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Research Article

Impact of Administration Age of Probiotic *Lactococcus plantarum* on the Intestinal Microflora and Performance of Broilers

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Abstract

Objective: A trial was conducted to evaluate the effects of age at administration of *Lactococcus plantarum* isolates from virgin coconut oil processing waste on the number of Lactic Acid Bacteria (LAB) in the intestine and the growth performance of broilers. **Methodology:** The research used 160 day old cobb broilers divided into 4 treatment groups: T0 (without LP), T1 (*Lactococcus plantarum* administered at 1 week of age), T2 (2 weeks of age) and T3 (3 weeks of age). The basal diet consisted of corn, rice brain, fish meal, soy bean meal, bone meal, vegetable fat and premix (21.1% crude protein and 3038 kcal kg^{-1} energy metabolism). Chickens were given *Lactococcus plantarum* only one time and were slaughtered every week until 5 weeks old. Variables included the number of LAB, *E. coli* and *Salmonella* in the intestine, thickness and length of the intestine, carcass weight, fat and cholesterol content of carcass, body weight, feed intake and feed efficiency. The data were evaluated using a one-way ANOVA. **Results:** The results showed that *Lactococcus plantarum* administration affected the balance of microflora in the gut and the length of the intestine. *Lactococcus plantarum* treatment significantly increased the number of LAB in the intestine ($p<0.01$) up to 2 weeks after administration, conversely, the number of *E. coli* and *Salmonella* decreased. When given at 2 and 3 weeks, the effect of *Lactococcus plantarum* increased intestinal length and broiler growth performance was highly significant ($p<0.01$). Probiotic treatment did not affect carcass percentage but affected both the abdominal fat and cholesterol of broiler meat. **Conclusion:** Optimal body weight, feed conversion ratio (1.78) and cholesterol content were observed when *Lactococcus plantarum* was given at 2 weeks of age.

Key words: Probiotic, age, microflora, intestine, performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lactic Acid Bacteria (LAB) has been historically known to be important to the food industry for fermentation processes. In recent years, the role of LAB has focused on its probiotic health benefits. Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit to the host[1]. The microbial populations in the gastrointestinal tract of poultry play a key role in the normal digestive processes and in maintaining animal health. Lactic acid bacteria play a major role in intestine of human and livestock because of their ability to reduce pH and produce antimicrobials. Similar results have been reported in other studies regarding the LAB functioning as an antibacterial and having a beneficial effect on the host[2,3]. These bacteria are called probiotics. According to Fuller[1], probiotics are a mixture of live and nonpathogenic bacteria that contribute to the health and balance of the host's intestinal tract. According to Reid and Friendship[4] and Patterson and Burkholder[5], the addition of probiotics to feed is an alternative and substitute for antibiotics.

The main effect of probiotics is their performance in the gastrointestinal tract. To be considered a probiotic candidate, the bacterium must survive when it reaches the intestine. The probiotics must have functional properties and beneficial effects for the host[6]. Husmaini *et al.*[7,8] reported that LAB isolates from virgin coconut oil processing wastes have functional properties and the ability to survive the conditions found in the small intestine. Furthermore, LAB was identified as *Lactococcus plantarum*. Husmaini *et al.*[9] found that the administration of *Lactococcus plantarum* as much as 1 mL (1.3×10^8 CFU mL^{-1}) can improve the performance of broilers by reducing abdominal fat and blood cholesterol levels.

Development of intestinal microflora is dynamic and it is influenced by many factors, including the environment and age. In birds, the gut microbial community composition fluctuates during the first 2-3 weeks of age before stabilizing at 5-6 weeks of age. Therefore, it is important to know when probiotics are most effective for the performance of poultry. This study aimed to evaluate the impact of the age of poultry administered probiotics on the performance and meat quality of broilers.

MATERIALS AND METHODS

The experiment was conducted using a completely randomized design using 1 day old cobb broilers. The chickens were divided into 4 treatment groups with 4 replicates. Each replicate contained 10 broilers. The experimental treatments, the age of administration of *Lactococcus plantarum* were as follows: T0 (without *Lactococcus plantarum*), T1 (*Lactococcus plantarum* administered at first week of age), T2 (2 weeks of age) and T3 (3 weeks of age). Broilers received equal culture treatment dosages orally. Chickens were given *Lactococcus plantarum* only one time and were slaughtered every week until the 5 weeks period. The broilers were reared at the UPT Animal Science House, Faculty of Animal Science. Broilers (doc) were fed a starter diet (commercial feed) until 7 days of age and treatment diets (Table 1) from 7-35 days of age.

Probiotics: The probiotic used in the experiment was pure lactic acid bacteria which was isolated from byproduct of virgin coconut oil[7]. A small amount of a colony from glycerol stock was transferred into a universal bottle containing MRS broth (Merck) and grown at 37°C during vigorous shaking. After 17 h, the culture was centrifuged (10,000 rpm for 5 min) and then pelleted cells were resuspended in fresh 0.85% NaCl. *Lactococcus plantarum* culture was placed in a suspension of a given optical density ($OD_{\lambda = 580}$) of 0.5 orally. The product had a total bacterial count, of approximately 1.3×10^8 CFU *Lactococcus plantarum* mL^{-1} of suspension.

Enumeration of microorganisms in ileum: Viable bacterial counts in the ileum of the broilers were determined every week from the 7th day until rearing on the 35th day. The broilers were slaughtered and the ileums were aseptically removed. Samples with suitable dilution were either poured on CHROMagar™ *Salmonella*, CHROMagar™ *E. coli* or MRS agar plates (Merck) containing 0.5% $CaCO_3$ to enumerate *Salmonella*, *E. coli* and LAB, respectively.

Body weight and feed intake: Body weight and feed intake of chickens were measured every week. Body Weight

Table 1: Composition of diet

Items	Composition
Ingredients (%)	
Corn	58.00
Rice bran	6.00
Soybean meal (40.5% protein)	18.00
Fish meal (56.78% protein)	14.70
Bone meal	0.30
Vegetable fat	2.50
Premix	0.50
Calculated analysis (per kilogram of diet)	
ME (kcal)	3038.71
CP (g)	21.11
Fat (g)	5.72
Crude fiber (g)	3.18
Methionine (g)	0.39
Lysine (g)	1.07

ME: Metabolic energy, CP: Crude protein

Gain (BWG) was calculated weekly. The consumed amounts of feed were recorded every week and the Cumulative Feed Intake (CFI) was calculated at the end of the experiment.

Carcass: At the end of the experiment, every chick in each replicate was weighed to obtain their live body weight and then sacrificed. After bleeding was complete, the feathers were plucked. Head, viscera and shanks were removed. The carcass was left for 1 h to drain excess water. Carcass and abdominal fat percentages were calculated from the live weight.

Statistical analysis: Data were evaluated by using one-way analysis of variance to verify significant differences in relation to treatments[10]. The significant difference among treatment mean values (p<0.01) were analyzed subsequent Duncan's multiple range test.

RESULTS AND DISCUSSION

The number of LAB, *E. coli* and *Salmonella* sp., in the ileum of broilers by observed age are presented in Table 2. Administration of *Lactococcus plantarum* at the age of 2 and 3 weeks caused the number of LAB to increase and the growth of pathogenic bacteria to decrease. As reported by Hidayat[11], the effectiveness of probiotics is strongly influenced by the adequacy of the administered dosage. *Lactococcus plantarum* probiotics showed a highly significant (p<0.01) effect on the length of the small intestine. Giving *Lactococcus plantarum* as the 2 weeks old treatment resulted in the longest intestines of the broilers among all the treatments. This condition will affect the amount of nutrients absorbed because the process of digestion and absorption of nutrients is dependent on intestine length.

The quantities of lactic acid bacteria, *E. coli* and *Salmonella* sp., in the ileum of broiler were highly significant (p<0.01) and affected by the chick age at the time of probiotic administration. Table 3 shows that the timing of the administration of *Lactococcus plantarum* on broilers shows highly significant results (p<0.01) for an increasing number of LAB, whereas the number of *E. coli* and *Salmonella* sp., decreased in the ileum. This study shows that the *Lactococcus plantarum* isolated from virgin coconut oil processing waste has contributed to regulating the balance of microflora in the gut. Haghighi *et al.*[12] also reported that probiotics reduced *Salmonella* colonization in the intestine. Savadogo *et al.*[13] explained that the activity of each LAB, as an antimicrobial, affected different species of pathogenic bacteria in different ways, which may be caused by different antimicrobial

Table 2: Number of lactic acid bacteria in the ileum of broiler ($\log_{10}$ CFU g^{-1})

	Treatments			
Age (days)	T0 (Control)	T1	T2	T3
3	3.522^a	3.547^a	3.585^a	3.697^a
7	6.798^a	6.862^a	5.803^b	5.848^b
14	8.366^b	10.765^a	10.402^a	8.376^b
21	8.249^c	9.574^b	11.261^a	10.237^b
28	8.255^b	8.415^b	11.114^a	11.217^a
35	7.114^d	8.138^c	9.478^b	10.137^a

Treatments with different letters are significantly different at p<0.05

Table 3: Number of lactic acid bacteria, *E. coli* and *Salmonella* sp., in ileum and length of small intestine of broiler at the end of observation

	Treatments			
Variables	T0	T1	T2	T3
Microbes ($\log_{10}$ CFU g^{-1})				
Lactic acid bacteria	7.114^d	8.138^c	9.478^b	10.137^a
E. coli	5.732^a	4.987^b	3.633^c	3.376^c
Salmonella sp.	1.973	1.241	nd	nd
Length of intestine (cm)	165^c	167^c	187^a	176^b

Treatments with different letters are significantly different at p<0.05, Nd: Not determined

components produced by each isolate. According to Arief *et al.*[14], the mechanism of inhibition of lactic acid on bacterial cells involved hydrophobic properties, thus facilitating diffusion in the form of protons into the cell through the cell membrane. As a result, intracellular pH is higher than extracellular pH. In this study, antimicrobial activity of LAB was caused mainly by lactic acids produced as a result of glucose metabolism. Savadogo *et al.*[15] explained that the other possible factors that reduce pathogenic bacteria are that some bacteriocins produced by LAB caused low pH. Pal *et al.*[16] and Savadogo *et al.*[15] noted that the bacteriocins can be degraded by protease enzymes in the digestive tract. Bacteriocins are irreversible, easy to digest and show positive effect on health and remain active at low concentrations. According to Ogunbanwo *et al.*[17], the antimicrobial mechanism of bacteriocin begins with entry into target cells by forming pores in the cell membrane that are sensitive to lower potential or pH gradients, which causes damage to the cellular material that could inhibit the growth of target cells.

According to Tannock[18], the population and distribution of microbes in the intestines of chickens, which occur naturally are lactobacilli and *E. coli* whose microbial populations are 10^8 and 10^4 CFU g^{-1} (wet weight), respectively. The digestive tract is an organ that acts as a defensive barrier system against pathogenic bacteria. This barrier is often damaged by pathogenic bacteria that dominate the gut composition. At this time, the role of probiotic bacteria was to gradually eliminate the influence of pathogenic bacteria. Probiotic treatment causes the amount of lactic acid bacteria in the gut

Table 4: Performance of 35 days old broiler

Variables	Treatments T0	T1	T2	T3
Performance				
Feed intake (g/b)	3020[a]	3021[a]	3064[a]	3083[a]
Weight gain (g/b)	1578.32[b]	1600.36[b]	1765.66[a]	1759.83[a]
Body weight (g/b)	1757.50[b]	1780.00[b]	1945.67[a]	1940.38[a]
Feed conversion	1.91[b]	1.89[b]	1.78[a]	1.81[a]
Carcass				
Carcass relative (%)	69.75[a]	73.55[a]	76.15[a]	74.61[a]
Abdominal fat (%)	3.87[a]	2.1[b]	0.54[d]	1.19[c]
Cholesterol content of meat (mg g^{-1})	87.9[a]	48.1[b]	33.4[d]	38.7[c]

to increase, thus increasing the acidity of the intestine as well as the production of secondary metabolites. This causes stunted growth of pathogenic bacteria and can even deactivate them. Jans[19] explained that the naturally occurring number of pathogenic microbes in the digestive tract can be reduced by the presence of native microflora in the gut and white blood cells.

The age at which the chickens were given cultured *Lactococcus plantarum* did not affect feed intake (p>0.05) but showed high significance (p<0.01) for increased broiler performance. Highest weight and the lowest feed conversion were achieved if probiotics were given at 2 or 3 weeks, which led to increased feed efficiency of 6.94 and 5.47%, respectively, when compared with T0 (without probiotics). This result is closely related to the function of LAB as probiotics. According to Fuller[1] increase in the number of probiotic bacteria in the small intestine cause the number of pathogenic bacteria in the intestinal mucosa to be reduced (translocation) and the absorption of nutrients to increase. Then, body weight increases with higher feed efficiency, as reported by previous researchers[20,21]. Giving probiotics at 1 week of age did not generate the same feed conversion performance as T2 and T3 due to the smaller increase in the amount of LAB at 4 weeks old (Table 4).

Carcass broilers were not affected by the time of cultured *Lactococcus plantarum* given to chickens (p>0.05) but abdominal fat and cholesterol content was greatly influenced. Giving *Lactococcus plantarum* at 1 week of age was highly significant (p<0.05), causing reduced abdominal fat and cholesterol content in the meat. Giving *Lactococcus plantarum* at 2 weeks of age caused the abdominal fat percentage and cholesterol content of meat to reduce by 0.54 and 33.4 mg g^{-1}, respectively. According to Huang *et al.*[22] feeding probiotics increased T cell frequencies compared to the control group in the intestinal mucosa of broilers at 7 days of age. Thus, probiotic feedings are likely to affect the immune functions in the intestine. It is important to obtain conditions for optimal digestion and absorption to improve performance. Husmaini *et al.*[7] also reported that LAB isolated from virgin coconut oil processing waste has the ability to survive and grow in extreme pH and bile salts. Furthermore, pathogenic bacteria such as *E. coli* and *Salmonella* will be reduced even be undetectable if given sufficient amount of probiotics. The decline in the number of pathogenic bacteria, such as *E. coli* and *Salmonella* sp., showed the role of probiotics in the gut, creating conditions conducive for healthier chickens and improving feed utilization efficiency. Sissons[23] reported that some mechanisms of the absorption of fats, carbohydrates and protein can be influenced by the presence of intestinal microflora. Lactic acid bacteria produce Bile Salt Hydrolase (BSH), this enzyme will break down bile acids that are poorly absorbed by the small intestine so that they may be excreted through the feces. The lack of bile in the digestive tract, causing cholesterol to be present in the blood, is synthesized into bile acids and released back into the digestive tract. In addition, lactic acid bacteria also bind to cholesterol in the digestive tract so that cholesterol is reduced. Noh *et al.*[24] explained that most of the cholesterol assimilated into the bacterial cell membrane is grown on the media. Voet *et al.*[25] also explained that a reduction of cholesterol in poultry occurs because the metabolites produced by the microbes compete with HMG CoA to bind to the enzyme HMG-CoA reductase. According to Usman[26], *Lactobacillus*, which is able to reduce cholesterol in the blood stream, is taken to the heart and used to form bile acids, which are then released by the gall bladder and then taken to the small intestine to be discarded along with the feces.

CONCLUSION

The *Lactococcus plantarum* administration affected the balance of microflora in the intestine and the length of the intestine. The number of LAB increased up to 2 weeks after administration, it decreased *E. coli* and *Salmonella*, whereas the intestinal trace becomes longer. *Lactococcus plantarum* administration affected both the abdominal fat and cholesterol content of broiler meat. Optimal body weight

(1945.67 g/b), feed conversion ratio (1.78) and cholesterol meat content (33.4 mg g^{-1}) were observed when *Lactococcus plantarum* was given at 2 weeks of age.

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