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Evaluation on Isolates of Lignochloritic Bacteria as Probiotics of Broilers

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ABSTRACT

The research objective was to produce a product assembly and feed technology and natural additives to improve food quality and sustainable food security. The results of the first year obtained isolates of lactic acid bacteria and lignochloritic bacteria that have potential as probiotics for livestock, namely growth, high production, and enzyme activity. Isolates of lignochloritic bacteria grow well in the intestine, but lower in the gizzard and ventricles. Isolates as probiotics tend to increase feed intake, growth, the production of eggs, meat and egg protein but lower feed conversion, meat fat, and egg cholesterol.

Key words: feed additive, food additive, probiotic, antibiotic, antioxidant

INTRODUCTION

Feed additive is frequently used as extra feed for livestock in order to improve its health, food digestion and absorbance, growth, post harvest preservation, and product quality improvement. Feed additive source is derived from synthetic source since it is easily obtained and can be produced in mass quantities. Another significant aspect is the provision of feed additive in livestock and crop cultivation processes where the additive is given excessively without proper measurement which affects human body health due to the high content of residues of antibiotics, pesticides, and other synthetic additives. Therefore, the provision of natural feed additive becomes a strategic issue that is to produce healthy and safe feed sources. The easiest application of feed additive is through feeding. Feed additive supplementation commonly occurs in livestock food and the most potentially developed feed additives are probiotic, prebiotic, and natural antibiotic.

A study on the natural resources of the feed additive from microbiology, biological or animal has been done and it resulted in microbes, enzymes, and bioactive concorsum potential for natural feed additives materials. Some studies have produced microbes to generate probiotics and enzymes, bioactive for antioxidants, dyes or preservatives, natural antibiotics from biological sources for animals and humans.

Probiotic is a product consisting non-patogen living microorganism which is added into the feed that may affect living growth, production, feed delivery efficiency, feed digestion, and livestock health through the improvement of balanced microorganism in digestive system **(Bahlevi, 2001).**

Probiotic for livestock can be given in feed mix or through drinking water, or in the form of probiotic that contains one strain or in mixed strains of microbes namely "probiolac" or "protexin". Several advantages of the use of probiotic for animals/livestock are the increase in growth, production, the betterment of feed conversion, health control such as to avoid digestive problem especially for young livestock, pre-digestive anti-nutrition factors such as trypsin, phytat acid, glucosinolates inhibitors and others (Karyadi, 2003). Several study results showed that the addition of probiotic treatment in animal feed has been able to significantly increase production, quality, and productivity of livestock. By manipulating the composition of bacteria in the digestive tract of cattle, probiotics

can improve digestibility of feed consumed by livestock (**Barrow**, 2008).

Prihatini (2007) found that lignochloritic bacteria have a high ability to degrade lignin and organochlorin residue. Each microbe also has different lignin biodegradable ability which is influenced by the composition and structure of lignin in the walls of the plant cells and the oxidative ability of lignolitic enzymes produced by microbes. Lignochloritic bacteria have facultative anaerobic nature and fast growth, so they can be used as probiotics and can be introduced into the rumen to improve rumen's ability to digest lignocellulosic materials.

According to Linder (1992), probiotics present in the digestive tract is useful to neutralize toxins produced by pathogenic bacteria, inhibiting the growth of pathogenic bacteria and boosting immunity, whereas lignochloritic bacteria have the ability to degrade lignin and organochlorin. If the content of lignin and organochlorin are kept, the level of ruminant digestibility will certainly decrease so that the synthesis of rumen microbes are obstructed, resulting in a decrease of nutrient digestibility in the rumen. Hawksworth (2002), the bacterial culture addition functioning as probiotics may stimulate digestive enzyme synthesis in order to improve nutrients utilization. The application of Starbio probiotics as much as 2.5 gram/kg of feed would boost a better performance and efficiency for laying hens. The dose of probiotic application derived from *katuk* leaves by 0.5% as media is capable of growing Bacillus spp bacteria doubling in three hours.

An evaluation research of lignochloritic isolates as probiotics is majorly aimed to characterizing and potential testing the lignochloritic bacterial isolates as probiotics on gastrointestinal and productivity of poultry that include the viability of bacterial cells in the gastrointestinal tract and the performance of broiler livestock, including consumption, body weight gain, conversion and efficiency, protein quality, fat and meat cholesterol.

RESEARCH METHODOLOGY

The materials are broilers aged 1 day and laying hens aged 19 weeks. The broilers are placed in individual cage equipped with food and drink corners. These chickens are raised communally and the number of each flock is suited with the treatment and repetition. The feed necessity will be based on its age and body weight. The main feed is commercial concentrates which are commonly given by the farmer.

Research Variable

The observed research variables are:

Broilers' tests : Feed consumption, body weight gain, feed efficiency and conversion, meat quality and cattle health

Laying hens' tests: Feed consumption, egg production, feed efficiency and conversion, and cattle health

Experimental Design

The livestock were classified into control group (P0), experimental group with 0.5% probiotics (P1), 1% (P2), and 1.5% (P3) mixed with drinking water. Each treatment was repeated for 6 times. For broilers, each flock used 6 chickens. The measurement of hematocrit and the total of immunoglobulin plasma were conducted at the end of the experiment.

RESULTS AND DISCUSSION

Feed Consumption

The study results for bacteria isolates potential for probiotics in feed consumption are presented in Table 1 and Figure 1. The results of variance analysis are presented in Table 2.

Table 1. The Average Consumption of Broiler Feed CP 707 for 32 Days (g/head/day)

Treatment	Repetition						Total	Average
	R1	R2	R3	R4	R5	R6		
P0	87.24	86.06	83.61	86.34	83.36	87.32	513.93	85.66
P1	85.78	84.47	84.33	85.53	83.93	84.81	508.85	84.81
P2	84.55	84.18	85.09	83.9	85.05	84.86	507.63	84.61
P3	84.89	86.46	84.72	87.15	86.04	88.7	517.96	86.33
Total							2048.37	85.35

The study result showed that feed consumption average with 0.15 % LC liquid probiotic in drinking water signified the high consumption rate compared to that of the control group; however, the administration of less than 0.15% material was proven to generate lower consumption rate compared to the control group.

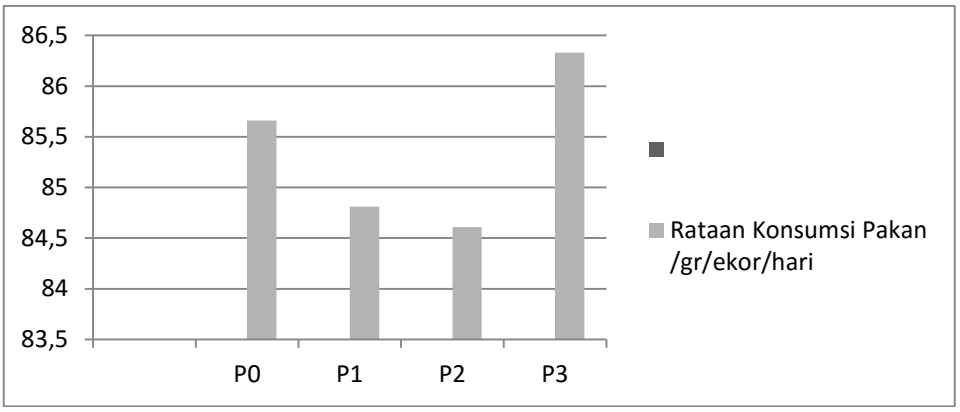

Figure 1. Average Feed Consumption

Table 2. The Analysis Result of Feed Consumption Variance for Broiler Chickens CP 707 in 32 Days (g/head/day)

Variance Sources	Freedom Degree	Sums of Squares	Central Squares	F. Calculation	F. Table	
					5%	1%
Treatment	3	11.39	3.797	2.52 ^{ns}	3.10	4.94
Galat	20	30.18	1.509			
Total	23	41.54	-	KK= 1.44%		

Note :^{ns} = No Significant Influence (P > 0.05)

The results of variance analysis showed no significant effect of treatments on feed consumption. Consumption among the treatments did not show significant differences even though the average score portrayed some differences in height in Treatment 3 (P3). The result indicated that the level of provision was not optimally given; that was only 0.15% or 1.5 liter per 1000 liter of drinking water with the total cells of 1.5 x 10^9 cfu. On the other hand, the optimum result from bacteria isolates activity could be seen in its minimum by 5 x 10^9 cfu. However, these results have not shown the overall performance of livestock nutrition needs. It should also be seen in the results of Body Weight Gain, as well as the conversion of health and quality of meat being produced.

Body Weight Gain

The result of the research for bacteria isolate potential test as prebiotic on body weight gain is presented in Table 3 and Figure 2. The variance analysis result is displayed in Table 4.

Table 3. The Average Body Weight Gain Data for Broiler Chickens CP 707 in 32 Days (g/head/day)

Treatments	Repetition						Total	Average
	R1	R2	R3	R4	R5	R6		
P0	57.36	56.76	59.32	57.37	57.74	60.87	349.42	58.24
P1	64.25	57.61	59.95	62.71	58.07	59.85	362.44	60.41
P2	62.9	57.79	56.92	53.38	58.91	57.97	347.87	57.98
P3	59.03	58.68	58.03	56.39	60.85	65.38	358.36	59.73
Total							1418.09	59.09

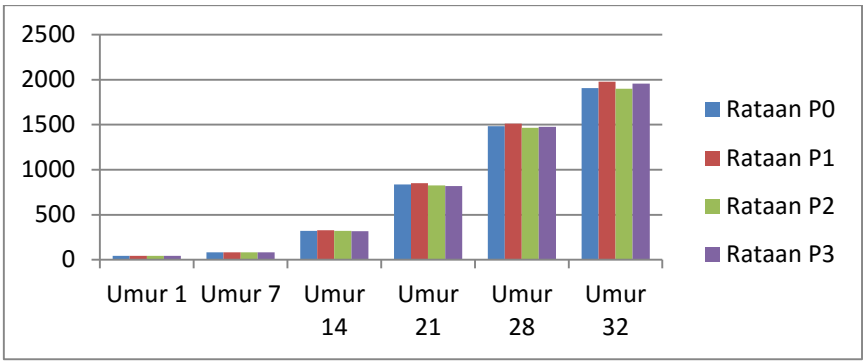

Figure 2. Weekly Body Weight Gain for Broilers

The study result represented by Figure 2 indicated the growth of chicken's body weight which was relatively similar between the treatments and the high growth level was noted by the age of 14 up to 28 days. These results demonstrated that the treatment day 14-28 was a phase of the highest growth in chickens and it could be optimized by giving probiotics to provide high growth acceleration so that the raising time is faster with optimal weight performance. The results in Figure 2 also signified the end of rising phase which was 32 days faster than the manufacturer's recommended raising period that was 40 days. This demonstrates that probiotic addition helps chickens to achieve the body weight gain growth more quickly than normal.

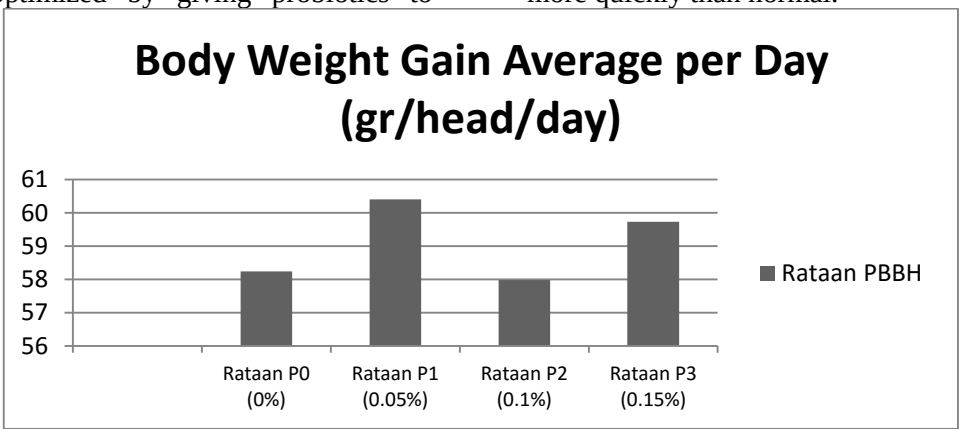

Figure 3. Average Body Weight Gain per-Day of the Broilers

Study result of body weight gain per day of broiler chickens showed that P1 treatment gained more weight compared to that of the control and other treatments although the variance result did not give significant influence towards body weight gain per day. However, the result proved different proportion of body weight gain, where P1 indicated the highest gain, while P3 projected the highest consumption rate. To conclude, probiotic provision in a certain level would increase body weight gain without increasing feed consumption, so that feed conversion might be lower.

Table 4. Result Analysis of Body Weight Gain Variance of Broilers CP 707 in 32 days (g/head/day)

Variance Sources	Freedom Degree	Total Square	Central Square	F. Calculation	F. Table	
					5%	1%
Treatment	3	24.62	8.206	1.155^{ns}	3.10	4.94
Galat	20	142.14	7.106			
Total	23	166.76	-	KK= 5.51%		

Note ^{ns} = No Significant Influence (P > 0.05)

Feed Conversion

Research result on bacteria isolates potential as probiotic on feed conversion is presented in Table 5 and the results of variance analysis are presented in Table 6.

Table 5. Data of Feed Conversion Average of Broilers CP 707 in 32 days (g/head/day)

Treatment	Repetition						Total	Average
	R1	R2	R3	R4	R5	R6		
P0	1.52	1.52	1.41	1.50	1.44	1.43	8.83	1.47
P1	1.34	1.47	1.41	1.36	1.45	1.42	8.43	1.41
P2	1.34	1.46	1.49	1.57	1.44	1.46	8.78	1.46
P3	1.44	1.47	1.46	1.55	1.41	1.36	8.69	1.45
Total							34.73	1.45

According to the results of research on consumption and body weight gain, the conversion average showed that P1 treatment gave the lowest conversion by 1.41. Although the results of the analysis showed no significant effect of treatments and showed no difference among treatments, these results indicated the best conversion results compared to the other studies and conversion recommendations from companies that was 2.1.

Table 6. Result Analysis of Feed Conversion Variance by Biological Additive Treatment for Broilers CP 707 in 32 days (g/head/day)

Variance Sources	Freedom Degree	Total Squares	Central Squares	F. Calculation	F. Table	
					5%	1%
Treatment	3	0.013	00044	1.206^{ns}	3.10	4.94
Galat	20	0.073	0.0036			
Total	23	0.086	-	KK= 4.17%		

Note :^{ns} = No Significant Influence (P > 0.05)

Meat Protein Content

The result of bacteria isolates potential as probiotic in meat protein content is displayed in Table 7 and Figure 4. The variance analysis result is available in Table 8.

Table 7. The Average of Broilers' Meat Protein (%)

Treatment	Repetition						Total	Average
	R1	R2	R3	R4	R5	R6		
P0	18.52	18.89	17.96	18.68	18.72	18.89	111.66	18.61
P1	19.36	19.24	18.94	19.38	19.23	19.17	115.32	19.22
P2	19.43	19.79	19.69	19.63	19.88	19.59	118.01	19.67
P3	20.19	20.67	20.32	20.03	20.26	20.42	121.89	20.32
Total							**466.88**	**19.46**

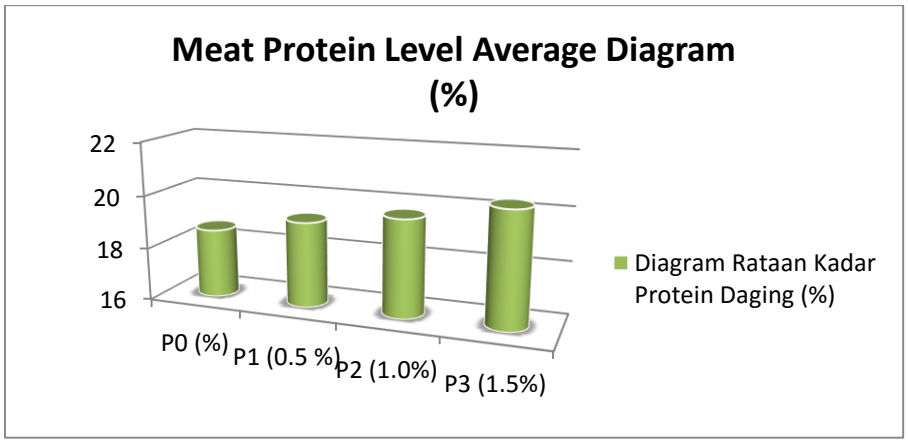

Figure 4. The Influence of Biological Additive Provision towards the Average Level of Protein Differences

The study signified that the highest average protein level for broiler chickens was in P3 by 20.32% compared to the P0 (control flock) and other treatments. This result underpinned the fact that the higher the liquid probiotic use in drinking water, the more increase in protein synthesis in body tissue and produce higher feed energy which was significant for meat protein synthesis occurred. These results were consistent with the character of the LC probiotics isolates that was efficient in protein synthesis by utilizing the available metabolites in tissue metabolism pool.

Table 8. Variance Analysis Calculation of Broiler's Protein level (%)

Variance Resource	Freedom Degree	Total Squares	Central Squares	F. Calculation	F. Table	
					5%	1%
Treatments	3	9.326	3.109	56.826**	3.10	4.94
Galat	20	1.093	0.055			
Total	23	10.429	-	KK= 1.20 %		

Note: ** = Significant Influence

Results of variance analysis also showed a significant influence on the treatment of liquid probiotics towards the protein content of chicken meat. The LSD indicated that the higher the levels of probiotics provision, the higher the levels of protein produced and there is no significant difference between P1 and P2. However, it exhibits different results between P3 to P2 and P1.

Table 9. The LSD Test Result of Each Treatment towards Meat Protein Content

Treatments	Mean	P0	P1	P2	P3
		18.61	19.22	19.67	20.32
P0	**18.61**	-	0.61 *	1.06**	1.71**
P1	**19.22**	-	-	0.45^{tn}	1.1**
P2	**19.67**	-	-	-	0.61*
P3	**20.32**	-	-	-	-

Note * = different **= significantly different tn= no significant different

Fat Content of Meat

The results of the study as a test of the potential of probiotic bacteria on meat fat is presented in Table 10 and the results of analysis of variance are projected in Table 11.

Table 10. The Average Result of Fat Content in Broiler's Meat

Treatments	Repetitions						Total R	Average
	R1	R2	R3	R4	R5	R6		
P0	25.91	26.14	25.55	25.42	26.31	26.19	155.52	25.92
P1	25.77	25.24	25.46	26.14	25.44	25.11	153.16	25.53
P2	24.88	25.19	24.98	24.86	24.93	25.2	150.04	25

P3	24.43	24.08	23.97	24.51	25.01	24.38	146.38	24.4
Total							**605.1**	**100.85**
Average							**151.275**	**25.21**

Table 10 and Figure 11 exhibit LC liquid probiotic provision in drinking water which reduced fat content in meat. The result was quite different from the protein content test which proved the opposite result that was in line with the characteristic of probiotic isolates. Probiotic isolates would synthesize proteins more efficiently and use energy for proteins synthesis, so that the excess energy turning into fat became lower. These results went hand in hand with the analysis of variance indicating a significant influence of probiotic usage towards fat level. In addition, LSD test results signified that the more probiotic treatment was given, the lower the fat level would exist in broiler's meat. P3 treatment had the lowest fat content compared to any other treatments.

These findings gave a notion that probiotic is efficient to improve product and quality where the intake nutrients in the metabolism pool are used not only to increase meat production, but also to maintain product quality and meat's health as the food source with higher protein and lower fat content.

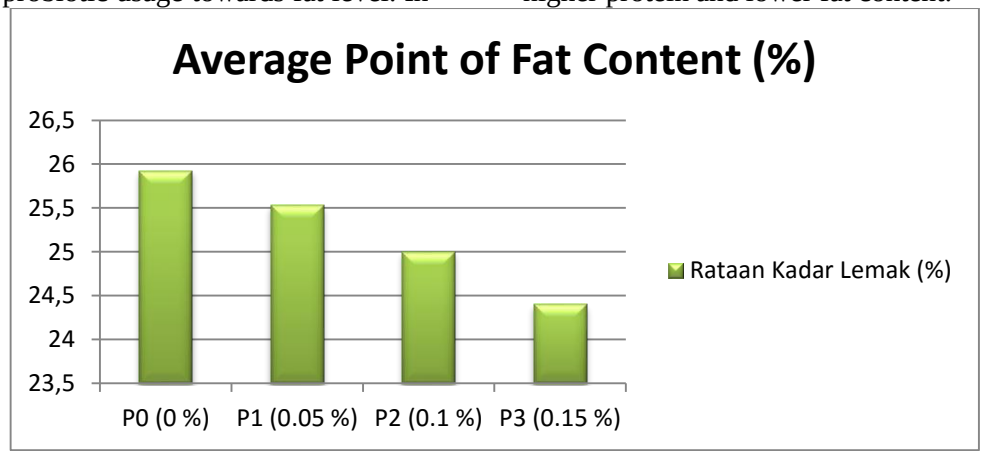

Figure 5 The Influence of Biological Additive Provision towards Average Level of Fat Content Difference.

Table 11. Analysis Result of Fat Content Variance in Broiler's Breast Meat (%)

Variance Sources	Freedom Degree	Total Squares	Central Squares	F. Calculation	F. Table	
					5%	1%
Treatment	3	7,85	2,615	24,363**	3,10	4,94
Galat	20	2.148	0.107			
Total	23	9,995	-	KK= 1,30 %		

Note: ** = Significantly Influenced

Table 12. Test Result of Treatment Difference towards Fat Content of Meat.

Treatments	Average	P0	P1	P2	P3
		25,92	25,53	25	24,4
P0	**25.92**	-	0.39^{tn}	0.92**	1.52**
P1	**25.53**	-	-	0.53*	1.13**
P2	**25**	-	-	-	0.6^{tn}
P3	**24.4**	-	-	-	-

Notes * = Different **= Significantly Different tn= No Difference

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