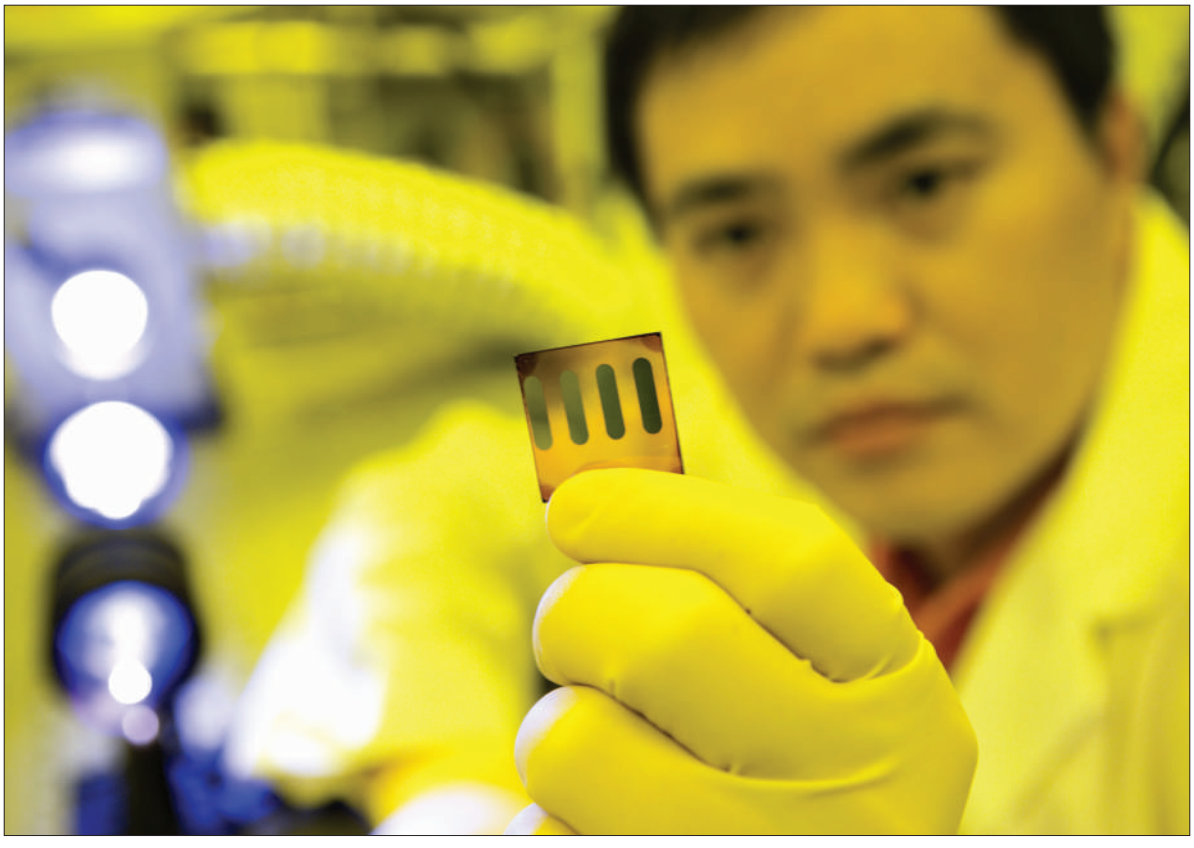

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Research Article
Effect of Probiotics on Digestive Enzymes and Immuno-stimulation in Animal Model (Broilers)

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Abstract

Background and Objective: Avian Leukocytes serve as the first line of defense against invading microorganisms. Infected non-treated group showed leukocytosis, lymphocytosis then lymphopenia and heterophilia. The present study was conducted to isolate *Lactobacillus* spp. from human breast milk and determine their probiotic properties and to evaluate these *Lactobacilli* as probiotics in broiler chicken. **Materials and Methods:** Human milk samples were obtained from healthy volunteers for probiotic isolation and screened their efficiency on *in vivo* system by standard methods. **Results:** Infected non-treated group showed leukocytosis, lymphocytosis then lymphopenia and heterophilia. Probiotic (P23) supplemented groups showed leukocytosis and lymphocytosis. The increased lymphocyte population (4%) and DTH reaction showed in the probiotic supplemented chickens than non-supplemented chickens. Respect to antibody titers against *V. cholerae* antigen probiotic supplemented groups showed HI antibody titers higher than those of probiotic non-supplemented groups. Abnormal increase in serum levels of AST (68.2 U L^{-1}), ALP (77.7 U L^{-1}) and ALT (71.2 U L^{-1}) was observed in infected non-treated group. It may imply liver damage due to infection on the host. Therefore, the relatively stable levels of serum enzymes in probiotic supplemented group may associate with hepato-protective effects of the probiotic isolate (P23). Increased levels of digestive enzymes amylase and protease in the intestinal contents and in pancreatic tissue was observed in probiotic supplemented groups, but lipase activity was not observed in probiotic supplemented groups. **Conclusion:** This finding indicated that the probiotics secrete digestive enzymes that aid in better digestion and absorption of nutrients, there by contributing to promotion of growth.

Key words: Avian leukocytes, probiotics, poultry, *Vibrio cholerae*, immunological study

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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

Poultry industry has been considered one of the most dynamic and ever expanding sectors in the world. It helps to fill the gap between requirement and availability of high quality protein for human consumption. Poultry industry has always been confronted with challenges in the form of various diseases[1], which led to increased use of antibiotics for therapeutic, prophylactic and growth promotion purposes. The presence of antibiotic residues in poultry meat and eggs may have deleterious effects on human consumers. It can cause resistance of human flora and pathogenic microbes to those groups of antibiotics[2]. The most important advantage of a probiotic is that it neither has any residues in animal products, nor exerts any antibiotic resistance by consumption. Microflora regulation may serve to improve feed conversion, weight gain and also improve the intestinal health and immune competence of the chickens.

Direct fed microbials maintain intestinal health by competitive exclusion to prepare population of live obligate and facultative anaerobic bacteria, originating from normal, and healthy adult individuals of an avian species, which is free from specific pathogenic microorganisms. This treatment is intended to reduce the populations of pathogens in the alimentary tract. However, probiotics were found to inhibit tumor growth, enhance systemic antibody production as well as activate innate immunity and systemic immunomodulatory effects were proposed to be an important mechanism for probioitc function. Consequently, probiotic bacteria have been shown to enhance humoral immune responses and thereby promote the intestine's immunologic barrier[3-5]. The probiotics are effective therapeutics agents against the gastrointestinal diseases. Probiotic supplementation aids in repairing the deficiencies in gut flora, balancing intestinal microflora which in turn enhance the resistance against infections[6,7]. Therefore, the aim of this study was to examine the effect of feeding probiotic *L. acidophilus* through drinking water on improving the changes induced by experimental infection of *V. cholerae* on hematological, biochemical and immunological parameters in broiler chicks.

MATERIALS AND METHODS

Study area: Human milk samples were obtained from 10 healthy mother volunteers from different location in Madurai, Tamil Nadu (9.9197°N, 78.1194°E), India, during January, 2013. For sample collection, mothers were asked to carefully clean the mammary areola and breast skin with soap and rinse several times with sterile water. Mother exerted slight pressure on their breast and the first 500 µL of breast milk were discarded collecting the following 2 mL in sterile tubes and stored on ice until delivery to the laboratory. Once deliver to the laboratory, one part of the samples were used for isolation of bacteria and remaining samples were stored in -20°C until further analysis.

From samples probiotic strains were isolated on MRS agar medium. Pure colonies of probiotics were obtained by purification method[8]. *In vitro* assessment of potential probiotic bacterial was done by bile salt resistance (0.3 and 0.5 w/v), tolerance to acidic pH (2.0, 3.0, 4.0 and 5.0) and tolerance to stimulated gastric transit. The antimicrobial activities of the isolate probiotic strains were screened against 16 bacterial organisms and 3 fungal organisms by measuring the diameter of inhibitory zones (mm). One virulent pathogen was selected against potent probiotic strain according to their antimicrobial activity. The selected potent probiotic was administrated to animal model for the period of 21 days and used for further analysis.

Collections of blood samples for clinico-pathological investigation: Blood samples of 2 mL from chicks of each group were collected at weekly intervals. Two blood samples were taken from each bird (Wing Vein). The first blood samples were anticoagulated by di-potassium salt of Ethylene Diamine Tetra Acetic acid (EDTA) and used for evaluating hemogram. The second blood samples were collected in a clean centrifuge tube and allowed to clotting, then centrifuged at 3000 rpm for 10 min for serum separation. The clear non-hemolysed supernatant serum was harvested for biochemical studies and Haemagglutination Inhibition (HI) test for determining serum antibody titers.

Preparation of whole cell bacterial antigen: About 24 h culture of *V. cholerae* was centrifuged at 3000 rpm for 30 min, pellet was washed with PBS and then packed cells were resuspended to desired concentration (2.5×10^5 cells mL^{-1}) in PBS after counting in haemocytometer. This bacterial antigen was further used for serum antibody titer and delayed type hypersensitivity response in broilers.

Antibody titration: Quantification of serum antibodies were carried out by antibody titre plate technique containing respective (*V. cholerae*) antigens. Twenty micro liter of physiological saline was added into all wells of microtiter plate and then 25 µL of antiserum added into the first well of microtiter plate, the antiserum was serially diluted in the well

of the first row till the 11th well of the microtiter plate leaving the 12th well as negative control. Then 25 µL of 1% test antigen was added to all the wells of the microtiter plate. The plate was hand shaken for the effective mixing of reagents and incubated for 1 h at 37°C. The highest dilution of serum samples which shows detectable agglutination was recorded and expressed in log22 of the serum antibody.

Haematological investigation: Total and differential counts of blood cells in chicks were stained with Leishman's strain to show the following appearance to differentiate the blood cells and also confirm the sample stained with Coomassie brilliant blue. Polymorph nuclear neutrophil shows a pinkish cytoplasm filled with nearly uniform tiny granules which take a pink color. The nucleus was usually divided irregularly into five lobes which were commented by five lands. It was a round cell with a distinct nuclear membrane. Eosinophil was distinguished by compact coarse granules with color, circular shape and blobbed nucleus looks like spectacles. Basophil contains purplish granules which were usually intermediate in size between those of the proceeding tubes of cells and were less refractive then the eosinophil granules. Heterophils were characterized by brick red to pale blue granules, elongated intra cytoplasmic granules and bilobed nuclei. Monocytes were larger than a large lymphocyte and the nucleus appears like a kidney and twisted. The cytoplasm has a frostily appearance of five granules. Large lymphocytes were having rounded nucleus and clean baraphilic cytoplasm. Small lymphocytes were having round deeply staining nucleus which almost fills the cell leaving a rim of strongly basophilic cytoplasm.

Delayed Type Hypersensitivity (DTH): The DTH test was performed by the method described by Vaghasiya *et al.*[9] after 6 weeks of experiment. Treated and control birds were injected intradermally in the right footpad with 0.1 mL *V. cholarae* antigen (75 µg bird) diluted 1:1 with sterile PBS as the eliciting challenge the left footpad was injected with PBS alone. The thickness of both footpads at the site of challenge was measured using a vernier caliper prior to challenge. The thickness of each footpad was measured after 24, 48 and 72 h after challenging.

Lymphocyte Migration Inhibition Test (MIT): Blood samples were collected from different group of broilers and was introduced into a sterile conical flask/beaker containing (4-5) sterile glass beads. It was then continuously swirled until no sounds heard from vessel. This indicates that all fibrins have adhered to the beads. This blood was considered as defibrinated blood and diluted with equal volume of physiological saline. Lymphoprep solution (3 mL) was taken in a centrifuge tube using Pasteur Pipette care was taken so that FICON layer of the lymphoprep solution present in the centrifuge tube was then centrifuged at 1600 rpm for 20 min. The interphase (containing Lymphocytes) was removed using pipette. The cells were washed with 1 mL saline and excess FICON was removed. The cells after washing 3 times in hangs balanced salt (HBSS) containing heparin (5 mL) were suspended in eagles's minimum essential medium with 10% borin serum. The viability of the cells were checked by trypan blue dye exclusion method and the concentration have to be adjusted to 1×10^7 cells mL^{-1}. The cells were packed in capillary tubes and fixed in Petri dish to which added medium containing specific antigen then incubated overnight for migration:

$$\text{Migration inhibition index} = \frac{\text{Distance migration with antigen}}{\text{Distance migration without antigen}} \times 100$$

Estimation of immunoglobulin level: The serum sample was centrifuged at 10000 rpm for 30 min at 4°C. The cell debris was discarded and the supernatant was used. The dialyses supernatant was collected into a clean test tube and centrifuge at 15000 rpm for 60 min at 4°C. The pellet was dissolved in 5 mL of borate buffer. The columns were prepared (100×2.5 cm) with sephacryls-200 and equilibrate with borate buffer. The dialyzed supernatant was loaded and allows flow into the column the immunoglobulin was eluted with borate buffer. The fraction was collected and the absorbance was taken at 280 nm using a uv spectrometer. At 280 nm an absorbance of 1.0 is equivalent to an immunoglobulin concentration of 0.74 mg mL^{-1}.

Estimation of serum enzymes: The ALT catalyses the transfer of an amino group from L-alanine to 2-oxoglutarate to form pyruvate and L-glutamate. An increase in pyruvate concentration corresponds with the levels of serum Aspartate Aminotransferase (AST) and estimation of serum alanine aminotransferase (ALT) activities. The pyruvate concentration was determined spectrophotometrically in the form of hydrazone, which was produced by reaction with 2, 4-dinitrophenylhydrazine in an alkaline medium. The pyruvate hydrazone absorbs at 510 nm more than 2-oxoglutarate hydrazine[10].

Two test tubes were taken and marked as test and blank. Both the tubes, 0.5 mL of substrate ALT and 0.5 mL of distilled

water was added and kept for a few minutes at 37°C to attain the room temperature. To the tube marked as test, 0.2 mL of serum was added. Both the tubes were incubated at 37°C for 30 min and 0.5 mL of 2,4-dinitrophenylhydrazine was added and allowed to stand for 20 min followed by the addition of 0.5 mL of 0.4 N NaOH and the colour was developed and read at 510 nm.

Estimation of serum aspartate aminotransferase (AST): Estimation of serum aspartate aminotransferase (AST) was done by Reitman and Frankel[10] method. The AST catalyses the transfer of an amino group from L-aspartate to 2-oxoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate spontaneously decarboxylates to form pyruvate under the strongly acidic conditions. Two test tubes were taken and marked as test and blank. Both the tubes, 0.5 mL of substrate AST and 0.5 mL of distilled water were added and kept for a few minutes at 37°C attain the room temperature. To the tube marked as test, 0.2 mL of serum was added. Both the tubes were incubated at 37°C for 60 min and 0.5 mL of 2,4-dinitrophenylhydrazine were added and allowed to stand for 20 min followed by the addition of 0.5 mL of 0.4 N NaOH and the colour was developed and read at 510 nm.

Simultaneously a set of standards were prepared as follows. To 8 different test tubes 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.70, 0.8 and 0.9 mL of standard pyruvate solution was added. All the tubes were make up to 1 mL with buffer and 0.2 mL of distilled water was added. To all the tubes add 0.5 mL of 2, 4-dinitrophenylhydrazine add 0.5 mL of 0.4 N NaOH. The colour developed was read at 510 nm. A graph was plotted by taking percentage activity along the X-axis and OD along the Y-axis. The activity of enzyme was read directly from the graph.

Estimation of alkaline phosphatase (ALP): Estimation of alkaline phosphatase (ALP) was done by King and Armstrong[11] method. The phosphate present in serum or substrate, disodium phenyl phosphate to yield phenol as a product. In the presence of alkaline oxidizing agents 4-aminoantipyrine gives a purple color with phenol which was read at 520 nm in a colorimeter. Two test tubes were taken and marked as test and blank. Two milliliters of buffered substrate (Disodium phenyl phosphate) was added to the each tube. To the tube marked as test 0.1 mL of serum was added and to the second tube 0.1 mL of distilled water was added. The tubes were incubated exactly for 15 min, simultaneously a serious of standard containing 0.2, 0.4, 0.6 and 0.8 mL of buffered substrate were taken and make up to 1 mL with phenol standards. To all the test tubes 0.8 mL of NaOH and 1.2 mL of $NaHCO_2$ and 1 mL of potassium ferricyanide and 1 mL of 4-aminoantipyrine were added. The colour developed was read at 520 nm using a colorimeter. A standard graph was plotted with percentage of enzyme activity taken in X-axis and OD in Y-axis. The activity of enzyme was read directly from the graph.

RESULTS AND DISCUSSION

Impact of Probiotics on immunomodulation: Differential blood cell counts of control and treated animals were estimated by standard methodology and recorded in Table 1. A significant increase in total leukocytes and lymphocytes count (87) (leukocytosis and lymphocytosis) were observed in probiotic treated group (G2) without change in heterophils count (7) when compared with the control group (G1). Concerning to leukogram, there was significant increase in total leukocyte count (leukocytosis and lymphocytosis) when probiotic was used, this may be attributed to immunostimulatory and immunomodulatory effect of probiotic isolate P23.

Probiotic supplemented (G2) group showed highest level of RBC and WBC when compared to control (G2) group. Increased in RBC and WBC count was observed in probiotic group (G2) and infected treated group (G4) then control group (G1) and infected group (G3), respectively (Fig. 1a-b). Decreased in RBC count (23) showed normochromic anemia in infected non treated group (G3) which may be due to *V. cholerae* endotoxins that suppress to the bone

Table 1: Enumeration of differential blood cells count in different groups of broilers

Differential counts (%)					
Intervals	Lymphocytes	Monocytes	Heterophil	Eosinophil	Basophil
Group 1					
14	71	4	7	2	2
28	73	4	7	2	3
42	73	4	7	2	3
Group 2					
14	72	6	7	0	2
28	80	6	7	0	3
42	87	7	7	1	3
Group 3					
14	68	4	7	3	3
28	89	3	9	3	4
42	70	6	12	5	4
Group 4					
14	68	4	8	2	2
28	71	3	9	3	4
42	74	4	8	2	3

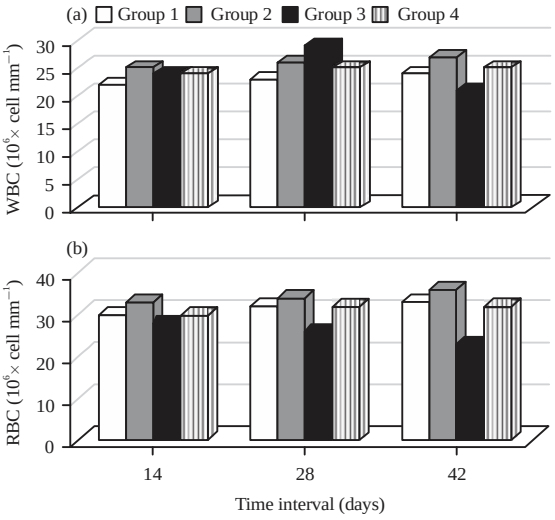

Fig. 1(a-b): Enumeration of blood cells, (a) WBC and (b) RBC in different groups of broilers

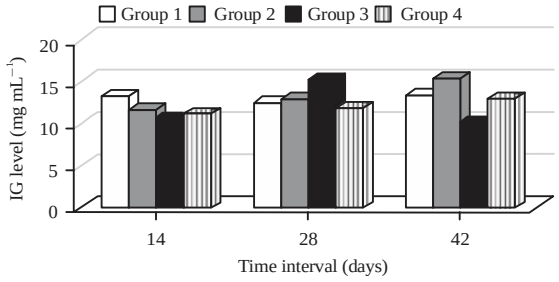

Fig. 2: Enumeration of immunoglobulin levels in different groups of broilers

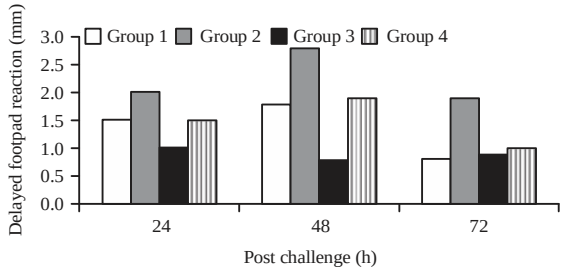

Fig. 3: Evaluation of DTH response in different groups of broilers

marrow. Supplementation of probiotic to infected treated group (G4) returned the phenomena to normal state as, increased in erythrocyte number, hemoglobin and hematocrit value in probiotic treated groups. The results of differential count were shown in Fig. 1a-b.

Table 2: Enumeration of antibody titre in different groups of broilers

	Antibody titre (log22) at different days		
Groups	14	28	42
1	3.2	6.2	7.3
2	3.9	8.0	9.8
3	3.3	4.5	3.4
4	3.9	4.3	5.6

Table 3: Results of migration inhibition test in different groups of broilers

	Migration index (mm) at different days		
Groups	14	28	42
1	0.8	0.7	0.6
2	0.8	0.8	0.3
3	0.9	0.7	0.6
4	0.8	0.7	0.4

Probiotics, particularly may modulate the systemic response of antibodies against antigens in poultry. Supply of probiotics increases the blood and intestinal number of antibodies against different poultry antigens. With respect to the antibody titers against *V. cholerae*, G2 and G4 showed HI antibody titers higher than those of G1 and G3, respectively at 2nd week of age till the end of experimental period (Table 2). As seen in the present study, the serum immunoglobulin level was increased in group G3 and group G4 when compared to control at the end of 42 days (Fig. 2).

Result of cell mediated immunity by migration inhibition test gave an inhibition of migration of leukocyte with significal index and this inhibition was increase in probiotic supplemented group (G2) then control group chicks. At the same time infected treated group (G4) showed increased lymphocyte migration inhibition index than the infected non- treated group. Antigen sensitized lymphocytes secrete cytokine called migration inhibility factor in response to re-challenge with the same antigen. Migration inhibitory factor inhibits the migration of normal monocytes/macrophages (Table 3). The DTH requires the specific recognition of a given antigen by activated T-lymphocytes which subsequently proliferate and release cytokines. Maximum enhancement of DTH response to antigen was observed with probiotic supplemented group (G2). From the results showed in Fig. 3, it was clear that probiotic *L. acidophilus* induced immune modulating effect in the test animal.

Impact of probiotics on digestive enzymes: Aspartate Aminotransferase (AST) and Alkaline phosphatase (ALT) activities of the serum may indicate the liver function and health of the hosts. Cellular injury in liver may increase the

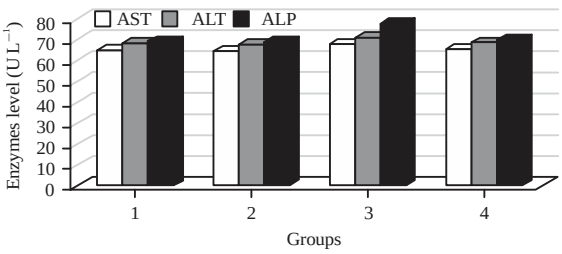

Fig. 4: Estimation serum enzymes in different group of broilers, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase and ALP: Alkaline phosphatase

level of these enzymes in serum. ALT was principally found in the liver and was regarded as being more specific than AST for detecting liver cell damage (Fig. 4).

The increased WBC count could be attributed to improvement in the immune system of the chickens brought about by improved stimulation of different subset of cytokines induced by *Lactobacilli*[12]. The obtained results in agreement with El-Boshy *et al.*[13] reported that, the absolute lymphocyte count values on both 28 and 42 days were significantly increased in supplemented group with probiotic indicating better immune response that could be attributed to immunostimulatory effect of probiotics. The increased lymphocyte populations at 28 days may be indicative of higher activity of humoral immunity in chicks fed probiotic supplemented diet[14]. Infected non-treated group (G3) showed leukocytosis at the 28 day of the experimental period. The leukocytosis detected at group (G3) was characterized by an increase in lymphocyte and heterophil (9) counts, these results were in agreement with El-Boshy *et al.*[13]. This stimulates a strong immune response and induces a type of antigen-antibody reaction that was responsible for the clinical signs. At the same time, the possible presence of the bacterium in target organs such as; the liver, spleen, kidneys, thymus and heart, may stimulate the production and release of leukocytes into the blood stream. But at the end of 42 days, a significant decrease in total leukocyte count (21) and heterophils count (12) (heterophilia) and decrease in lymphocyte count (70) (lymphopenia) were observed in infected non treated group (G3) as compared to the control one. On the other hand, there was significant increase in lymphocyte count (74) and significant decrease in heterophils count (8) was observed in infected treated birds with probiotic (G4) compared to infected non treated group.

These findings agree with those reported by Gheith[15], observed lymphocytic leukocytosis in bacterial infected broiler chicks and probiotics administrated. Leukocytosis could be attributed to the effect of probiotics in stimulation of bone marrow to produce more leukocytes. Also probiotics stimulate immunity to infection by boosting interferon production immunoglobulin concentration and macrophage activity. On the other hand, lymphocytes were the bulky leukocyte in the peripheral blood of most normal chicken that play a major role in the humeral and cell mediated immunity of bird. Therefore, lymphocytosis was suggestive of immunogenic stimulation[16].

Antibody titers against *V. cholerae*, G2 and G4 showed HI antibody titers higher than those of G1 and G3, respectively at 2nd week of age till the end of experimental period. This results were agreed with probiotics had stimulatory effect of humeral immunity. On the same trend Khaksefidi and Ghoorchi[17] detected that antibody production against pathogen in a group of broiler chicks treated with probiotics was significantly higher in that in an untreated group. It was well known that immunoglobulins was usually used to evaluate the immune status of birds due to their important roles in immune function. Serum immunoglobulin level was increased in group G3 and group G4 when compared to control at the end of 42 days. This may be attributed to the effect of probiotics in stimulation of immune system and production of immunoglobulins[18]. Serum immunoglobulin contents were reduced in group G3 when compared to other groups at the end of the 42 days, But immunoglobulin level was increased at the day of 28 in infected group G3 of broilers. The increase in immunoglobulin levels, as verified in the present study during the infection, demonstrates the importance of this immunoglobulin in the elimination of the microorganism through opsonization, complement fixation and promoting the phagocytosis of the bacterium.

Good protection against *V. cholerae* requires as much immunity mediated by T cells as humoral immunity. Data of group G2 and G4 showed significant increase in immunoglobulin level at 6th week of age. Increase of immunoglobulins in these groups may be related to the stimulatory effect of probiotic P23 in increase of humeral immunity. Improvement of liver lesion in group G2 and group G4 by the action of probiotic P23 may be the main cause of increase of immunoglobulins. Gheith[15] achieved increase in immunoglobulins in probiotics administrated broiler chicks. These results agree with probiotic stimulation of the immune system manifested by increased production of immunoglobulins, increased activity of macrophages and lymphocytes and stimulate the production of interferon[19].

Results of this study consistent with those of previous experiments using migration inhibition test as a criterion of cell-mediated reaction in chickens and inhibition of monocyte/macrophage migration[20]. The Delayed Type

Hypersensitivity (DTH) reaction was measured as an indicator of T-cell mediated immunity. DTH was characterized by large influx of nonspecific inflammatory cells, mainly macrophages and it was a part of the process of graft rejection, tumor immunity and most importantly immunity to many intracellular infectious microorganisms, especially those causing chronic diseases[21].

On administration of probiotic an enhanced and visible DTH responses were observed in groups G2 and G4 then control group. Broilers in infected treated group (G4) showed higher DTH response than infected non-treated group (G4) of broilers. The significant difference in the DTH response observed in experimental animals indicates that the *L. acidophilus* has a stimulatory effect on lymphocytes and accessory cells required for the expression of the reaction and thus increases cell mediated immunity. Simultaneously, several investigators demonstrated the potential effect of probiotic on immunomodulation[22].

As showed in Fig. 4, the biochemical analysis revealed that *V. cholerae* infection in chicks resulted in liver damage manifested by increase enzymes activities of AST , ALP and ALT comparing with control group. This was resulted by increase lipid peroxidation of hepatocytes as pathogen induces extensive damage to a variety of organs, including liver due to the increased production of reactive oxygen intermediates[23]. Benzer *et al.*[23] showed a significant increase in the AST and ALT enzyme activities in the infected group at the 5th and 6th weeks of the experiment which may reflect development of hepatic lesions at that time.

The activities of AST, ALP and ALT in probiotic supplemented group G2 were the same as in the control group G1 which indicate that probiotics has no side effect as it not alter biochemical parameters. This result might be due to improvement in the physiological and morphological condition of the liver. In this study, no significant differences between control group and probiotic supplemented groups were observed in the activity of AST and similar results were observed in broiler chickens fed probiotic supplemented diet[24].

The main benefits of probiotics may occurred by preventing production and uptake of lipopolysaccharides in the gut reducing levels of low grade inflammation. Abnormal increase in serum levels of AST, ALT and ALP in infected non- treated group (G3) of broilers may imply liver damage[25]. Therefore, the relatively stable levels of enzymes may be associated with hepato-protective effects of the probiotic *L. acidophilus*.

From the obtained results it was concluded that, probiotic *L. acidophilus* did not induce any harmful effect on the host. Probiotic improve hematological, biochemical and immunological parameters. Probiotic *L. acidophilus* can be considered as an immune-potentiators due to stimulation of immune system and it has the abilities to reduce the adverse effect of *V. cholerae* infection in broiler chicks.

CONCLUSION

This study discovers the potential protease enzyme secreting probiotics isolated from human breast milk that can be beneficial for gain body weight and innate immunity. Improvements in feed conversion efficiency recorded on supplementation with probiotic *L. acidophilus* and their combination of probiotic and pathogen could be due to the same factors responsible for improvement in body weight gain in broiler chickens. Due to restriction in using antibiotic in poultry industry, probiotics represent an alternative tool for antibiotics. Probiotics are known as live microorganisms including bacteria and yeast that have a beneficial effect on the host health by improving its intestinal microbial balance.

SIGNIFICANCE STATEMENT

Probiotics can be effective as antibiotics, they have high efficacy in reducing colonization of *Salmonella*, modulating immunological response and suppress inflammatory reactions in the intestinal walls preventing tissue damage. This study will help the researcher to uncover the critical areas of probiotic prevent attachment of pathogenic bacteria by forming physical barrier on intestinal mucosa, also produce antibacterial compound and enzymes and increase phagocytic population that many researchers were not able to explore. Thus a new theory on prebiotic and probiotic helps better synthesis of vitamins, release of unidentified growth factors, acting as source of nutrients, alteration in morphology, counteraction of toxins, improved immune response, competitive exclusion of pathogenic enteric bacteria and alleviation of stress may be arrived at.

REFERENCES

1. Premalatha, M. and P. Dhasarathan, 2011. Probiotic action of lactobacillus isolated from the milk sample against some human pathogens. Res. J. Immunol., 4: 31-37.
2. Pelicano, E.R.L., P.A. de Souza, H.B.A. de Souza, F.R. Leonel, N.M.B.L. Zeola and M.M. Boiago, 2004. Productive traits of broiler chickens fed diets containing different growth promoters. Braz. J. Poult. Sci., 6: 177-182.

3. Isolauri, E., Y. Sutas, P. Kankaanpaa, H. Arvilommi and S. Salminen, 2001. Probiotics: Effects on immunity. Am. J. Clin. Nutr., 73: 444s-450s.
4. Sujatha, M., P. Dhasarathan and B. Karthick, 2010. Antibiotic susceptibility and immunomodulatory potential of chosen bacterial pathogens. Am. J. Immunol., 6: 15-19.
5. Sujatha, S., S. Tamilselvi, K. Subha and A. Panneerselvam, 2013. Pathogenicity of bacterial isolates to *Catla catla*. Int. J. Curr. Microbiol. Applied Sci., 2: 575-584.
6. Vanderhoof, J.A., 2001. Probiotics: Future directions. Ann. J. Clin. Nutr., 73: 1152S-1155S.
7. Saravanan, S., P. Dhasarathan, V. Indira and R. Venkatraman, 2011. Gastro protective and antioxidant activity of *solanum nigrum* Linn. against aspirin and cold restraint stress induced ulcerated rats. Res. J. Immunol., 4: 1-11.
8. Awan, J.A. and S.U. Rahman, 2002. Microbiology Manual. Unitech Communications, Faisalabad, Pakistan.
9. Vaghasiya, Y.K., V.J. Shukla and S.V. Chanda, 2011. Acute oral toxicity study of *Pluchea arguta* Boiss extract in mice. J. Pharmacol. Toxicol., 6: 113-123.
10. Reitman, S. and S. Frankel, 1957. Photometric methods of estimating serum transaminases. Am. J. Clin. Pathol., 28: 56-61.
11. King, E.J. and A.R. Armstrong, 1980. Method of King and Armstrong. In: Practical Clinical Biochemistry, Volume 1, Varley, H., A.H. Gowenlock, M. Bell (Eds.). 5th Edn., Heinemann Medical Books Ltd., London, UK., ISBN-13: 9780433338055, pp: 897-899.
12. Christensen, H.R., H. Frøkiær and J. Pestka, 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol., 168: 171-178.
13. El-Boshy, M.E., A.M.M. El-Ashram and N. Abdel-ghany, 2008. Effect of dietary β-1,3 glucan on immunomodulation on diseased *Oreochromis niloticus* experimentally infected with Aflatoxin B_1. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-14, 2008, Cairo, Egypt, pp: 1109-1127.
14. Paryad, A. and M. Mahmoudi, 2008. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. Afr. J. Agric. Res., 3: 835-842.
15. Gheith, E.M., 2008. Clinicopathological studies on effect of probiotics on broiler. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.
16. Thrall, M.A., D.C. Baker and E.D. Lassen, 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins, Philadelphia, USA., ISBN-13: 9780781768504, Pages: 618.
17. Khaksefidi, A. and T. Ghoorchi, 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. J. Poult. Sci., 43: 296-300.
18. Elmer, G.W., 2001. Probiotics: "Living drugs". Am. J. Health-Syst. Pharm., 58: 1101-1109.
19. Yang, Y., P.A. Iji and M. Choct, 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J., 65: 97-114.
20. Chubb, R., V. Huynh and R. Bradley, 1988. The induction and control of delayed type hypersensitivity reactions induced in chickens by infectious bronchitis virus. Avian Pathol., 17: 371-383.
21. Pitsios, C., E.C. Stefanaki and A. Helbling, 2010. Type IV delayed-type hypersensitivity of the respiratory tract due to budesonide use: Report of two cases and a literature review. Primary Care Respir. J., 19: 185-188.
22. Nayebpor, M., P. Farhomand and A. Hashemi, 2007. Effects of different levels of direct fed microbial (*Primalac*) on growth performance and humoral immune response in broiler chickens. J. Anim. Vet. Adv., 6: 1308-1313.
23. Benzer, F., A. Kilic, S. Yilmaz, M. Erisir, N. Timurkaan and H.B. Ertas, 2009. Influence of enrofloxacin administration on oxidative stress and antioxidant enzyme activities of experimentally infected broilers with *Salmonella enterica* serovar enteritidis. e-J. New World Sci. Acad. Vet. Sci., 4: 24-33.
24. Oyetayo, V.O., F.C. Adetuyi and F.A. Akinyosoye, 2003. Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. Afr. J. Biotechnol., 2: 448-452.
25. Yalcin, S., K. Uzunoglu, H.M. Duyum and O. Eltan, 2012. Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) and black cumin seed (*Nigella sativa* L.) on performance, egg traits, some blood characteristics and antibody production of laying hens. Livestock Sci., 145: 13-20.