IMMUNO-MODULATORY EFFECTS OF LACTOBACILLUS IN *SALMONELLA GALLINARUM* INFECTED BROILER CHICKS

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ABSTRACT: *Lactobacillus* is a direct fed microbial which helps to protect organisms against pathogens by discouraging their colonization in intestine. This project had been designed to evaluate the effect of *Lactobacillus* in broiler chicken experimentally infected with *S. gallinarum*. A total of 100 day old chicks divided into 4 equal groups (A, B, C & D) comprising 25 chicks in each group. Birds of groups B and D were infected with *S. gallinarum* orally at day 19th of age. Group A was kept as control negative and group C and D were supplemented with *Lactobacillus.* Disease combat efficiency in each group was determined by monitoring weight gains, morbidity & mortality rates, immune parameters and histopathological findings. Analysis of variance (ANOVA) technique was used to compare results by using MSTAT C statistical software. The results revealed that morbidity and mortality rates were more in group B and significantly low in group C (*Lactobacillus* supplemented) and D (Treatment group). The antibody response was found to be highest in group C followed by group D. Body weight gains and relative organ weight gains were also higher in treated groups with maximum in the group C followed by group D. Histopathology studies revealed that treated groups showed less lesions and untreated infected group showed all classical lesions of the disease. The study concluded that *Lactobacillus* can be very efficient as probiotic for prophylactic immune-stimulation to replace antibiotics being used irrationally as prophylaxis.

Key words: Immuno-modulatory, *Lactobacillus*, Probiotics, *Salmonella gallinarum*, Broiler chicks.

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INTRODUCTION

Poultry sector is one of the most vibrant sub-sectors of livestock. The current investment in poultry industry is more than Rs.700 billion. This industry is growing at the rate of 8% per annum over the last few years. Pakistan is the 11th largest producer in the world with 1.163 million broilers annually and also provides employment to more than 1.5 million people (Anonymous, 2019-2020). A person requires 102.7 grams of protein on daily basis but almost 66% of Pakistani population is deficient in animal protein source (Abedullah, 2007). Poultry industry is vulnerable to many infectious and non-infectious diseases which are the main obstacle in the development of poultry industry. Among communicable diseases, Fowl Typhoid is an important disease which is caused by *S. enteric* serovar *Gallinarum* (*S. gallinarum*). *S. gallinarum* is facultative anaerobe, non-motile, rod shaped and gram-negative bacterium which is host specific in nature. The most prominent clinical signs in Fowl Typhoid are sulpher colored diarrhea, dehydration and in layers it leads to sudden drop in egg production (Dey *et al*., 2016).

The accessibility of antibiotics to be used for treatment of infective diseases has considerably improved the human health and animal welfare. The massive misuse of antibiotics causes the development of antimicrobial resistance in commensal and pathogenic bacteria (Carattoli 2008, Depoorter *et al*., 2012). Antibiotic residues in the poultry meat and drug resistance are basic threats for antibiotic use for curative and preventive purposes. Now it is much needed to use alternative of antibiotics, not as prophylactic measure but also for therapeutic purpose. Use of prebiotics, probiotics, organic acids and plant extracts can overcome this problem (Griggs *et al*., 2005). Probiotics can improve growth of beneficial bacteria by inhibiting the multiplication of pathogenic organisms (Li *et al*., 2018).

Lactobacillus supports intestinal epithelial tissues of the host against pathogenic bacteria (Klaenhammer *et al*., 2000). *Lactobacillus* is a micro-aerophilic anaerobe and gram positive bacteria which has

ability to proliferate at low pH. It has beneficial effects on gut of animal by competitive exclusion of pathogenic microbes and by producing lactic acid. It can also promote the production of antibodies by stimulating host humoral immune response and can trigger T-cells of immune system (Talebi *et al*., 2008). In poultry, *Lactobacillus* heat killed strains like LAH7, LAP5, LAF1 have the ability to adhere with GIT epithelium and protect the host from infectious micro-organism. In return it inhibits the growth of *Salmonella* and *E. coli* (Jin *et al*., 1998). Other strain such as LF33 also has the ability to adhere with intestinal cells and inhibit the activity of *E. coli*, *Staphylococcus aureus* and *S. typhimurium*. Thus the issue of the antimicrobial resistance and antibiotic residues in the poultry can be minimized by the use of probiotics in poultry.

The current project was designed to use *Lactobacillus* as probiotic in poultry feed to determine the efficacy of *Lactobacillus* against *S. gallinarum* infection, immunomodulatory action of *Lactobacillus* supplementation and pathological changes in broiler chicks.

MATERIALS AND METHODS

Lactobacillus samples were collected by mixing 1 ml of yogurt with 10 ml of normal saline. For its propagation, MRS (de Man, Rogosa, Sharpe agar) media was used which is selective for *Lactobacillus*. For MRS agar 62 grams of MRS powder was mixed in one liter of water. Ten ml of this mixture was poured in petri dish. MRS broth was prepared by mixing 52 grams of MRS powder in one liter water in Pyrex flask. Ten ml of this mixture was poured in test tube. Media was sterilized at 121^{o}C for 20 minutes at 15lbs atm. Yogurt mixture was streaked on four plates from every batch and kept at 37^{o}C and 25^{o}C respectively. After 72 hours plates were examined and absence of growth showed sterility (Cruickshank, 1975).

For identification of *Lactobacillus* bacterial smear was prepared by applying a drop of bacterial suspension on a glass slide. It was flooded with 1.0% of crystal violet followed by dipping in 5% $NaHCO_3$ solution for 3 minutes. Gram iodine solution was poured on slide for 2 minutes followed by decolorisation with acid alcohol for 10 seconds. Glass slide was tilted and counter stain i.e. safranin was used for 30 seconds, slide was washed, air dried and observed under 40X and 100X oil immersion lense.

A total of 100 day old chicks were purchased from hatchery and divided into four groups (A, B, C & D) comprising 25 chicks in each group. All chicks were vaccinated against ND, IBV, IBD (D78, 228E) and HPS. Feed and water were provided ad libitum. The birds in group A were kept as negative control, group B as positive control, group C infected with *Lactobacillus* at the dose rate of @10^9 Colony Forming Units (CFU) and group D infected with *Lactobacillus* and *S. gallinarum* at dose rate of @10^9 CFU and 10^8 CFU respectively on 19th day of experiment through oral route. Afterwards infection was induced, seven birds were culled weekly from each group and blood was collected for hematological evaluation. For histopathological studies organs were preserved in 10% buffered formalin solution (Bancroft and Gamble., 2007).

Broiler birds were kept under observation for 6 weeks. Clinical signs, gross lesions and organs weight were observed. At 21st, 28th, 35th and 42nd days, 6 birds were slaughtered from each group and organs having lesions were recorded. For histopathological studies organs including spleen, bursa of fabricius, thymus, liver and heart were preserved in 10% buffered formalin (Bancroft and Gamble., 2007).

Immunological parameters including IgG, IgM and IgA were measured by commercial ELISA kit. EDTA added blood samples were collected from each group, hematological parameters including total erythrocyte counts (TEC), total leukocyte counts (TLC), packed cell volume (PCV) and hemoglobin concentration (Hb) were estimated.

Statistical analysis: Analysis of variance technique and means were compared by DMR, ANOVA test by using MSTAT C statistical software.

RESULTS

Isolation and Identification of Bacteria: The colonies of *Lactobacillus* were observed which were raised, whitish in color and had sand like appearance. Microscopically rod shaped, purple colored and gram positive bacteria were seen (Fig. 1).

Feed intake: Feed intake was significantly increased in the birds of control negative, treatment group and supplemented with *Lactobacillus* alone as compared to the birds of control positive group (Table 1).

Morbidity and Mortality Percentage: Clinical signs like depression, diarrhea, ruffled feathers and anemia were observed in infected and treated groups (Fig. 2). Percentage of clinical signs were higher in infected group as compared to the treatment. Group A was kept as control group, no infection was given and it was kept in a separate room. Morbidity and mortality were not seen in this group. Chicks of group B showed highest morbidity (52%) and mortality (48%) due to *S. gallinarum* infection as compared to all other groups. No morbidity and mortality were observed in group C. In the birds of group D 28% morbidity and 24 % mortality were observed (Table 2).

Hematological Parameters: Total erythrocytes count, Hemoglobin (Hb) concentration and Packed cell volume percentage were significantly decreased in infected birds as compared to other treated groups. There was non-significant relationship between control negative (group A) and supplemented group (group C). Total leukocytic count was significantly increased in infected group as compared to other treated groups and there was no significant difference between control negative group A and Lactobacillus supplemented group C (Table 3).

Body Weight and Organs Weight Parameters: Body weight gain was significantly decreased in infected group as compared to treated group but there was non-significant relationship between control negative and supplemented group. Absolute and relative weight of spleen (Table 4), thymus (Table 5), bursa (Table 6), kidney (Table 7) and liver (Table 8) were significantly increased in infected group (control positive) as compared to the treated group (group D) while non-significant relation between control negative and supplemented group were recorded.

Immunomodulatory Effects: *Lactobacillus* was tested as a probiotics with possible immunomodulatory effects to combat *S. gallinarum* infection. The IgG and IgA levels were found to be higher in *Lactobacillus* treated groups rather than control groups (Table 9).

Gross Lesions: Seven birds from group A were culled on every week. Birds of this group were non infected and non-supplemented. Thus no postmortem lesions were seen in this group. Postmortem was organized for mortality birds to examine lesions of fowl typhoid. Group B showed severe lesions in heart (pericarditis), liver (perihepatitis) and spleen (splenomegaly). In group C supplemented with *Lactobacillus*, mild postmortem lesions were seen. In this group only pericarditis was seen. In group D, pericarditis and perihepatitis were seen during postmortem of chickens at 7^{th}, 14^{th} and 21^{st} day post infection (Fig. 3).

Histopathology: Birds of group B and D exhibited microscopic changes on histopathological examination. Lymphocyte proliferation and degenerative changes were seen under microscope. Hemorrhagic spots and congestion were also seen in the infected birds. Birds of control positive and treatment plus infected groups showed microscopic alterations upon histopathological examination in lungs. Marked increase in congestion due to rupture of inter alveolar septa was observed (Fig. 4).

Table 1. Feed intake (gm) of broiler birds supplemented with *Lactobacillus* and infected with *S. gallinarum*.

Weeks	Group A	Group B	Group C	Group D
1st	18.60±6.83[a]	17.91±6.05[a]	18.31±6.92[a]	18.66±6.88[a]
2nd	54.56±13.55[a]	52.2±10.05[a]	56.59±18.91[a]	52.66±14.55[a]
3rd	105.48±10.84[bc]	92.16±6.88[c]	122.27±8.44[a]	113.51±14.88[ab]
4th	152.88±15.20[a]	95.01±10.98[b]	159.14±18.21[a]	158.66±11.05[a]
5th	177.80±4.29[b]	120.10±3.05[c]	192.45±7.40[a]	188.67±6.97[a]

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infection with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 2. Percentage morbidity and mortality in experimental groups.

Group	Morbidity		Mortality	
	No.	%	No	%
A	-	-	-	-
B	13	52	12	48
C	-	-	-	-
D	7	28	6	24

A= No Infection (Control –Ve)
B= Infection with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 3. Red blood cells count (10^6), Total leukocyte Count (10^3), Pack cell volume (%) and Hemoglobin concentration (g/dl) in *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus*

Days post Infection	Hematology Parameters	Groups			
		A	B	C	D
7	RBC	3.12±0.011^{bc}	2.50±0.098^{e}	3.38±0.075^{a}	2.90±0.026^{d}
	TLC	22.55±0.074^{gh}	32.85±0.139^{a}	21.85±0.711^{h}	28.66±0.186^{b}
	PCV	35.99±0.19^{bc}	26.79±0.49^{g}	37.67±0.33^{a}	33.21±0.26^{d}
	Hb	11.53±0.081^{def}	8.97±0.115^{g}	12.56±0.149^{ab}	11.45±0.185^{ef}
14	RBC	3.08±0.014^{bc}	2.33±0.043^{f}	3.17±0.049^{b}	2.90±0.025^{d}
	TLC	25.47±0.158^{e}	29.17±0.115^{b}	24.67±0.309^{ef}	25.81±0.273^{de}
	PCV	32.63±0.10^{de}	23.76±0.13^{h}	36.81±0.41^{b}	31.20±0.30^{f}
	Hb	12.16±0.092^{bc}	7.986±0.118^{h}	12.68±0.109^{a}	11.34±0.243^{f}
21	RBC	3.01±0.018^{cd}	2.32±0.041^{f}	3.05±0.031^{bc}	2.88±0.043^{d}
	TLC	23.81±0.106^{fg}	28.20±0.141^{bc}	22.81±0.346^{gh}	27.03±1.501^{cd}
	PCV	30.54±0.10^{f}	22.97±0.09^{h}	32.11±0.35^{e}	35.75±0.45^{c}
	Hb	11.66±0.108^{def}	7.13±0.163^{i}	11.88±0.142^{cd}	11.86±0.184^{cde}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control–Ve)
B= Infection with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*
D= Challenged with *S. gallinarum* and challenged with *Lactobacillus*

Table 4. Absolute and relative weight (gm) of Spleen of *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus.*

Days Post Infection	Weight (gm)							
	A		B		C		D	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
7	1.40±0.216^{fg}	0.193±0.011^{bc}	2.84±0.453^{b}	0.413±0.026^{a}	1.56±0.127^{ef}	0.177±0.007^{bcd}	11.69±0.090^{de}	0.194±0.006^{bc}
14	1.49±0.135^{efg}	0.121±0.006^{cd}	2.96±0.270^{b}	0.264±0.015^{b}	1.37±0.364^{fg}	0.097±0.010^{d}	1.21±0.168^{g}	0.097±0.007^{d}
21	1.93±0.206^{cd}	0.097±0.006^{d}	3.69±0.406^{a}	0.219±0.018^{b}	2.057±0.151^{c}	0.094±0.003^{d}	2.17±0.180^{c}	0.109±0.006^{cd}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infection with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*10^9 CFU orally
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 5. Absolute and relative weight of Thymus (gm) in *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus.*

Days Post Infection	Weight (gm)							
	A		B		C		D	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
7	1.94±0.270 ^{cd}	0.269±0.017 ^{b}	1.64±0.172 ^{f}	0.243±0.009 ^{b}	2.29±0.241 ^{a}	0.260±0.011 ^{b}	2.34±0.215 ^{a}	0.271±0.007 ^{b}
14	2.03±0.099 ^{bcd}	0.164±0.009 ^{b}	1.80±0.064 ^{def}	0.163±0.012 ^{b}	1.89±0.248 ^{de}	0.131±0.005 ^{b}	1.69±0.177 ^{ef}	0.133±0.005 ^{c}
21	2.16±0.223 ^{abc}	0.109±0.006 ^{b}	1.40±0.216 ^{g}	0.083±0.007 ^{b}	2.35±0.418 ^{a}	0.110±0.008 ^{a}	2.20±0.141 ^{ab}	0.111±0.008 ^{a}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infected with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*10^9 CFU
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 6. Absolute and relative weight of Bursa (gm) in *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus*

Days Post Infection	Weight (gm)							
	A		B		C		D	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
7	1.84±0.140 ^{bc}	0.253±0.00 ^{8b}	1.46±0.151 ^{e}	0.213±0.010 ^{b}	1.787±0.205 ^{bc}	0.203±0.00 ^{8b}	1.74±0.151 ^{cd}	0.197±0.005 ^{b}
14	1.91±0.195 ^{bc}	0.154±0.005 ^{b}	1.54±0.190 ^{de}	0.139±0.012 ^{b}	1.84±0.162 ^{bc}	0.129±0.003 ^{b}	1.56±0.207 ^{de}	0.126±0.011 ^{c}
21	2.16±0.223 ^{a}	0.109±0.006 ^{b}	1.40±0.216 ^{e}	0.083±0.007 ^{b}	2.15 ±0.127 ^{a}	0.099±0.001 ^{a}	1.97±0.302 ^{ab}	0.099±0.001 ^{a}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infected with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*10^9 CFU
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 7. Absolute and relative weight of Kidney (gm) in *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus*

Days Post Infection	Weight (gm)							
	A		B		C		D	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
7	7.87±0.515 ^{b}	1.090±0.030 ^{abc}	9.21±0.438 ^{b}	1.351±0.029 ^{ab}	8.23±0.457 ^{b}	0.937±0.027 ^{abc}	8.26±0.680 ^{b}	0.951±0.015 ^{abc}
14	8.70±0.480 ^{b}	0.709±0.034 ^{bc}	10.53±0.559 ^{b}	0.941±0.053 ^{abc}	7.47±0.640 ^{b}	0.521±0.017 ^{bc}	22.79±34.958 ^{a}	1.757±0.997 ^{a}
21	14.14±2.031 ^{ab}	0.716±0.024 ^{bc}	16.91±1.783 ^{ab}	0.990±0.055 ^{abc}	9.90±0.305 ^{b}	0.457±0.004 ^{c}	11.27±0.846 ^{b}	0.566±0.038 ^{bc}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infected with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*10^9 CFU
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

Table 8. Absolute and relative weight of liver (gm) in *S. gallinarum* infected broiler chicks supplemented with *Lactobacillus.*

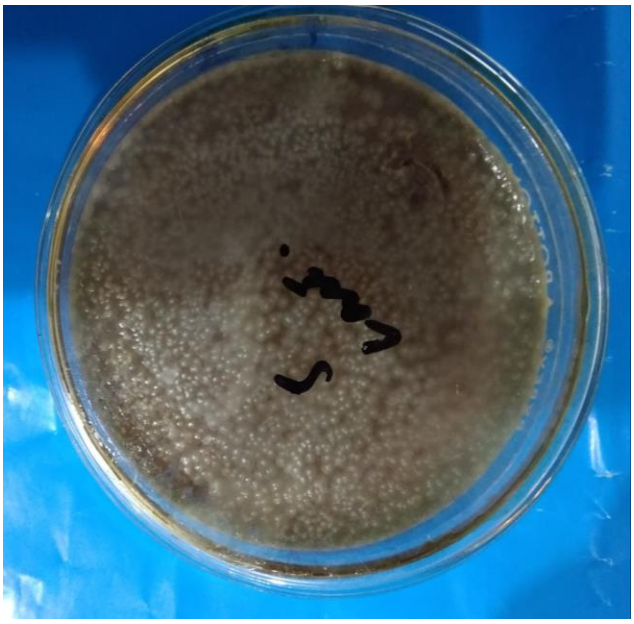
Days Post Infection	Weight (gm)							
	A		B		C		D	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
7	35.44 ± 0.736^{def}	4.904 ± 0.141^{b}	46.26 ± 1.310^{c}	6.783 ± 0.181^{a}	31.98 ± 0.337^{f}	3.646 ± 0.094^{bcd}	33.17 ± 0.170^{ef}	3.830 ± 0.104^{bc}
14	36.67 ± 0.733^{de}	2.971 ± 0.114^{cde}	53.00 ± 2.93^{b}	4.711 ± 0.273^{b}	38.84 ± 0.563^{d}	2.719 ± 0.079^{cde}	35.53 ± 1.705^{def}	2.800 ± 0.076^{cde}
21	50.59 ± 1.413^{bc}	2.569 ± 0.049^{cde}	75.86 ± 3.865^{a}	4.440 ± 0.289^{b}	48.43 ± 0.95^{bc}	2.237 ± 0.034^{e}	47.17 ± 0.922^{c}	2.354 ± 0.100^{de}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infection with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*10^9 CFU
D= Infected with *S. gallinarum* and Supplemented with *Lactobacillus*

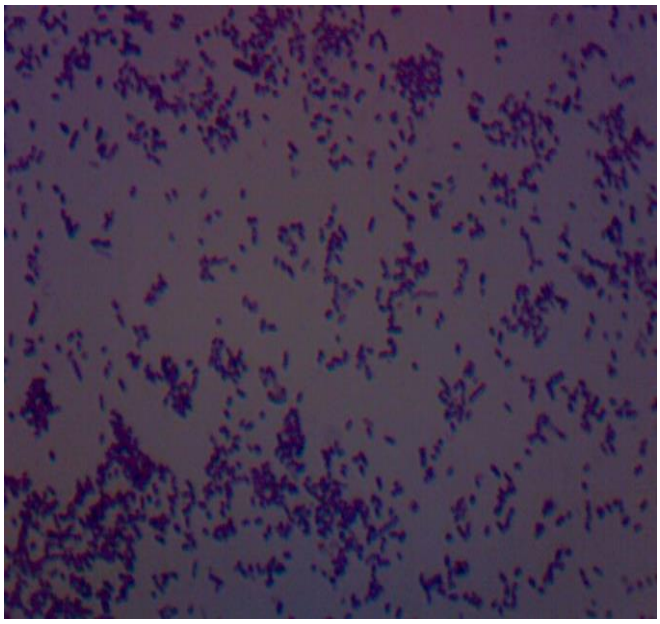
Table 9. Immunoglobulin A (IgA) and Immunoglobin G (IgG) in broiler chicks supplemented with *Lactobacillus* and infected with *S. gallinarum.*

Days Post Infection	Group A		Group B		Group C		Group D	
	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG
7^{th}	0.39 ± 0.007^{ef}	4.25 ± 0.056^{cd}	0.28 ± 0.007^{g}	2.89 ± 0.095^{e}	0.49 ± 0.08^{ab}	5.18 ± 0.048^{bc}	0.35 ± 0.007^{cd}	3.34 ± 0.052^{de}
14^{th}	0.49 ± 0.006^{cd}	5.28 ± 0.066^{bc}	0.035 ± 0.005^{ef}	3.26 ± 0.034^{de}	0.56 ± 0.008^{abc}	6.19 ± 0.082^{ab}	0.48 ± 0.010^{ab}	4.22 ± 0.048^{cd}
21^{th}	0.60 ± 0.008^{ab}	6.39 ± 0.105^{ab}	0.46 ± 0.007^{de}	4.28 ± 0.114^{cd}	0.64 ± 0.007^{cd}	7.14 ± 0.084^{a}	0.54 ± 0.013^{bcd}	5.48 ± 0.274^{bc}

Mean ± SE having similar alphabets in a row are statistically non-significant (P> 0.05)
A= No Infection (Control –Ve)
B= Infected with *S. gallinarum* (Control +Ve)
C= Supplemented with *Lactobacillus*
D=Challenged with *S. gallinarum* and supplemented with *Lactobacillus*



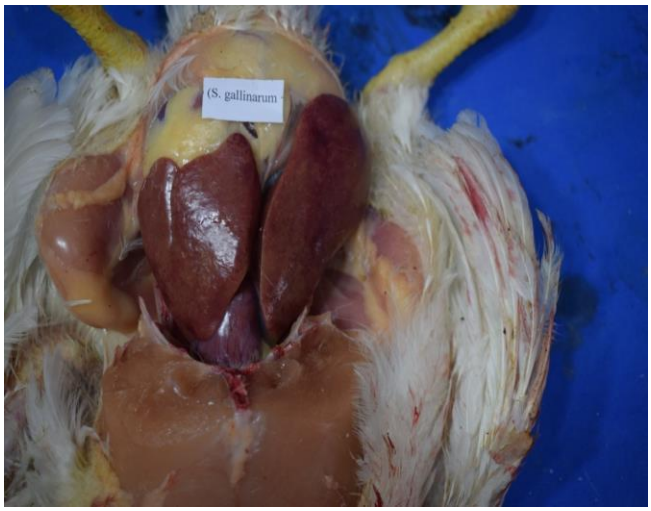
White opaque colored colonies of *Lactobacillus* on MRS media



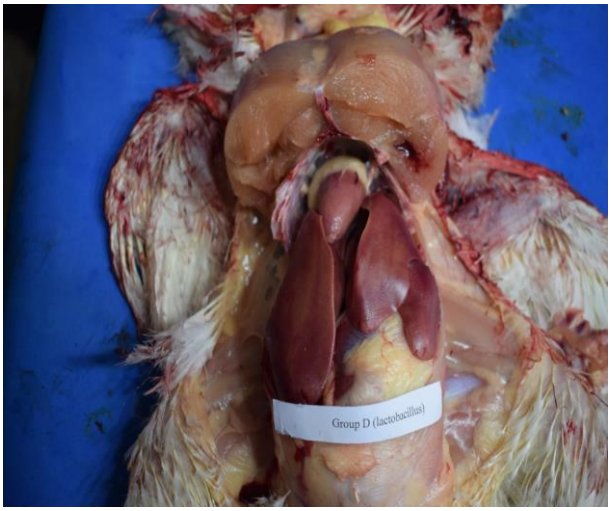
Purple stained *Lactobacillus* by Gram staining

Figure1. Isolation and identification of *Lactobacillus* .

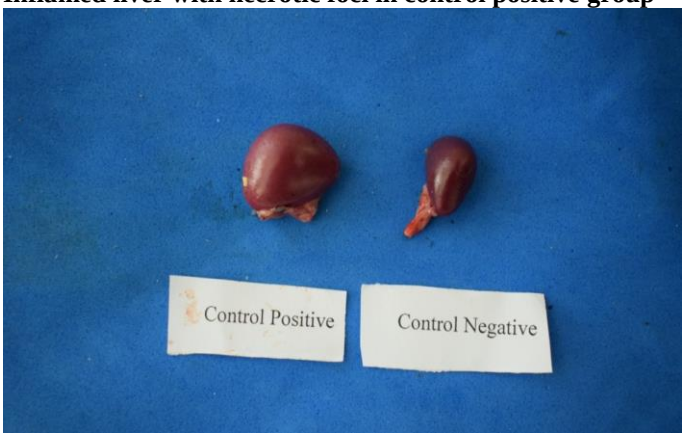
Figure 2. Depressed birds with ruffled feathers from treatment group and healthy group.



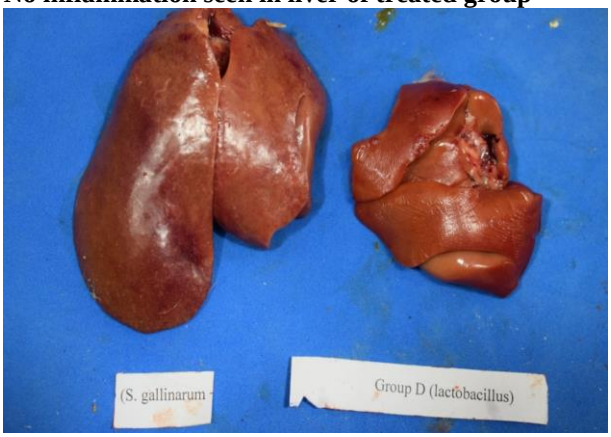
Inflamed liver with necrotic foci in control positive group



No inflammation seen in liver of treated group

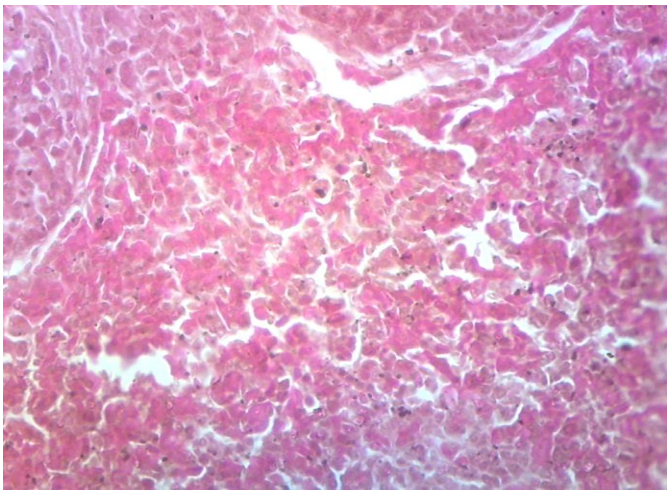


Difference in size of spleen in control positive and control negative group

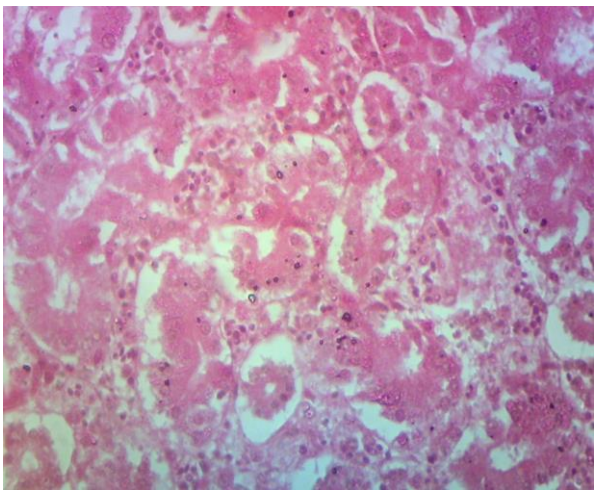


Difference of liver size in control positive and treatment groups

Figure 3. Lesions seen in different organs.



Photomicrograph of liver showing diffuse hemorrhage and inflammatory cellular infiltration



Photomicrograph of lung tissue showing inflammatory cellular infiltration.

Figure 4. Histopathological changes in liver and lung.

DISCUSSION

Poultry industry is one of the largest agro based sector of Pakistan established in 1962. A total of 45-50 percent meat demand is being fulfilled by poultry. Therefore it is necessary to develop poultry industry on commercial basis to meet the baseline requirement of meat and eggs. Poultry industry is exposed to many infectious and non-infectious diseases which are the main obstacle in the development of poultry industry (Hussain *et al*., 2015). Among infectious diseases fowl typhoid is a major disease caused by *Salmonella gallinarum* and it causes lesions on multiple visceral organs. The disease is responsible for high morbidity and mortality rates in chicken flocks worldwide (Riaz and Aslam, 2016).

Recently farmers are using antibiotics irrationally to overcome their fear of loss of their chicken flocks. Now the concept of antibiotic free meat has obtained strength and the demand for antibiotic free chicken has increased. Therefore stress is being given on finding alternatives to antibiotics and major breakthrough are prebiotics and probiotics (Patterson and Burkholder, 2003). Probiotics provide a rational alternative to antibiotic use. Among probiotics, the *Lactobacillus* species are most common, found in feed such as yogurt, cheese, beer and fermented food etc. Many probiotics along with their feasibility are safe and effective for treatment. Tellez *et al*. (2012) reported *Lactobacillus* to be very safe by not causing super-infections and also found to elicit up to 201 genes for immune stimulation response.

The objective of this trial was to evaluate the effects of Lactobacillus supplementation on weight gain, pathology, hematology and immune response of broiler chicks infected with field isolate of the *S. gallinarum.* Feed intake and body weight of *Lactobacillus* supplemented groups were significantly increased as compared to *S. gallinarum* infected groups. Probiotic (*Lactobacillus*) has beneficial effect on weight gain of broiler chickens and similar findings were recorded by AN *et al.* (2008). The results are also in accordance with the findings of Marota et al. (2019) who illustrated the beneficial effects of Probiotics on public health.

The infected groups showed clinical signs including off feed, ruffled feathers, depression, lethargy, lameness and being unable to stand. While in group D (treatment group), dull physical appearance and leg weakness were prominent signs. No clinical signs were observed in group A (control negative) and group C (only *Lactobacillus* supplemented group). Mild clinical signs were seen in group D because that was supplemented with *Lactobacillus*. Similar results were reported by Griggs and Jacob (2005) that supplementation of *Lactobacillus* as probiotic in chicken led to significant decrease in clinical signs against Salmonellosis.

There was decrease in the morbidity and mortality rates in all of *Lactobacillus* treated groups as compared to the control group. Same findings were seen by Khan *et al.* (2010) who reported about the supplementation of 40 mg/kg of *Lactobacillus* against *S. gallinarum* infected broiler chicks and observed decrease in mortality rate.

Absolute weight of liver, spleen and heart were significantly decreased in treated groups which were supplemented by *Lactobacillus* as compared to other non-treated groups. Similar findings were reported by Huff *et al*. (2006) that after 25 days of *Lactobacillus* supplementation, absolute weight of the heart, spleen and liver were decreased against *S. gallinarum* infected broiler birds.

Birds which were supplemented by *Lactobacillus* and challenged by *S. gallinarum*, WBCs were increased as compared to the control negative group. Same results were observed by Dong *et al*. (2013) who reported that WBCs count was significantly increased from 4.08 to 4.55 after the *Lactobacillus* supplementation. Hemoglobin concentration (Hb) was increased significantly in *Lactobacillus* supplemented birds. Same results were reported by Sharaity *et al.* (2017) who observed increase in hemoglobin concentration in probiotic supplemented birds.

There were severe hepatitis, nephritis and splenomegaly in broiler chicks of control positive (infected with *S. gallinarum*) group while in group A (non-infected) broiler chicks no lesions were observed. These findings were also in agreement with the results of Tonu *et al.* (2011). *Lactobacillus* was tested as a probiotics with possible immunomodulatory effects to combat *S. gallinarum* infection. The IgG and IgA levels were found to be higher in *Lactobacillus* treated groups rather than control groups. *Gill et al*. (2001) found similar results and reported immunomodulatory effects of probiotic (*Lactobacillus*) against *E. coli* infection given to mice.

Conclusion: The present study concludes that supplementation of *Lactobacillus* @10^9 CFU has positive effects on the growth performance, decreased morbidity and mortality rates of broiler chicks during fowl typhoid. At the same time *Lactobacillus* also helps to increase the immunoglobulin level in blood and improves the health of birds.

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