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RESEARCH ARTICLE

Probiotic Effect of *Limosilactobacillus fermentum* on Growth Performance and Competitive Exclusion of *Salmonella* Gallinarum in Poultry

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A B S T R A C T

Fowl typhoid, an acute or chronic systemic infection of poultry is caused by *Salmonella* Gallinarum. It causes considerable economic losses in poultry in different countries including Pakistan. The aim of this study was to evaluate the effect of two previously characterized indigenous *Limosilactobacillus fermentum* strains (PC-10 and PC-76) on competitive exclusion of *Salmonella* Gallinarum in poultry gut. Day old chicks (n=90) were equally divided into six groups. Group 1 was kept as negative control and group 2 was positive control. Birds in group 3, 4 and 5 were administered with *Limosilactobacillus fermentum* strains PC-10, PC-76 and commercial probiotic, respectively from day 1-35. Group 6 was treated with antibiotic (florfenicol 30mg/kg) following the post infection of *S.* Gallinarum. All experimental groups except the negative control were challenged with *S.* Gallinarum (10^8CFU/bird) on day 21. The effect of probiotics on gut microbiota; *Lactobacillus*, total coliform and *Salmonella* were enumerated before and after the administration of challenge organism. Weight gain, feed conversion ratio and immune response to Newcastle disease and infectious bursal disease vaccine were determined. The results showed that *Limosilactobacillus fermentum* PC-10 and PC-76 significantly ($P<0.05$) decreased the growth of *S.* Gallinarum (3.92±0.37 vs 3.99±0.22 $\log_{10}$CFU/g) in comparison to positive control group (6.88±0.2$\log_{10}$ CFU/g) on day 35. The administration of these probiotics led to a significant increase in *Lactobacillus* count (>2log10) and reduction in coliforms count (1-2log10) in broilers. The probiotic fed groups exhibited less lesion scores and mortality rates compared to positive control group. Moreover, the broiler bird fed with *Limosilactobacillus fermentum* PC-10 and PC-76 group exhibited higher weight and immune response. Based on these findings, it was concluded that *Limosilactobacillus fermentum* PC-10 and PC-76 may be used as potential probiotics for targeted mitigation of *S.* Gallinarum in broilers.

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INTRODUCTION

Fowl typhoid a septicemic infection in poultry, is caused by *Salmonella* enterica subspecies enterica serovar Gallinarum. It causes significant economic losses in poultry sector due to high morbidity and mortality (Spickler, 2019). However, the global burden of this disease still remains unknown (Zhou *et al.,* 2022). In developed countries including United States and Canada, fowl typhoid has been eradicated from commercial poultry through the implementation of biosecurity measures, maintaining the hygienic practices, and screening and removal of infected flocks (Desin *et al.*, 2013). However, it remains endemic in many developing countries including South America and Asia where inadequate control measures and favorable climatic conditions facilitate the spread of *Salmonella* Gallinarum in environment (Barrow and Neto, 2011).

The *Salmonella* Gallinarum is host restricted pathogen of poultry that has less zoonotic importance (Andino and Hanning, 2015). It primarily caused infection in chicken while seldom cause infection in other hosts such as pheasants, quails and guinea fowl (Shivaprasad, 2000). It is highly prevalent in commercial chicken

particularly in brown layers and breeders (Penha *et al.,* 2018). The infection in poultry cause anemia, depression, anorexia, difficulty in breathing, drop in egg production and diarrhea causing adherence of feces to the vent (Anonymous, 2018).

Antibiotics are commonly employed as preventive and therapeutic measures to control bacterial infections such as fowl typhoid. However, the excessive use of antibiotics has led to the increasing development of antibiotics resistant strains including the multidrug resistance *Salmonella* Gallinarum (Yasmin *et al.*, 2019; Zhou *et al.,* 2020). To overcome this issue, various alternatives to antibiotics are available such as probiotics, prebiotics, synbiotics, bacteriophages, organic acids, essential oils, cinnamaldehyde, chitosan, vaccines and nanoparticles. These alternatives can be used for prevention or control of fowl typhoid in poultry (Al-Razem *et al.*, 2022; El-Saadony *et al.*, 2022). Among these options, probiotics have gained significant attention as safe and efficient alternative to antibiotics (Chen *et al.,* 2020).

Probiotics are live microorganisms when administered in sufficient amount provide health advantage to host (FAO/WHO, 2002). Probiotics are utilized as feed additives in poultry feed to promote the growth of beneficial bacteria, enhance the growth performance of birds and reduce the level of enteric pathogens in broiler (El Jeni *et al.*, 2021; de Souza *et al.,* 2022). The microbial genera include *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Streptococcus* and *Bacillus* are commonly used as probiotics in poultry (Gadde *et al.*, 2017; Rashid *et al.*, 2023). Among these, *Lactobacillus* has the advantage of being Generally Regarded as Safe and are naturally occurring in intestinal tract (Robyn *et al.,* 2012). They inhibit the growth of pathogenic bacteria through different mechanisms such as binding to epithelial cell, modulation of immune system and secretion of antimicrobial compounds (Alonso *et al.*, 2019).

The *Limosilactobacillus fermentum* has gained significant recognition as one of the most promising probiotics (Rodríguez-Sojo *et al.*, 2021). It is commonly found in gastrointestinal of humans and animals, as well as in plant tissues and dairy products. The beneficial effects of this species are strain specific (Dos Santos *et al.*, 2021). The *Limosilactobacillus fermentum* strains (PC-10 and PC-76) isolated in our previous study have *in-vitro* probiotic properties and possess the antimicrobial activity against *Salmonella* Gallinarum (Mehmood *et al.,* 2023). This study was designed to analyze the effect of these isolates on weight gain, immune response, feed conversion ratio, and gut morphology and inhibition of *Salmonella* Gallinarum in broiler.

MATERIALS AND METHODS

Growth conditions of bacterial strains: Previously characterized *Limosilactobacillus fermentum* PC-10 (ON819856) and PC-76 (OP703611) were cultured on De Man Rogosa and Sharpe agar (Mehmood *et al.*, 2023). The test strain, *Salmonella* Gallinarum (CP116616) isolated from field outbreak was cultured on Salmonella shigella agar.

Experimental design: Day old chicks (n=90) were purchased from commercial hatchery and housed in experimental shed of the Institute of Microbiology, for a period of 35 days. They were divided into 6 different groups with 15 birds kept in each group. Group 1 served as negative control and did not receive any bacterial growth. Group 2 was positive control and only challenged with *S.* Gallinarum (10^8CFU/bird) on day 21. Birds in group 3 and 4 were orally administered *Limosilactobacillus fermentum* strains PC-10 and PC-76, respectively through drinking water (10^8CFU/bird) from day 1 to day 35 and challenge of *S.* Gallinarum on day 21. Group 5 received commercial probiotic HAN-LACVET (0.6g/15 birds) from day 1 to day 35 and was challenged with *S.* Gallinarum on day 21. Group 6 was challenged with *S.* Gallinarum on day 21, following the infection, started receiving antibiotic florfenicol (30mg/Kg body weight) for five days as given in Table 1. Mortality rate and symptoms of birds in each group were recorded on daily basis.

Enumeration of *Salmonella*, coliform and lactobacilli: On the arrival of chicks and before the *S.* Gallinarum challenge (day 21) cloacal swabs were collected to ensure the absence of *S.* Gallinarum. Subsequently, on alternate days following post *S.* Gallinarum infection, fresh poultry droppings samples were collected (at day 23, 25, 27, 29, 31, 33, 35) from each group to evaluate the effect of different treatments on *Salmonella*, coliform and *Lactobacillus* count in broiler. The *Lactobacillus* count was determined on MRS agar, coliforms were enumerated on MacConkey agar and *Salmonella* counting was done on SS agar. The bacterial count was calculated as CFU/gram and converted into log10 values. The mean ± standard deviation of log10 values were calculated for comparison among different groups. The log reduction of coliform was calculated by subtracting the log10 values from negative control group while reduction of *Salmonella* was calculated by subtracting log10 values from positive control group. The increase in lactobacilli count was calculated by subtracting the log10 values from negative control group (Shi *et al.*, 2022).

Effect of lactobacilli on weight gain: Feed conversion ratio and live weight of birds were recorded on different days (1, 7, 14, 21, 28, and 35) with a one-week interval.

Immune response against NDV and IBD vaccine: The immune modulatory effect of *Limosilactobacillus fermentum* PC-10 and PC-76 against Newcastle Disease Virus (NDV) and Infectious Bursal Disease (IBD) virus was determined. Chickens in all experimental groups were immunized with NDV "Lasota" (Medion, Bandung Indonesia) via eye drop on day 5 and booster dose was given on day 17 of broiler age. The vaccine for IBD was given to bird on day 11. Blood samples were collected from wing vein of birds (n=5) at different age of birds (14, 21, 28 and 35 days) for determination of antibody titer against ND and IBD (Beard *et al.,* 1975). Serums from blood samples were separated and stored at -20ºC till for further analysis. Antibody titer against NDV was determined by Heamagglutination Inhibition (HI) assay and IBD vaccine was determined by commercially purchased ELISA kit (Allan *et al.,* 1978).

Table 1: Experimental design for *in vivo* evaluation of the probiotic properties of *Limosilactobacillus fermentum* in broiler chicks.

Groups	Treatment
Negative control	No treatment
Positive control	Challenge of *S.* Gallinarum at day 21
Limosilactobacillus fermentum (PC-10)	Supplementation of probiotic (PC-10) from day 1 to 35 + challenge of *S.* Gallinarum at day 21
Limosilactobacillus fermentum (PC-76)	Supplementation of probiotic (PC-76) from day 1 to 35 + challenge of *S.* Gallinarum at day 21
Commercial probiotic	Supplementation of HAN-LACVET from day 1 to 35 + challenge of *S.* Gallinarum at day 21
Antibiotic	Challenge of *S.* Gallinarum at day 21 + Treatment with antibiotic for five days

Statistical analysis: The data of bacterial counts and weight gain was analyzed by one ANOVA and significant difference between groups was analyzed by post hoc Tukey's multiple comparison tests.

RESULTS

Lactobacilli effect on *Salmonella* count in broiler gut: The probiotic effects of *Limosilactobacillus fermentum* PC-10 and PC-76 on broiler gut were determined by *Salmonella* count, coliform count and total lactobacilli count following the post infection of *S.* Gallinarum in broilers. The results revealed a significantly ($P<0.05$) higher *Salmonella* count in positive control group (log10 6.88±0.2 CFU/g) at day 35 compared to other experimental groups. However, the groups administered with *Limosilactobacillus fermentum* PC-10 and PC-76 exhibited the significantly less in the growth of *S.* Gallinarum (log10 3.92±0.30 vs log10 3.99±0.22 CFU/g) compared to commercial probiotic (log10 4.90±0.70 CFU/g) and antibiotic treatment group (log10 5.17±0.1 CFU/g) as given in Fig. 1.

On day 35, there was no significant difference in coliform count between groups infected with *S.* Gallinarum (positive control) and negative control (log10 7.35±0.10 vs log10 7.15±0.10) as shown in Table 2. The coliform count was reduced significantly in all probiotic groups while maximum reduction was obtained in the group fed *Limosilactobacillus fermentum* PC-10 ($P<0.05$) among all other experimental groups.

The groups fed with *Limosilactobacillus fermentum* PC-10 and PC-76 had significantly higher ($P<0.05$) *Lactobacillus* counts (log10 9.37±0.2 vs log10 9.42±0.44) than negative control group (log10 7.46±0.52) as mentioned in Table 2. The *Lactobacillus* counts decreased progressively in chicken treated with antibiotic and positive control group at the end of experiment on day 35.

Antibody response to IBD and ND virus in birds challenged with *S.* Gallinarum: The geometric mean titers of broilers were significantly different in all experimental groups on day 21, 28 and 35 compared with control groups. As indicated in Fig. 2 the higher antibody titer was obtained in group 3 (114.10) and group 4 (110.29) at day 28 as compared to negative control group (90.25). The maximum antibody titer was obtained in group 4 on day 28 against IBD vaccine as given in Fig. 3.

Effect of lactobacilli on growth performance of broiler: The mean weight of birds was calculated in all experimental groups. As given in Fig. 4, the significant difference in body weight was recorded on day 21, 28 and 35 of broiler age. At day 35, the groups fed with probiotics PC-10, PC-76 and commercial probiotics had significantly ($P<0.05$) higher mean body weight (2032.091±89.16g, 1968.385±95.82g vs 1934.23±78.23g, respectively) compared to other negative control, positive control and antibiotic group.

Feed conversion ratio (FCR) was calculated on weekly basis. There was non-significant difference ($P>0.05$) in FCR obtained at day 1, 7, and 14 among all

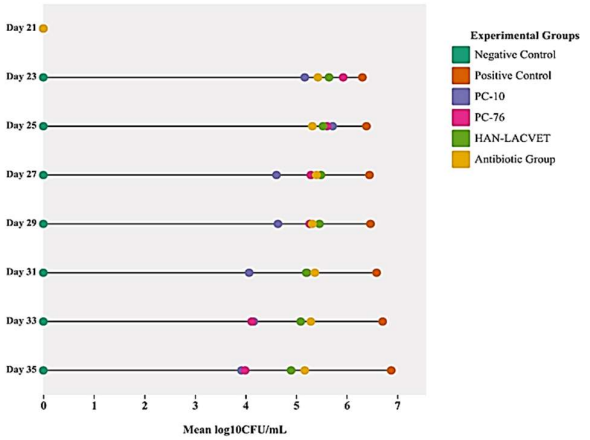

Fig. 1: *Salmonella* count in different experimental groups before and after challenged with *S.* Gallinarum

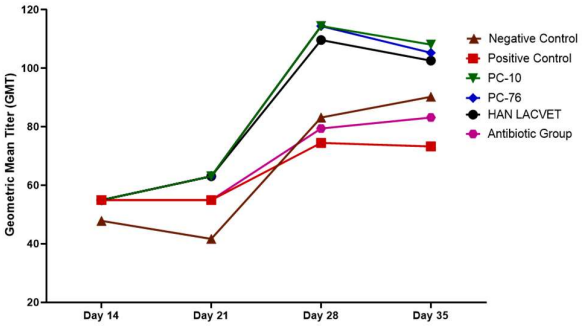

Fig. 2: Geometric mean titer of birds in different experimental groups against NDV vaccine at different days

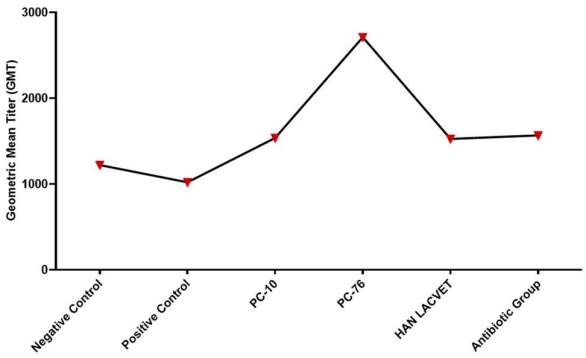

Fig. 3: Antibody titer of birds in different experimental groups against IBD vaccine at day 28: A positive result (titer greater than 396) indicates birds are vaccinated with IBD vaccine.

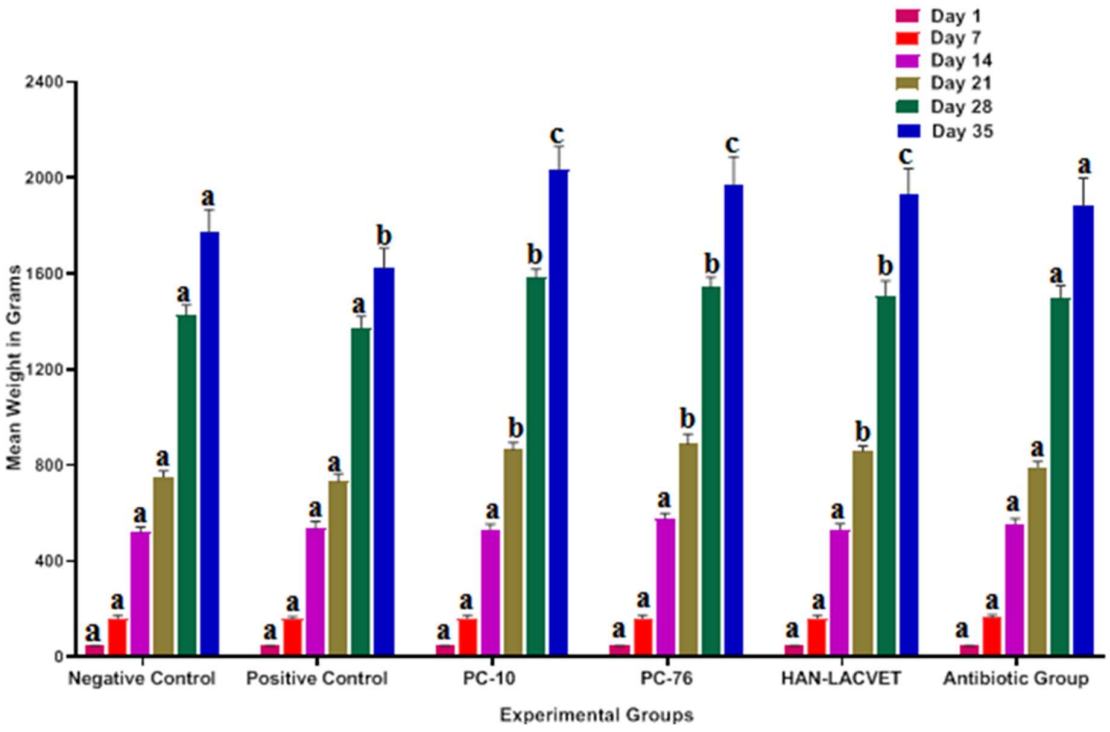

Fig. 4: Mean weight (g) of broilers in different experimental groups at different days.

Table 2: Effect of selected lactobacilli on coliform count in broiler gut at day 35.

Groups	Coliform count			*Lactobacillus* count		
	Before infection (mean log10CFU/mL)	After infection (mean log10CFU/mL)	Log reduction	Before infection (mean log10CFU/mL)	After infection (mean log10CFU/mL)	Increase in Log 10
Negative control	7.05±0.35[a]	7.15±0.10[d]	-	7.20±0.12[a]	7.46±0.52[b]	
Positive control	7.24±0.19[a]	7.35±0.10[d]	-0.2	7.20±0.21[a]	6.87±0.32[a]	-0.59
PC-10	7.27±0.46[a]	5.18±0.40[a]	1.97	7.18±0.19[a]	9.37±0.23[d]	1.91
PC-76	8.78±0.16[b]	6.01±0.05[b]	1.14	7.99±0.08[b]	9.42±0.44[d]	1.96
Commercial Probiotic	7.99±0.05[a]	6.90±0.07[c]	0.25	7.20±0.17[a]	8.70±0.16[c]	1.24
Antibiotic-Group	7.27±0.19[a]	5.92±0.50[b]	1.23	7.18±0.07[a]	7.39±0.27[b]	0.07

Table 3: Feed conversion ratio determined on weekly basis in different experimental groups.

Groups	Age of broiler					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Negative control	0.22	0.93	1.10	1.24	1.38	1.56
Positive control	0.22	0.93	1.08	1.61	1.53	1.91
PC-10	0.22	0.91	1.09	1.36	1.32	1.61
PC-76	0.22	0.94	0.94	1.33	1.37	1.67
Commercial Probiotic	0.22	0.94	1.03	1.37	1.39	1.70
Antibiotic-Group	0.22	0.93	0.98	1.49	1.40	1.74

experimental groups. The groups fed *Limosilactobacillus fermentum* (PC-10 and PC-76) exhibited no significant effect on FCR when compared to negative control, commercial probiotic and antibiotic group, while the positive control group exhibited the higher FCR on day 21, 28 and 35 as given in Table 3.

Clinical findings and mortality: Birds in all experimental groups were monitored twice a day for any types of clinical signs and mortality. In control group of broilers not infected with *S.* Gallinarum, no clinical signs and mortality were recorded throughout the experiment. In positive control and antibiotic group, initial signs of dullness and decreased feed intake were observed on the 3rd day of post infection. Subsequently, on 5th to 6th days after infection, these groups exhibited the additional clinical signs such as droopy wings, ruffled feathers and yellowish green diarrhea. Higher mortality was recorded in positive control group (46.66%) due to *S.* Gallinarum infection in comparison to all other groups.

DISCUSSION

Lactobacilli have been used widely as an alternative to antibiotics to prevent *Salmonella* infection in chicken (Wang *et al.,* 2023). They are main residents of gastrointestinal tract where they protect the host by competing for nutrients and releasing substances that inhibit the growth of harmful pathogens (Chen *et al.,* 2007). In a previous study, we isolated the *Limosilactobacillus fermentum* strains (PC-10 and PC-76) from poultry caeca which showed >99% reduction of *S.* Gallinarum *in vitro* in co-culture assay. In this study, we investigated the *in vivo* effect of these strains on reducing the growth of *S.* Gallinarum in broiler and further analyzed their effect on weight gain, feed

conversion ratio and increase in immune response against ND and IBD vaccine. The efficacy of these strains was compared with commercial probiotic product (HAN LACVET) and antibiotic (florfenicol) routinely used in poultry farms to prevent the *Salmonella* infections.

The study findings demonstrated the significant decrease ($p<0.05$) in *Salmonella* in fecal dropping of broilers that were administered with *Limosilactobacillus fermentum* PC-10 and PC-76 at day 35. Our results are in line with previous findings, indicating that administration of probiotics via oral route in poultry inhibited the attachment of *Salmonella* Enteritidis in poultry gut (Shi *et al.*, 2022). This inhibitory effect is likely attributed to production of various metabolites by lactic acid bacteria such as bacteriocins, lactic acid and hydrogen peroxide that inhibit the growth of pathogenic bacteria. Moreover the lactic acid bacteria can competitively exclude the pathogen in gut by reducing the pH in gut and competing them for nutrients and attachment (Kulkarni *et al.,* 2022).

The Lactic Acid Bacteria (LAB) belong to *Lactobacillus* play a key role in regulating and maintaining the intestinal microbial flora and exert their beneficial effects by inhibiting the adhesion of pathogenic bacteria and facilitating the colonization of beneficial microorganisms (Hai *et al.*, 2021). Probiotic administration on daily basis helped to increase the population of beneficial microbes in GIT (Khan *et al.,* 2019). The current study also reported the higher microbial count of lactobacilli in experimental groups fed with potential lactobacilli on daily basis. A significant increase in lactobacilli (~2log10CFU/mL) was observed in probiotics groups PC-10, PC-76 and commercial probiotic (HAN-LACVET) supplemented group (day 01 to 35). These probiotics groups exhibited a significant difference (P value<0.05) compared to other experimental groups. Challenge control group which was only administrated with *S.* Gallinarum reported less lactobacilli count (6.87±0.2) at day 35.

Binding of probiotics to gut prevent the colonization of pathogenic bacteria and coliform via competitive exclusion process. Newly hatched chicks have incomplete intestinal microbiota. So, early administration of lactobacilli helped to establish the dominant microbiota and prevent the colonization of non-host specific pathogens (Chen *et al.*, 2020). Previous research has also highlighted that using probiotics for prevention rather than treatment can offer better health benefit to host (Chen *et al.,* 2020). The current study findings are similar to previous study, administration of probiotics in new hatched chicks decrease the coliform in broiler gut. In comparison to this, coliform count (7.14±0.36 $\log_{10}$ CFU/g) was high in negative control group and is more than coliform count (5.07±0.15 $\log_{10}$ CFU/g) in PC-10 and other probiotic groups.

Growth performance and weight gain of birds in all experimental groups were monitored on weekly basis. Significant difference in weight was recorded at 3rd week of broiler age and it was observed till the completion of experimental trial. Meanwhile, various previous studies have also reported the similar results (Asghar *et al*., 2016). In our study the *Limosilactobacillus fermentum* strain PC-10 and PC-76 significantly improved weight gain of broilers at day 35 groups in comparison to negative control group. Enhancement of weight was also analyzed in probiotic supplemented groups in broilers in comparison with the negative control group (Forte *et al.*, 2018). A related study reported that supplementation of *L. fermentum* in broiler increased the weight of broiler (Mustafa *et al.*, 2022). Various previous evidences have reported the probiotic effect on weight gain in chicken (Asghar *et al.*, 2016; Khan *et al.,* 2019; Chen *et al.,* 2020).

Probiotics also play an important role in immune modulation in poultry and also enhanced the immune cells to produce natural antibodies (Azad *et al.*, 2018). Probiotics also stimulate the cell mediated and humoral immune response in chicken. *L. salivarius* is a natural microflora of chicken gut and in mononuclear cell it induced the proliferation of cytokines (Brisbin *et al*., 2011). *Lactobacillus gallinarum* PL-53 has improved the immune status of chicken (Khan *et al*., 2019). In present study immune response of broiler was assessed with elevated antibody titer against NDV vaccine. Probiotics have a good influence on immune response in broiler. This study reported that GMT against NDV and IBD was higher in probiotic groups in comparison with those groups which did not receive probiotics. Hedayati *et al.* (2022) recorded higher antibody titer against IBD and infectious bronchitis (IB) virus in broiler fed with lactobacilli spores. Immune response of probiotics supplemented groups and control groups against NDV were determined on weekly basis and is mentioned Fig. 2. The highest GMT titer in this study was recorded by *Limosilactobacillus fermentum* (PC-10) in comparison to all experimental groups at day 35. The results of this experiment are also in agreement with Khan *et al.* (2019). This study reported that antibody titer was increased at 4th week of broiler age in probiotics groups in comparison with groups challenge control and antibiotic group.

In control group of broilers, which was not infected with *S.* Gallinarum, no clinical signs and mortality was recorded in throughout the experiment. Birds in positive control and antibiotic group were represented the initial signs of dullness and decrease of feed intake on 3rd day of post infection. Some of the birds died suddenly due to the acute form of illness. Higher mortality was recorded in positive control group (46.66%) after the post infection of *S.* Gallinarum. Abbas *et al.* (2021) reported the 48% mortality rate in chicks only challenged with *S.* Gallinarum and less mortality 24% in birds infected with *S.* Gallinarum and lactobacilli. Birds in probiotic groups represent the mild sign of dullness after the post challenge of *S.* Gallinarum and no mortality was recorded in 6 days of post challenge. These results are similar to another study reported, *Lactobacillus* as probiotic supplementation in poultry reduced the clinical sign against *Salmonella* infection (Abbas *et al.,* 2021).

Conclusions: This study demonstrated that indigenous *Limosilactobacillus fermentum* strains (PC-10 and PC-76) have potential probiotic properties, regulate intestinal microbiota of poultry, and competitively exclude *S.* Gallinarum in poultry gut. Moreover, these isolates have significant effect on weight gain and immune response in broiler. The *Limosilactobacillus fermentum* strains (PC-10 and PC-76) may be used as probiotic to mitigate *S.* Gallianrum in poultry.

Ethics approval and consent to participate: The study was conducted according to the guidelines and protocols approved by "Ethical Review Committee" of University of Veterinary and Animal Sciences Lahore, Pakistan (vide letter no. DR/1103, Dated: 11 October 2017). Informed consent was obtained from the owners or an authorized agents of poultry farms for the collection of intestinal samples from poultry birds for this study.

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Authors contribution: MN, MR and MHM designed the research project. AM collected samples, performed experiments and MN analyzed the data. AM prepared the manuscript. All authors contributed to manuscript revision and approved final version for submission.

REFERENCES

Abbas A, Rizvi F, Hussain S, *et al.,* 2021. Immuno-modulatory effects of *Lactobacillus* in *Salmonella* Gallinarum infected broiler chicks. Pak J Sci 73(1):77.

Allan WH, Lancaster JE and Toth B, 1978. Newcastle disease vaccines: their production and use. Food and Agriculture Organization of the United Nations, Rome. pp 163.

Alonso S, Carmen Castro M, Berdasco M, *et al.,* 2019. Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. Probiotics Antimicrob Proteins 11:569-579.

Al-Razem F, Al-Aloul H, Ishnaiwer M, *et al.,* 2022. Isolation and partial characterization of *Salmonella* Gallinarum bacteriophage. Saudi J Biol Sci 29(5):3308-3312.

Andino A and Hanning I, 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. Sci World J :2015:1-16.

Anonymous, 2018. Fowl typhoid and pullorum disease. OIE Terrestial Manual. Chapter. 3(2):719-735.

Asghar S, Arif M, Nawaz M, *et al.,* 2016. Selection, characterisation and evaluation of potential probiotic *Lactobacillus* spp. isolated from poultry droppings. Benef Microbes 7(1):35-44.

Azad MAK, Sarker M and Wan D, 2018. Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int 2018:1-10

Barrow P and Neto OF, 2011. Pullorum disease and fowl typhoid—new thoughts on old diseases: a review. Avian Patho 40 (1):1-13.

Beard CW, Hopkins SR and Hammond J, 1975. Preparation of Newcastle disease virus hemagglutination-inhibition test antigen**.** Avian Dis 19:692-699.

Brisbin JT, Gong J, Orouji S, *et al.,* 2011. Oral treatment of chickens with lactobacilli influences elicitation of immune responses. Clin Vaccine Immunol 18(9):1447-1455.

Chen C, Li J, Zhang H, *et al.,* 2020. Effects of a probiotic on the growth performance, intestinal flora, and immune function of chicks infected with *Salmonella* pullorum. Poult Sci 99(11): 5316-5323.

Chen X, Xu J, Shuai J, *et al.,* 2007. The S-layer proteins of *Lactobacillus crispatus* strain ZJ001 is responsible for competitive exclusion against *Escherichia coli* O157: H7 and *Salmonella typhimurium*. Int J food Microbiol 115(3):307-312.

de Souza OF, Vecchi B, Gumina E, *et al.,* 2022. Development and evaluation of a commercial direct-fed microbial (zymospore®) on the fecal microbiome and growth performance of broiler chickens under experimental challenge conditions. Animals 12(11):1436.

Desin TS, Köster W and Potter AA, 2013. *Salmonella* vaccines in poultry: past, present and future. Expert Rev Vaccines 12(1):87-96.

Dos Santos CI, Campos CD, Nunes-Neto WR, *et al.,* 2021. Genomic analysis of *Limosilactobacillus fermentum* ATCC 23271, a potential probiotic strain with anti-*Candida* activity. J Fungi 7(10):794.

El Jeni R, Dittoe DK, Olson EG, *et al.,* 2021. Probiotics and potential applications for alternative poultry production systems. Poult Sci 100(7):101156.

El-Saadony MT, Salem HM, El-Tahan AM, *et al.,* 2022. The control of poultry salmonellosis using organic agents: an updated overview. Poult Sci 101(4):101716.

FAO/WHO, 2002. Agriculture Organization of the United Nations and World Health Organization Working Group: Joint FAO/WHO Working Group report on drafting guidelines for the evaluation of probiotics in Food.

Forte C, Manuali E, Abbate Y, *et al.,* 2018. Dietary *Lactobacillus acidophilus* positively influences growth performance, gut morphology, and gut microbiology in rurally reared chickens. Poult Sci 97(3), 930-936.

Gadde U, Kim W, Oh S, *et al.,* 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev 18(1):26-45.

Hedayati M, Khalaji S and Manafi M, 2022. Lactobacilli spp. and Zataria multiflora essence as antibiotic substituent on broiler health and performance parameters. Ita J Anim Sci 21(1):1-7.

Hai D, Kong L, Lu Z, *et al.,* 2021. Inhibitory effect of different chicken-derived lactic acid bacteria isolates on drug resistant *Salmonella* SE47 isolated from eggs. Lett Appl Microbiol 73(1): 54-63.

Khan M, Anjum AA, Nawaz M, *et al.,* 2019. Effect of newly characterized probiotic lactobacilli on weight gain, immunomodulation and gut microbiota of *Campylobacter jejuni* challenged broiler chicken. Pak Vet J 39(4):473-478.

Kulkarni RR, Gaghan C, Gorrell K, *et al.,* 2022. Probiotics as alternatives to antibiotics for the prevention and control of necrotic enteritis in chickens. Pathogens 11(6):692.

Mehmood A, Nawaz M, Rabbani M, *et al.,* 2023. *In Vitro* Characterization of Probiotic Potential of *Limosilactobacillus fermentum* against *Salmonella* Gallinarum Causing Fowl Typhoid. Animals 13(8):1284.

Mustafa A, Nawaz M, Rabbani M, *et al.,* 2022. Characterization and evaluation of anti-*Salmonella enteritidis* activity of indigenous probiotic lactobacilli in mice. Open Life Sci 17(1):978-990.

Penha RAC, Zancan FT, Almeida AMd, *et al.,* 2018. Protection of chickens against fowl typhoid using field vaccine programs formulated with the live attenuated strain *Salmonella* Gallinarum ΔcobSΔcbiA. Arq Inst Biol 84:1-5.

Rashid S, Tahir S, Akhtar T, *et al.,* 2023. Bacillus-based Probiotics: An antibiotic alternative for the treatment of salmonellosis in poultry. Pak Vet J 43(1):167-173.

Robyn J, Rasschaert G, Messens W, *et al.,* 2012. Screening for lactic acid bacteria capable of inhibiting *Campylobacter jejuni* in *in vitro* simulations of the broiler chicken caecal environment. Benef Microbes 3(4):299-308.

Rodríguez-Sojo MJ, Ruiz-Malagón AJ, Rodríguez-Cabezas ME, *et al.,* 2021. *Limosilactobacillus fermentum* CECT5716: mechanisms and therapeutic insights. Nutrients 13(3):1016.

Shi S, Zhou D, Xu Y, *et al.,* 2022. Effect of *Lactobacillus reuteri* S5 Intervention on intestinal microbiota composition of chickens challenged with *Salmonella enteritidis*. Animals 12(19):2528.

Shivaprasad H, 2000. Fowl typhoid and pullorum disease. Revue scientifique et technique (International Office of Epizootics). 19(2):405-424.

Spickler AR, 2019. Fowl typhoid and pullorum disease. http://www.cfsph.iastate.edu/diseaseInfo/factsheet.

Wang M, Hu J, Yu H, *et al.,* 2023. *Lactobacillus fermentum* 1.2133 display probiotic potential *in vitro* and protect against *Salmonella* pullorum in chicken of infection. Lett Appl Microbiol 76(1):ovac041.

Yasmin S, Nawaz M, Anjum AA, *et al.,* 2019. Antibiotic susceptibility pattern of Salmonellae isolated from poultry from different Districts of Punjab, Pakistan. Pak Vet J 40:98-102.

Zhou C, Liang J, Jiang W, *et al.,* 2020. The effect of a selected yeast fraction on the prevention of pullorum disease and fowl typhoid in commercial breeder chickens. Poult Sci 99(1):101-110.

Zhou X, Kang X, Zhou K, *et al.,* 2022. A global dataset for prevalence of *Salmonella* Gallinarum between 1945 and 2021. Scientific Data 9(1):495-505.