatment was given 4 replications, each replication consisted of 6 layers, so in the study using the total layers as much as 144 heads. The treatment in this research is as follows:

B1L0: Basal Feed + 0% liquid probiotic (Control)
B2L0: Basal Feed + 0% powder probiotics (Controls)
B1L1: Basal feed + probiotic liquid form concentration 0, 4% (v / w)
B2L1: Basal feed + probiotic powder form concentration 0, 4% (w / w)

B1L2: Basal feed + probiotic liquid form concentration 0, 6% (v / w)
B2L2: Basal feed + probiotic powder form concentration 0.6% (w / w)

Table 1 Composition and content of the basal feeding agent

Feed Material [a)]	**Amount (%)**
Corn	52,99
Soybean meal	22,5
Rice bran	9
Limestone	8
MBM	6,5
Premix	0,5
D.C.P	0,25
Salt	0,2
D, L-*methionine*	0,06
Total	**100**
Content of Food Substances [b)]	**Amount**
Metabolic Energy (kkal/kg)	2869,3
Dry Matter (%)	87,1
Crude Protein (%)	18,86
Crude Fat (%)	3,93
Crude Fiber (%)	3,43
Ca (%)	3,9
P (%)	0,65
Methionine (%)	0,96
Lysine (%)	1,07

Source: a) Proportion of basal feed arrangement used by farmers
b) Based on the calculation of Blitar District Livestock Laboratory
Parameters measured in this study were calculation of total intestinal microflora using TPC (Total Plate Count) method, the number of colonies observed included Lactic Acid Bacteria (LAB), Eshericia coli, Salmonella sp.

Statistical Analysis Data
The data obtained were analyzed using Variance and if there was any difference between treatments followed by Duncan's Multiple Range Test test by Steel and Torrie (1993).

III. Result And Discussion

3.1 The Influence of Probiotic Form on Microorganisms of Layer Intestines

Table.2 Mean influence of probiotic forms on intestinal microflora of layer

3.1.1

Variabels	**Average Probiotic Form**	
	B1 (CFU/ml)	**B2 (CFU/g)**
Lactic Acid Bacteria (LAB)	7,54 ± 1,15	9,13 ± 1,56
Salmonella sp	4,29 ± 0,95	4,11 ± 1,05
E.coli	5,20 ± 1,32	4,87 ± 1,40

Lactic Acid Bacteria (LAB)

According to Table 2 it shows that the amount of TPC LAB of probiotic treatment of liquid form B1 (9,13 ± 1,56 CFU / ml) is higher than the treatment of probiotic powder form B2 (7,54 ± 1,15 CFU / g) probiotics in both liquid and powder forms have the same effect on the number of LAB colonies. This occurs because the probiotics are administered on antibiotic-free feeds so that no inhibitors cause a decrease in the number of LAB colonies.

The concentrate free feed used in this study helps the probiotic performance to be better because there is no suppression of the amount of LAB occurring by the presence of antibiotics contained in the concentrate and the administration of probiotics in both liquid and powder forms has a positive effect in determining the number of LAB colonies. The results showed that the probiotic in powder form (B2) has a higher value than the liquid form treatment (B1), this is due to the difference in the amount of feed consumption that occurs. Feed with B2 treatment is preferred by layer rather than feed with treatment B1, so that the difference of consumption will influence the amount of probiotics that enter into the chicken body resulted in differences in the number of colonies found in intestines of layer this is in accordance with the opinion Chesson (2001) the number of microbes will increase if the consumed feed increases because the microbes will have more time to interact with the feed.

3.1.2 Salmonella sp

Based on Table 2, it showed that the number of bacterial colonies of Salmonella sp treatment B1 (4.29 ± 0.95 CFU / ml) was higher than that of B2 treatment (4.11 ± 1.05 CFU / g) indicating that administration of probiotics both liquid and powder form give the same effect on the amount of TPC of Salmonella sp. In this case

the number of colonies of pathogenic bacteria can be affected by the presence of antibiotic substances that can kill these bacteria, but in this study did not use antibiotics so that the results of the number of colonies tend to be the same. The presence of probiotics can make the condition become more acidic in the digestive tract so as to suppress the growth of pathogenic bacteria, because pathogenic bacteria will die if the environment has high acid levels. This is consistent with Dildey's (1988) statement that the presence of probiotics can balance the microflora in the intestine.

3.1.3 Escherecia coli

Based on Table 2, the TPC Escherichia coli treatment B1 (5.20 ± 1.32 CFU / ml) was higher than the B2 treatment (4.87 ± 1.40 CFU / g). The result of statistical analysis in Appendix 8 shows that the probiotic form gives no significant effect (P> 0,05) to TPC of E.coli bacteria. This study showed that administration of liquid or powder probiotics in antibiotic-free foods had the same effect in colonies of TPC amounts of E. coli bacteria. Probiotic bellows can be said to be able to replace the role of antibiotics in suppressing the growth of pathogenic bacteria E. coli in the digestive tract of layer. Powder probiotic is better at suppressing the amount of E.coli bacteria in Table 4. It has a value (4.87 ± 1.40 CFU / g) which means that the smaller the TPC value of E.coli bacteria, the better the chicken's health condition, because in the gastrointestinal tract not found much amount of harmful bacteria such as E.coli.

3.2 Effect of Probiotic Level on Microorganisms of Layer Chicken Intestines

Table.3 Influence of probiotic level addition to intestinal microflora Layer

Treatment	**Levels**	**LAB**	***Salmonella* sp**	***Eschericia coli***
		(log cfu/ml)		
B1 (Liquid)	L0	$6,28 \pm 0,19^{a}$	$5,32 \pm 0,19^{b}$	$6,40 \pm 0,79^{b}$
	L1	$8,06 \pm 0,91^{b}$	$4,20 \pm 0,75^{b}$	$5,59 \pm 0,21^{b}$
	L2	$8,28 \pm 0,89^{b}$	$3,36 \pm 0,42^{a}$	$3,63 \pm 0,62^{a}$
		(log cfu/g)		
B2(Powder)	L0	$7,12 \pm 0,36^{a}$	$5,33 \pm 0,20^{b}$	$6,20 \pm 0,46^{b}$
	L1	$9,79 \pm 0,62^{b}$	$3,99 \pm 0,65^{b}$	$5,34 \pm 0,37^{b}$
	L2	$10.4 \pm 0,34^{b}$	$3,01 \pm 0,20^{a}$	$3,09 \pm 0,12^{a}$

Information:

- Different superscripts show that the treatment is very significant

3.2.1 Lactic Acid Bacteria (LAB)

Based on Table 3 it can be seen that the lowest LAB TPC value (6.28 cfu / ml) in treatment B1 with the addition of liquid probiotic 0% (B1L0), while the highest LAB TPC value (10.48 cfu / g) B2 with the addition of probiotic powder form with the addition of 0.6% (B2L2). Successively LAB TPC values contained in Table 4 ranging from lowest to highest are B1L0, B2L0, B1L1, B1L2, B2L1, B2L2. Effect of probiotic addition level of Lactobacillus sp of liquid form at treatment of B1 to TPC LAB respectively from lowest to highest B1L0, B1L1, B1L2 while the effect of probiotic addition of Lactobacillus sp powder form at treatment of B2 BTP gradually TPC LAB successively starting from the lowest until the highest is B2L0, B2L1, B2L2. This shows that probiotics are able to survive in the digestive tract of poultry. The higher the level of probiotic addition, the LAB value in the digestive tract also increases.

Probiotics contained in the digestive tract can improve the absorption process in the intestine. The addition of these probiotics can provide an acidic atmosphere that is unfavorable for the growth of pathogenic bacteria. The mechanism is probiotics break down simple carbohydrates into lactic acid, acetic acid, carbon dioxide, H2O2, bacteriocin, reuterin, and others (Soeharsono, 2010). According to Joerger (2003) bacteriocin composed of 17-37 amino acids produced from lactic acid bacteria, consisting of the substance of secreted proteins from probiotic bacterial cells that have antibacterial properties.

The presence of probiotics will form colonization of probiotics in the digestive tract, resulting in nutritional competition and the location of adhesion (attachment) between probiotics and other bacteria, especially pathogens. The growth of probiotics will also produce various anti-bacterial components (organic acids, hydrogen peroxide, and bacteriocins that can suppress the growth of pathogens) (Collado, Isolauri, Salmien, and Sanz, 2009).

Increased acid conditions cause the environmental pH to be low and cause pathogenic bacteria do not grow. If Lactobacillus colonizes on the surface of the gastrointestinal tract then this will inhibit colonization of unfavorable bacteria, the activity referred to as competitive exclusion (CE). Lactobacillus that has grown and attached to intestinal epithelial cells forms a colony. In the growth of these colonies require nutrients needed also by pathogenic bacteria, so there was competition of nutrients, this is not beneficial for the development of pathogenic bacteria (Soeharsono, 2010).

3.2.2 Salmonella sp

Based on Table 3, the lowest number of Salmonella sp TPC (3.01 cfu / g) was treated with B2 with a 0.6% powder probiotic addition (B2L2). While the highest value (5.33 CFU / g) is in the treatment of B2 with the addition of probiotic powder form 0% (B2L0). The mean values of Salmonella sp TPC from the lowest to the highest (B2L2, B1L2, B2L1, B1L1, B1L0, B2L0) The influence of liquid probiotic addition level on feed treatment B1 to the amount of TPC Salmonella sp from lowest to highest consecutive are B1L2, B1L1, B1L0, while the effect of probiotic additive level on feed treatment of B2 to the number of TPC of Salmonella sp from lowest to highest is B2L2, B2L1, B2L0.This result tends to decrease the value of TPC Salmonella sp as the increasing level probiotics. Powder probiotics in the treatment of B2 feed is more effective because of the influence of high viability of chicken so that the consumption of feed mixed with powder probiotics can suppress the amount of Salmonella sp bacteria in the small intestine. Expression of probiotic consumption may cause the pH in the small intestine to be low and cause intestinal environment conditions become acid.

According to Hayden (2000) Salmonella sp can grow optimum at pH 6.8 - 7.2 alleged Salmonella sp bacteria can not release proton H + so that these microbes can't survive in acidic environment conditions. Protons such as organic acids produced by probiotics will disrupt many of the metabolic functions in the body of the bacteria and cause an increase in osmotic pressure. Lactic acid bacteria that produce bacteriocin can kill pathogenic bacteria. Increased organic acids entering the bacterial cell will destroy the bacterial membrane so that the metabolic reaction becomes decreased. Added by the opinion of Kabir (2009) that Lactobacillus probiotics 0.75% in feed can reduce the number of Salmonella enteritidis. A decrease in the number of Salmonella colonies can be caused by probiotics that increase pH ileum, so that low pH conditions can suppress pathogenic bacteria. Decrease in the number of Salmonella colonies indicates that there is dominance of non-pathogenic bacteria in intestinal microflora so that the balance of chicken gastrointestinal microbiota becomes stable.

3.2.3 Eschericia coli

Based on the mean value of Table.3 it can be seen that the lowest E.coli TPC (3.09 cfu / g) is in the treatment of B2 feed with the addition of probiotic powder form of 0.6% (B2L2). While the highest value of TPC E.coli (6.40 cfu / ml) on feed treatment B1 with probiotic addition level of 0% (B1L0). Successively TPC E.coli values ranged from lowest to highest is B2L2, B1L2, B2L1, B1L1, B2L0, B1L0. The effect of liquid probiotic addition level on feed treatment B1 to TPC E.coli ranging from the lowest to the highest respectively are B1L2, B1L1, B1L0. While the influence of the level of probiotic addition of powder at feed treatment B2 to TPC E.coli start lowest to highest respectively that is B2L2, B2L1, B2L0. From Table 4, there is a tendency to decrease the number of TPC E.coli as the addition of probiotic level increases. The probiotic preparation of powder forms has a lower value compared to the probiotic form of liquid. This suggests that powder-state probiotics are more effective in suppressing the amount of E.coli bacteria contained in the small intestine compared with probiotics of the liquid form.

According to Russell and Diez-Gonzales (1998) E.coli is known to live in the gastrointestinal tract from the cache to the small intestine of 102. This shows that E. coli can be more resistant to acidic conditions produced by LAB. the antibacterial ability of the production of organic acid by LAB is related to the ability of ion-ion to separate. While inside the organic acid cell releases the proton H + which results in a more acidic linkage due to the decrease in intracellular pH of E.coli bacteria. This process will affect microbial metabolism, inhibit enzyme performance and stimulate bacterial cells to release more energy to release excessive H + protons in the body of E. coli bacteria. With these conditions result in microbial deaths due to the microbes run out of energy. Therefore, the amount of LAB is higher than the amount of E.coli bacteria found in the chicken intestines.

IV. Conclusion

The addition of probiotic Lactobacillus sp of a liquid form has a lower mean value than the powder form probiotic. Different forms of probiotic administration in feed give a positive effect on the amount of TPC LAB, Salmonella sp, and Escherecia coli. While the addition of probiotic level does not have an effect on the intestinal microflora of layer.

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