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Effects of *Lactobacillus cultures* as Probiotic on Blood Parameters, Plasma Enzymes Activities and Mortality in Broiler Chicken

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Abstract: A study with four hundred, 1 day old male (Ross 308) broilers that were randomly assigned to 4 treatments with 5 replicate, 20 birds per each was conducted to determine the effect of diet supplementation with *Lactobacillus cultures* on some blood parameters, plasma enzymes activities and mortality of broiler chicken. For this purpose, the experiment carried out by feeding 4 levels of the probiotic (0, 0.05, 0.10 and 0.15%) to broiler chicks from days 1-42 of age. Hematological, biochemical tests were measured at days 21 and 42; Hemoglobin (HGB), Hematocrit (HCT), release of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Lactate Dehydrogenase (LDH), White Blood Cell (WBC), heterophils, lymphocytes, cholesterol and triglycerides. The results of the recent experiment showed that feeding diet with *Lactobacillus cultures* (0.15%) to chickens significantly ($p<0.05$) decreased, ALT and AST activity, cholesterol and triglycerides. Moreover, *Lactobacillus cultures* (0.15%) significantly ($p<0.05$) increased HCT, HGB and WBC day 42 of age. Moreover, *Lactobacillus cultures* (0.15%) significantly reduced chicken mortality compared to the other groups. There are no significant effects of *Lactobacillus cultures* on other blood parameters.

Key words: *Lactobacillus cultures*, plasma enzymes activities, blood parameters, broiler chicken, cell

INTRODUCTION

The use of antibiotics in food animals for growth promotion and disease prevention may cause antibiotic resistance in humans and animals (Corpet, 1996; Williams and Heymann, 1998) resulting in treatment failure when needed (Marshall and Levy, 2011). These problems are also increasing due to the misuse of antibiotics as growth promoters in animal feeds as well as the treatment of humans and animals. The antibiotic resistance can spread directly by contact and indirectly through the food chain, air, water and soil. As a consequence, several countries have restricted the use of antibiotic in livestock feeds to avoid the harmful impact on public health. Additionally, public pressure to reduce usage of antimicrobials has influenced development of alternative methods for reduction of pathogens including probiotics. Probiotics are beneficial bacteria that influence the host by improving intestinal health (Isolauri *et al*., 2001). The intestinal microflora population is a complex ecosystem composed of a large variety of bacteria. The metabolic capacity of microflora is extremely diverse and can produce positive and negative effects on gut physiology (Macfarlane and Cummings, 1991). There is, therefore, a great deal of interest in the possibility of altering the intestinal microbiota in a beneficial way with the aim of improving the health of the host.

Lactic acid bacteria have been considered potentially useful in this respect (Sanders, 1993). Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract. These microorganisms can influence the intestinal microbiota as well as host health and welfare in different ways such as competitive exclusion of pathogenic bacteria, lowering the pH through acid fermentation, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the immune system associated with the gut increasing production of short-chain fatty acids, increasing epithelial integrity, reducing epithelial cell apoptosis and stimulating the intraepithelial lymphocytes (Nurmi and Rantala, 1973; Fuller, 1977; Ng *et al*., 2009; Ferket, 2011).

Some lactic acid bacteria have been reported to produce soluble antimicrobial peptides, called bacteriocins which are postulated to contribute to their ability to improve intestinal health. An isolate of *L. acidophilus* has been reported to produce 2 bacteriocins which inhibited growth of 2 nonpathogens: Lactococcus and Pediococcus. These bacteriocins also inhibited growth of several pathogenic organisms *in vitro*, from genuses including *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Listeria*, *Clostridium* and *Bacillus* (Bogovic-Matijasic *et al*., 1998). Ocana *et al*. (1999) reported isolation of a bacteriocin from a *Lactobacillus salivarius* strain that inhibited Enterococcus and

Staphylococcus. Other isolates have also been reported to produce bacteriocins including *Lactobacillus delbruekii* whose bacteriocin only inhibited other strains of Lactobacillus which may confer an advantage during colonization. However, there is a dearth of information regarding the effects of bacteriocins *in vivo*, likely due to the difficulty of measuring these effects *in vivo*.

Several reports indicate that killed *Lactobacillus cultures* are capable of initiating changes in the immune system parameters. Sashihara *et al.* (2006) applied heat-killed *Lactobacillus plantarum* and *Lactobacillus gasseri* to cultures of splenocytes and mesenteric lymph node cells and observed an increase in production of IL-12. Administration of live or dead Lactobacillus GG to cultures of Caco-2 cells resulted in a decrease of tumor necrosis factor-α induced interleukin-8 production (Zhang *et al*., 2008).

In the present study, researchers investigated the effects of *Lactobacillus cultures* as probiotic on blood parameters, plasma enzymes activities and mortality in broiler chicken.

MATERIALS AND METHODS

Animals and diets: Four hundred, 1 day old male broiler chickens (Ross 308) were used in this experiment. Chickens allocated randomly in to 4 treatments groups with 5 replicates each and 20 chicks per replicate (per cage). All chicks were fed a basal corn-soybean meal diet including 22.04% CP and 3,200 kcal kg^{-1} of ME (1-21 days) or 20.26% CP and 3,200 kcal ME (22-42 days). From day 1, the diets were supplemented with diets were supplemented with 4 levels of the dried *Lactobacillus cultures* (0, 0.05, 0.10 and 0.15%). Feed and water provided *ad libitum* from days 1-42; all chicks were fed the basal diet.

Preparation of lactobacillus cultures: A mixture of strains of Lactobacillus isolated from chicken intestine and inoculated into MRS broth 2 and incubated at 37°C for 24 h. The bacterial cells were harvested by centrifugation at 2,000 g for 20 min at 4°C and the bacterial pellets were lyophilized and stored at -20°C until used. To obtain a concentration of $1\text{-}2\times10^9$ cells per gram, the *Lactobacillus* cultures were diluted with cornstarch and skimmed-milk powder, based on their original colony forming units per gram determined on MRS agar. These products, containing a mixture of Lactobacillus strains were stored at 4°C and mixed into the feed each day. The viability of the *Lactobacillus cultures* was checked biweekly to ensure that the viability of the cultures remained at $6\text{-}7\times10^7$ viable cells per gram.

Sampling procedure

Sampling: At day 21 and 42, one chick from each replicate was randomly chosen and after 3 h starvation, blood sampling from wing vein. Blood samples were collected and centrifuged and plasma was collected and stored at -80°C until measurement of the other enzymatic and chemical analysis. Hematological and biochemical tests were measured; Hemoglobin (HGB), Hematocrit (HCT), plasma activity of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Lactate Dehydrogenase (LDH), Wight Blood Cell (WBC), heterophils, lymphocytes, cholesterol and triglycerides. It is also, mortality was recorded daily and all of the dead birds inspected for diagnosis of death.

LDH, AST and ALT activity: Plasma LDH, AST and ALT enzyme activities were determined using an autoanalyzer (Autolab, K4500, Autoanalyzaer Medical System, Germany).

Statistical analysis: The data were analyzed based on a completely randomized design using the GLM procedure of SAS (SAS 9.1 Institute, 2002). Contrasts between treatments means were evaluated by Tukey's test at a significance level of 5%.

RESULTS AND DISCUSSION

In the experiment, *Lactobacillus cultures* (0.15%) supplementation significantly decreased ($p<0.05$) mortality of broilers during 0-42 days (Table 1). It is also, the WBC count was significantly higher in *Lactobacillus cultures* (0.15%) treatment, compared to other groups however, no significant difference was observed among the treatments in heterophils and lymphocyte of broiler chickens (Table 2).

An important feature of lactic acid bacteria is their ability to synthesize bacteriocins, substances that exhibit antibacterial activity (Klaenhammer, 1993; Joerger, 2003). Bacteriocins are protein substances produced and secreted by bacterial cells with bactericidal activity against microorganisms, consisting of only 17-37 amino acids (Joerger, 2003). They have been attributed with the ability to eliminate pathogenic bacteria from the digestive

Table 1: Effect of *Lactobacillus cultures* on mortality of broilers

Days	Treatments (*Lactobacillus cultures* in diets %)	Mortality (%)
42	0.00	5.10[a]
	0.05	4.50[a]
	0.10	4.90[a]
	0.15	3.10[b]
SEM		0.12

[a, b]Means within columns with no common superscript differ significantly ($p<0.05$)

tract. Many research studies have documented the inhibitory effect of probiotics on the development of pathogenic microorganisms (Clostridium, Shigella, Salmonella, *Escherichia coli*, *Listeria monocytogenes*) and rotaviruses, the associated incidence of diarrhoea and on ameliorating the course of diarrhoea (Gaenzle *et al*., 1999; Rolfe, 2000).

Slizewska *et al*. (2006) suggest that following bacterial fermentation of sugars, lactic acid is partly dissociated while the un dissociated form passes through lipid cell membranes and by dissociating within the cell, it acidifies cell contents and inhibits the growth of pathogenic microorganisms including putrefactive bacteria, Gram-negative bacteria and also some moulds. The same researchers hold that lactic acid can be further fermented into acetic acid which neutralizes electrochemical cell potential and can decrease the growth of putrefactive bacteria including those of the genera *Clostridium* and *Salmonella*.

With regard to Table 3 are shown, *Lactobacillus cultures* (0.15%) supplementation significantly decreased ($p<0.05$) cholesterol and triglycerides. Moreover, *Lactobacillus cultures* (0.15%) significantly increased ($p<0.05$) hematocrit, hemoglobin of blood in broilers (Table 3). This is in disagreement with the study done by Djouvinov *et al*. (2005) who found that the probiotic supplementation did not affect the blood constituents comprising, haemoglobin concentrations.

Table 2: Effects of *Lactobacillus cultures* on White Blood Cells (WBC), heterophils and lymphocyte of blood in broilers

Days	Treatments (*Lactobacillus cultures* in diets %)	WBC (account/mm^3)	Lymphocyte (%)	Heterophil (%)
21	0.00	15980	29.2	75.4
	0.05	16200	31.5	78.5
	0.10	16070	28.4	77.3
	0.15	16800	31.6	76.5
SEM		175	3.5	4.6
42	0.00	15700^{b}	32.2	81.5
	0.05	16600^{b}	34.5	80.2
	0.10	17900^{ab}	35.6	78.1
	0.15	19700^{a}	34.9	77.5
SEM		198	7.8	6.5

$^{a, b}$Means within columns with no common superscript differ significantly ($p<0.05$)

In contrast, the findings agree with of Cetin *et al*. (2005) findings, who observed that the probiotic supplementation caused statistically significant increase in the erythrocyte count, haemoglobin concentration and haematocrit values of Turkeys. The differences may be attributed to type and number of species of bacteria present in probiotics. Moreover, this observation is in agreement with numbers of earlier literature. Mohan *et al*. (1995) reported that probiotic supplementation resulted in lowering of the serum cholesterol level in white leghorn layers from 176.5-114.3 mg dL^{-1} serum. Also, Mohan *et al*. (1996) mentioned that chickens that received 75, 100 and 125 mg probiotic/kg diets had lower serum cholesterol content (93.3 mg/100 mL) compared to the control birds (132.2 mg/100 mL). Similar results were reported by Panda *et al*. (2006) who found that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic containing *L. sporogene* at 100 mg kg^{-1} diet. The significant reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al*., 1995, 1996). Also, it was speculated that *Lactobacillus acidophillus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al*., 1996). Lactobacillus has found to have a high bile salt hydrolytic activity which is responsible for deconjugation of bile salts (Surono, 2003). Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to excrete in faeces (Klaver and van der Meer, 1993). This could be the case in the present study as the probiotic utilized in the study (*Pediococcus acidilactici*) is acidophilic and it lowers the pH of the environment it occupies. Another explanation of the mechanism by which a probiotic can lower the serum cholesterol has been declared by Fukushima and Nakano (1995). Researchers demonstrated that probiotic microorganisms inhibit hydroxymethyl-glutaryl-coenzyme A; an enzyme involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis.

Table 3: Effects of *Lactobacillus cultures* on hematocrit, hemoglobin, cholesterol and triglycerides levels of blood in broilers

Days	Treatments (*Lactobacillus cultures* in diets %)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Hematocrit (%)	Hemoglobin (mg/dL)
21	0.00	105.5	164.5	32.1	8.21
	0.05	107.9	158.1	35.7	78.70
	0.10	104.6	155.4	34.1	7.95
	0.15	102.8	151.5	35.5	7.75
SEM		5.5	8.5	5.2	0.54
42	0.00	104.1^{a}	147.8^{a}	30.2^{b}	5.20^{b}
	0.05	106.2^{a}	132.8^{a}	32.3^{ab}	6.90^{b}
	0.10	96.8^{ab}	115.4^{b}	33.7^{ab}	7.01^{ab}
	0.15	85.9^{b}	110.5^{b}	34.5^{a}	8.01^{a}
SEM		3.7	4.5	2.5	1.20

$^{a, b}$Means within columns with no common superscript differ significantly ($p<0.05$)

Table 4: Effects of *Lactobacillus cultures* on LDH, AST and ALT activity of plasma in broilers

Days	Treatments (*Lactobacillus cultures* in diets %)	LDH (U/L)	AST (U/L)	ALT (U/L)
21	0.00	3200	215	3.10
	0.05	3100	214	2.80
	0.10	2980	217	3.00
	0.15	3050	211	3.20
SEM		215	17	0.21
42	0.00	3290	270[a]	5.15[a]
	0.05	3280	245[a]	4.95[a]
	0.10	3140	220[b]	4.70[ab]
	0.15	3290	215	3.10[b]
SEM		150	17	0.15

[a, b]Means within columns with no common superscript differ significantly ($p<0.05$)

Mohan *et al*. (1996) suggested that of lactic acid bacteria absorbed folic acid in intestine and broilers experience a deficiency in folic acid and due to increasing in hematorict and hemoglobin. Table 4 clearly demonstrated that the probiotic tested in the study significantly decreased ($p<0.05$) ALT and AST level in plasma at 42 days.

These results disagreement with research by Stanley *et al*. (1997) and Sarica *et al*. (2005) who reported that the addition of probiotics (Mannan Oligosaccharides) did not significantly affect AST level but these results is agreement with Yalcinkayal *et al*. (2008) who reported that the addition of probiotics (Mannan Oligosaccharides) significantly reduced ALT and AST of plasma. Lower ALT and AST correlate with better health in animals. *Lactobacillus cultures* reduced both of those enzymes to the range of normal levels which represent the non pathological metabolism of the liver and heart.

CONCLUSION

Conclusively, supplementation of the probiotic (*Lactobacillus cultures*) to broilers improves health and reduces decreased, ALT and AST activity, cholesterol and triglycerides in broiler chickens. The probiotic level of (0.15%) was found better than other levels. This indicates that increasing the probiotic level to 0.15%, in the ration does ensure the best performance.

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