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RESEARCH ARTICLE

Effect of Newly Characterized Probiotic Lactobacilli on Weight Gain, Immunomodulation and Gut Microbiota of *Campylobacter jejuni* Challenged Broiler Chicken

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A B S T R A C T

The aim of the present study was to determine the competitive exclusion of *Campylobacter jejuni* in poultry gut by three potential probiotic lactobacilli strains. A total of 135 birds of one day age were randomly divided into nine groups. The groups of prevention model received respective strains from day 1-35 and treatment model received lactobacilli from day 15-35 (after challenging with *C. jejuni*). These groups were given lactobacilli (~10^8 CFUs) while challenged with *C. jejuni* on day 14 by oral gavage (10^6 CFUs/bird). There were three control groups including A, B and I. Negative control (A) did not receive any treatment, *C. jejuni* was given to group B and group I was given Enrofloxacin formulation. Cloacal swabs were collected from birds of each group before and after challenge while the ceca were collected from birds after slaughtering (on day 35) for enumeration of aerobic bacteria, coliform, *C. jejuni*, lactobacilli and *Bifidobacterium* on selective agar plates. Effect of lactobacilli on weight gain and New Castle Disease vaccine (NDV) titer were also evaluated. Probiotic strain *Lactobacillus gallinarum* PL 53 considerably decreased $\log_{10}$ values of aerobic plate count (3.19±0.66), coliform count (2.83±0.22) and *C. jejuni* (3.98±0.77) in poultry. The probiotics also enhanced *Lactobacillus* and *Bifidobacterium spp* counts (~2-3 log increase). Treatment group C had maximum weight gain (1994±188.32g) and geometric mean titer (274.4) on day 28. The results of *in-vivo* experiments concluded that probiotic administration may be effective for targeted mitigation of *C. jejuni* in broiler birds.

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INTRODUCTION

Campylobacteriosis is one of the leading causes of zoonotic bacterial gastroenteritis in humans worldwide (Ghareeb *et al.*, 2012). *Campylobacter jejuni* (*C. jejuni*) is a normal inhabitant of poultry gut, so is a major source of poultry meat contamination (Kabir *et al*., 2005, Willis and Reid, 2008). It is known that *C. jejuni* is prevalent in poultry flocks and so poses a potential serious public health hazard. Thus, there is an urgent need to develop methods to reduce or eliminate *C. jejuni* from poultry flocks and thereby reduce risk of human cases Rosenquist *et al.*, 2003; (Kabir *et al.*, 2005). Previous control strategies included the use of antibiotics in poultry feed and water but this has led to the emergence of antibiotic-resistant strains (Johnson *et al.*, 2017). An alternative and effective approach to antibiotic administration to poultry is the use of probiotics that may potentially inhibit or reduce *C. jejuni* colonization in poultry (Kabir *et al.*, 2005; Santini *et al.*, 2010; Saint-Cyr *et al.*, 2017). This approach relies on preventing *C. jejuni* adhesion to and invasion of epithelial cells (Nishiyama *et al.*, 2014).

According to Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are "Live micro-organisms which when administered in adequate amounts can confer beneficial effects on host health." Probiotics have effectively reduced infections with foodborne pathogens such as *Salmonella*, *E. coli*, *Clostridium*, *Listeria* and *Campylobacter* in vivo (Hakkinen and Schneitz, 1996, Hakkinen and Schneitz, 1999, Stern *et al.*, 2001). Probiotics can competitively inhibit *C. jejuni* colonization and infection by several mechanisms: including competing for attachment sites, co-aggregation with the pathogen, production of

antimicrobial compound such as lactic acid and hydrogen peroxide Nishiyama *et al.*, 2014; (Bratz *et al.*, 2015) and by stimulation of the immune system (Smits, *et al.*, 2005). Lactobacilli also improve growth performances in broilers, mainly by increasing nutrient utilization along with counteracting foodborne pathogen contamination by preventing *C. jejuni* shedding at primary production level (Saint-Cyr *et al*., 2016).

The objective of this study was to isolate probiotic strains that could prevent *C. jejuni* colonization in the broiler gut and thus eliminate or reduce *C. jejuni* numbers in poultry. The effectiveness of probiotics at clearing bacterial infections and regulating intestinal flora was assessed in this study by evaluating aerobic plate count, total coliform count (TCC), Total lactobacillus count (TLC), *Bifidobacterium* count and *C. jejuni* count. The effect of lactobacilli on weekly weight gain and response to NDV live vaccine was also evaluated in this study.

MATERIALS AND METHODS

Microbial strains: Previously characterized lactobacilli strains *Lactobacillus gallinarum* PL 53, *Lactobacillus casei* PL 120 and *Lactobacillus gallinarum* PL 149 (unpublished data) were grown on MRS agar. *Campylobacter jejuni* ATCC 33291 was grown on Campylobacter Cefex agar supplemented with sheep blood and selective supplement (Himedia).

In vivo* exclusion of *Campylobacter jejuni

Experimental animals and housing: A total of 135 broiler chicks were procured from a commercial hatchery (Punjab chicks, Pakistan poultry breeder hatchery) at the day of hatch and reared for 35 days in the experimental room of Department of Microbiology, University of Veterinary and Animal Sciences, Lahore. The temperature of the experimental room was maintained at 33-25°C (varying according to the age of birds) and 65-70% humidity with adequate air exchange by proper ventilation of the room. The birds were housed in floor pens on wood shavings. Feed (Kausar Feed Mills, Pvt. Ltd., Lahore) and water were available ad libitum for the duration of the 35 days trial.

Experimental design: The chicks were randomly distributed across nine treatment groups with 15 birds in each group. The groups C, D and E were given probiotics (10^8 CFU) in drinking water from day 1 as a prophylactic and were then challenged with *C. jejuni* (10^6 CFU/bird) on day 14 (Ghareeb *et al.*, 2012). Groups F, G and H were given the *C. jejuni* challenge (10^6 CFU/bird) on day 14 and then given probiotics from day 15-35 as a therapeutic administration (Saint-Cyr *et al.*, 2016; Thomrongsuwannakij, *et al.*, 2016). Groups B and I were challenged with fresh culture of *C. jejuni* on day 14 (Rosenquist *et al.*, 2003; Kabir *et al.*, 2005). Group A, the negative control, did not receive any treatment. The study design is presented in Table 1. Chickens in all groups were vaccinated with New castle Disease Vaccine (NDV) live virus "Lasota" vaccine via eye drop route at day 5 of age followed by a booster dose 10 days of post exposure. Cloacal swabs (n=4 from each group) were collected from birds before challenging birds with *C. jejuni* (day 14) and then after challenge on days 15, 21, 28 and 35. On day 35, 4 birds from each group were slaughtered and ceca were collected for enumeration of the microbial load.

Weight gain: Birds from each group were weighed weekly on days 1, 7, 14, 21, 28 and 35. The weight gain was calculated and compared for different treatment groups.

Enumeration of bacteria: The cloacal samples were used to evaluate the effect of probiotics on aerobic, coliform, *C.jejuni*, *Lactobacillus spp*. and *Bifidobacteria* counts. The aerobic count was done on plate count agar (Kabir *et al.*, 2005; Kabir, 2009) and coliforms were counted on MacConkey agar (Kabir, 2009). Diluted samples were spread on respective plates and incubated at 37°C for 24 hours. *C. jejuni* was enumerated on Campylobacter Cefex agar containing sheep blood and supplements (cefoperazone and amphotericin B) and incubated in micro-aerophilic condition at 42°C for 48 hours (Willis and Reid, 2008). *Lactobacillus spp* was counted on MRS agar and incubated aerobically at 37°C for 48 hours while *Bifidobacterium* was counted on *Bifidobacterium* selective count agar containing propionic acid as a supplement and incubated anaerobically at 37°C for 48 hours (Hakkinen and Schneitz, 1999). After slaughtering of birds, ceca was excised and placed in a sterile container. The cecal content was also used to enumerate *C. jejuni* and lactobacilli on selective agar.

Table 1: Treatment group description

Grouping	Experimental plan	Treatment
A	Control groups	No treatment
B		*Campylobacter jejuni* supplementation
C	Prevention model	PL 53 + challenge afterwards (day 14)
D		PL 120 + challenge afterwards (day 14)
E		PL 149 + challenge afterwards (day 14)
F	Treatment model	Challenge (day 14) + later PL 53
G		Challenge (day 14) + later PL120
H		Challenge (day 14) + later PL 149
I		Antibiotic formulation+ challenge afterwards (day 14)

PL 53: *Lactobacillus gallinarum*, PL 120: *Lactobacillus casei,* PL 149: *Lactobacillus gallinarum, Campylobacter jejuni* ATCC 33291, Antibiotic: Enrofloxacin solution.

Bacterial colonies were enumerated and CFU/gram was converted into $\log_{10}$ values. The mean ± standard deviation (S.D) of $\log_{10}$ values were calculated and compared among groups. Log reduction of aerobic plate count and coliform count was calculated by subtracting log values from negative control group while log reduction of *C. jejuni* was calculated by subtracting log values of treatment group from *C. jejuni* control. Lactobacilli and Bifidobacteria log increase was also calculated by subtracting log values from negative control group.

Immunomodulatory effects against Newcastle disease vaccine: Immunomodulatory effect of *Lactobacillus* isolates in broiler chicks against NDV was determined on a weekly basis throughout the experiment. Blood was collected from chicks of all groups at 14, 21, 28 and 35 days of age. Blood samples (3mL) were collected using sterile 5mL syringes and serum was separated by allowing the blood to clot at room temperature. Serum was used for determining serum antibody titers against NDV by hemagglutination inhibition assay (Ghafoor *et al.*, 2005). The geometric mean titers were evaluated using the Brugh table (Brugh, 1978).

RESULTS

Mean body weight gain: Mean body weights of the broiler chicks in different treatment groups is presented in Fig. 1. The mean body weight of birds in different groups was significantly different on days 14, 21, 28 and 35 when compared with mean body weight of birds in the control groups. The highest weight gain was obtained in treatment group C (1994±188.32) on day 35 compared with that in the negative control group A (1545±191.49) and the pathogen group B (1352±115.12).

Effect of probiotic Lactobacilli isolates on gut microbiota of broiler chicks: The effect of probiotic lactobacilli isolates on the gut microbiota of broiler chicks determined by aerobic plate count, coliform count, total lactobacilli count and Bifidobacteria count is presented in Table 1. Our results show that, the maximum log_{10} reduction in aerobic plate count (3.19±0.66) and coliform count (2.83±0.22) was obtained in group C while the lowest log_{10} reduction in aerobic plate count was observed in group G (0.77±0.14). The least reduction in coliform count was also obtained in group G (1.06±0.22).

The maximum log_{10} increase in lactobacilli CFU/g (7.97±0.64) was observed in group F. Maximum log_{10} increase of Bifidobacteria was 2.76±0.33 in group E in cloacal swabs when compared with control groups and Bifidobacteria count was reduced in group B.

C. jejuni counts indicate that group C had maximum log_{10} reduction (3.98±0.77) on day 35 followed by group F (2.92±0.55) (presented in Table 3). The highest *C. jejuni* count expressed as log_{10} CFU/g of cloacal samples was observed in control group B (6.55±0.53).

The maximum *C. jejuni* log_{10} reduction in cecal content samples was obtained in group C (3.07±0.77 log_{10} CFU/g). The maximum lactobacilli count was obtained in group C (8.63±0.37) while the minimum count was observed in *C. jejuni* control group B (4.35±0.19) in per gram of cecal content.

Immunomodulatory effects against Newcastle disease vaccine: Mean titers of birds were significantly different for all different treatment groups on days 14, 21, 28 and 35 compared with control groups (Fig. 2). Highest NDV titer was obtained in treatment groups group C (274.4) on day 28 compared to negative control group A (104) and *C. jejuni* group B (29.9).

Table 2: Effect of probiotic lactobacilli isolates on gut microbiota of broiler chicks determined from cloacal swabs expressed as Mean log_{10} CFU/g±S.D

Groups	Aerobic plate count		Coliform count		Lactobacilli count		Bifidobacterium count	
	Mean Log±SD	Log_{10} reduction	Mean Log±SD	Log_{10} reduction	Mean Log±SD	Log_{10} increase	Mean Log±SD	Log_{10} increase
A	7.35±0.60		7.07±0.26		4.95±0.88		4.53±0.28	
B	7.04±0.33	0.31±0.02	7.20±0.21	-0.31	4.98±0.46	0.03±0.01	4.21±0.01	-0.31
C	4.16±0.58	3.19±0.66	4.24±0.21	2.83±0.22	7.84±0.46	2.89±0.22	7.23±0.62	2.7±0.22
D	5.12±0.45	2.23±0.33	5.23±0.59	1.84±0.18	7.71±0.46	2.76±0.21	7.15±0.49	2.62±0.28
E	4.86±0.36	2.49±0.18	4.83±0.43	2.24±0.33	7.73±0.75	2.78±0.33	7.29±0.05	2.76±0.33
F	6.32±0.56	1.03±0.08	5.77±0.46	1.3±0.07	7.97±0.64	3.02±0.11	6.33±0.86	1.8±0.08
G	6.58±0.53	0.77±0.14	6.01±0.13	1.06±0.22	7.65±0.44	2.7±0.33	6.19±0.52	1.66±0.11
H	6.45±0.92	0.9±0.01	5.82±0.78	1.25±0.27	7.44±0.73	2.49±0.27	6.24±1.32	1.71±0.33
I	5.86±0.19	1.49±0.02	5.55±0.38	1.52±0.04	6.22±0.58	1.27±0.22	6.14±1.08	1.61±0.17

Note: S.D = standard deviation; Data presented as mean of logarithms of 4 cloacal swab samples per group (log cfu/g); A: Group with no treatment; B: Group orally gavaged with *C. jejuni*; C: Group supplemented with PL 53 lactobacilli strain from day of hatch; D: Group supplemented with PL 120 lactobacilli strain from day of hatch; E: Group supplemented with PL 149 lactobacilli strain from day of hatch; F: Group supplemented with PL 53 lactobacilli strain after challenge (day 15-35), G: Group supplemented with PL 120 lactobacilli strain after challenge (day 15-35); H: Group supplemented with PL 149 lactobacilli strain after challenge (day 15-35); I: Group supplemented with antibiotic and later challenged.

Table 3: Effect of probiotic lactobacilli isolates on *C. jejuni* count of broiler chicks determined from cloacal swabs and cecal matter expressed as Mean log_{10} CFU/g±S.D

Groups	Cloacal swabs count									Ceca count	
	14 (Before challenge)	15		21		28		35		35	
	log_{10} mean ± S.D	log_{10} mean ± S.D	Log_{10} reduction	log_{10} mean ± S.D	Log_{10} reduction	log_{10} mean ± S.D	Log_{10} reduction	log_{10} mean ± S.D	Log_{10} reduction	log_{10} mean ± S.D	Log_{10} reduction
A	3.41±0.03	3.33±0.26	-	3.31±0.08	-	3.77±0.09	-	3.66±0.37	-	2.88±0.37	-
B	3.37±0.05	6.55±0.53	-	6.22±0.03	-	6.27±0.06	-	6.15±0.35	-	6.05±0.35	-
C	3.05±0.02	4.32±0.52	2.23±0.02	3.32±0.06	2.9±0.11	3.19±0.04	3.08±0.12	3.17±0.40	3.98±0.77	2.98±0.40	3.07±0.77
D	3.18±0.12	4.72±0.53	1.83±0.11	4.05±0.40	2.17±0.23	3.96±0.15	2.31±0.19	3.88±0.62	2.27±0.83	3.88±0.62	2.17±0.83
E	3.15±0.14	4.61±0.49	1.94±0.05	3.96±0.12	2.26±0.42	3.67±0.09	2.6±0.18	3.61±0.35	2.54±0.33	3.61±0.35	2.44±0.33
F	3.35±0.22	4.92±0.17	1.63±0.05	4.38±0.15	1.84±0.17	3.60±0.11	2.67±0.22	3.23±0.24	2.92±0.55	3.23±0.24	2.82±0.55
G	3.33±0.16	5.07±0.88	1.48±0.03	4.77±0.26	1.45±0.22	4.01±0.09	2.26±0.18	3.93±0.50	2.22±0.33	3.93±0.50	2.12±0.33
H	3.27±0.20	5.01±0.29	1.54±0.02	4.12±0.17	2.1±0.33	3.93±0.08	2.34±0.27	3.77±0.40	2.38±0.12	3.77±0.40	2.28±0.12
I	3.29±0.31	4.88±0.59	1.67±0.22	4.06±0.25	2.16±0.22	3.82±0.07	2.45±0.22	3.75±0.83	2.4±0.22	3.75±0.83	2.3±0.22

Note: S.D = standard deviation; Data presented as mean of logarithms of 4 cloacal swab and cecal matter samples per group (log cfu/g); A: Group with no treatment; B: Group orally gavaged with *C. jejuni*; C: Group supplemented with PL 53 lactobacilli strain from day of hatch; D: Group supplemented with PL 120 lactobacilli strain from day of hatch; E: Group supplemented with PL 149 lactobacilli strain from day of hatch; F: Group supplemented with PL 53 lactobacilli strain after challenge (day 15-35), G: Group supplemented with PL 120 lactobacilli strain after challenge (day 15-35); H: Group supplemented with PL 149 lactobacilli strain after challenge (day 15-35); I: Group supplemented with antibiotic and later challenged.

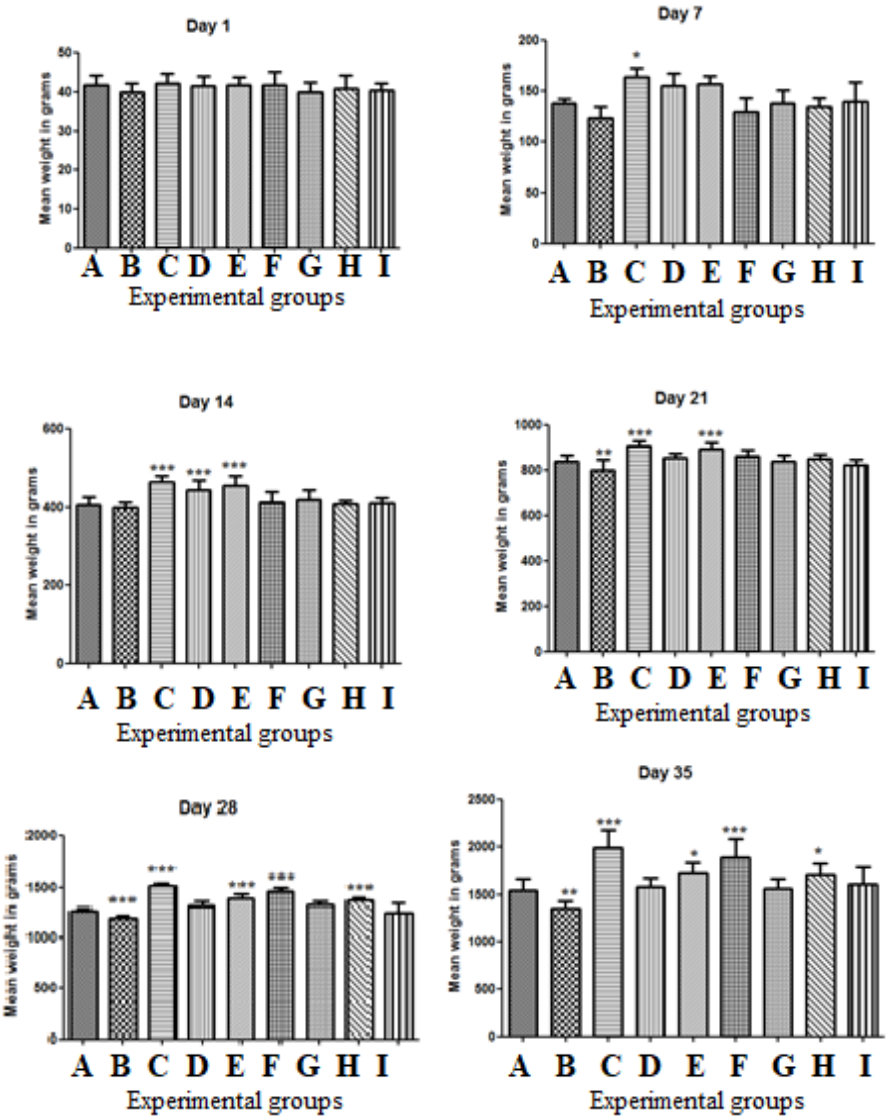

Fig. 1: Average weight (g) of chicken in various experimental groups at weekly intervals. A: Group with no treatment; B: Group orally gavaged with *C. jejuni*; C: Group supplemented with PL 53 lactobacilli strain from day of hatch; D: Group supplemented with PL 120 lactobacilli strain from day of hatch; E: Group supplemented with PL 149 lactobacilli strain from day of hatch; F: Group supplemented with PL 53 lactobacilli strain after challenge (day 15-35), G: Group supplemented with PL 120 lactobacilli strain after challenge (day 15-35); H: Group supplemented with PL 149 lactobacilli strain after challenge (day 15-35); I: Group supplemented with antibiotic and later challenged.

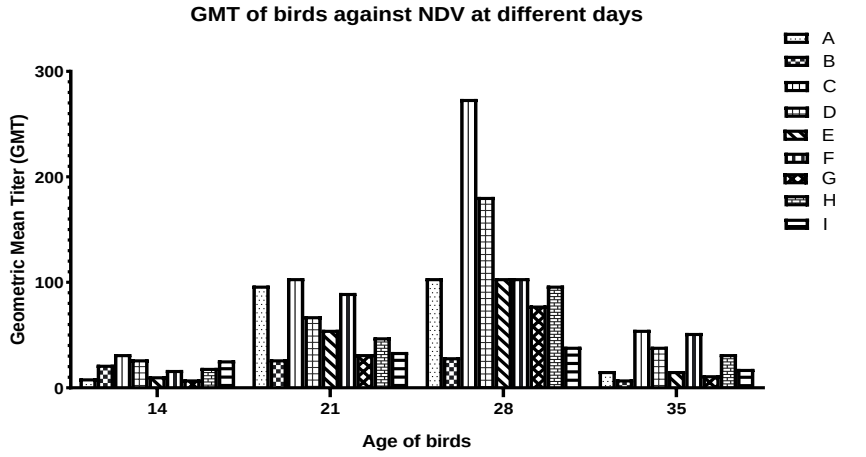

Fig. 2: Geometric mean titers (GMT) of chicken in various experimental groups against NDV at weekly intervals. Data presented as Geometric mean titer per group (GMT); A: Group with no treatment; B: Group orally gavaged with *C. jejuni*; C: Group supplemented with PL 53 lactobacilli strain from day of hatch; D: Group supplemented with PL 120 lactobacilli strain from day of hatch; E: Group supplemented with PL 149 lactobacilli strain from day of hatch; F: Group supplemented with PL 53 lactobacilli strain after challenge (day 15-35), G: Group supplemented with PL 120 lactobacilli strain after challenge (day 15-35); H: Group supplemented with PL 149 lactobacilli strain after challenge (day 15-35); I: Group supplemented with antibiotic and later challenged.

DISCUSSION

Campylobacter jejuni is the leading cause of gastroenteritis worldwide and it is common commensal of the intestinal tract of poultry (Beery *et al.*, 1988; Aguiar *et al.*, 2013). The use of probiotics to prevent colonization of *C. jejuni* at primary production level of broilers could be an effective approach to prevent enteric colonization (Beery *et al.*, 1988; Santini *et al.*, 2010; Aguiar *et al.*, 2013; Arsi *et al.*, 2015). Probiotic bacteria can prevent the enteric pathogens from adhering to the epithelial lining and can also produce antimicrobial compounds such as bacteriocins, lactic acids and hydrogen peroxides (Callaway *et al.*, 2008; Willis and Reid, 2008; Santini *et al.*, 2010; Neal-McKinney *et al.*, 2012; Nishiyama *et al.*, 2014; Shrestha, 2015).

According to a previous report, *C. jejuni* count was considerably reduced in broiler chickens fed with

probiotic formulation containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilus* and *Enterococcus faecium* (Willis and Reid, 2008; Kabir, 2009; Saint-Cyr *et al.*, 2017). Also, the efficacy of different strains of lactobacilli, *Lactobacillus acidophilus* NCFM, *Lactobacillus crispatus* JCM 5810, *Lactobacillus gallinarum* ATCC33199 and *Lactobacillus helveticus* CNRZ32 were evaluated for in vivo competitive exclusion after determining anti-campylobacter potential *in vitro* (Neal-McKinney *et al.*, 2012).

In the present study, three previously characterized indigenous isolates *L. gallinarum* PL 53, *L. casei* PL 120 and *L. gallinarum* PL 149 of poultry and human origin were used to evaluate their capacity to competitively exclude *C. jejuni* in vivo and other beneficial effects in day old broiler chicks. Groups C, D and E were administered to broiler chicks from day of hatch to 35 days daily (10^8 CFU/mL) in drinking water.

Comparing the results of enumeration of gut microbiota, minimum $\log_{10}$ values of aerobic plate count and coliform count was obtained in our probiotic supplemented group C. Previous reports showed that cecal contents of *Lactobacillus* treated group had significantly fewer coliforms and total viable count compared to control groups, suggestive of a healthier gastrointestinal tract with an improved overall balance in the intestinal microflora (Francis *et al.*, 1978; Watkins and Kratzer, 1983; Kabir, 2009). Maximum lactobacilli count and *Bifidobacteria* count was obtained in F and H groups respectively in cloacal swab samples. Maximum lactobacilli count was also obtained in group C in per gram of cecal content. The results were in accordance to previous reports, the probiotic administration results in increase in fecal counts of lactobacilli and *Bifidobacteria* (Bezkorovainy, 2001).

While comparing the *C. jejuni* count, significantly lower log values were obtained in groups supplemented with *L. gallinarum* PL 53 isolate on day 35 in cloacal swabs and cecal samples. Our results were comparable with a study where a significant reduction in *C. jejuni* count (2 log reduction) was observed in cecal content from birds in groups supplemented with *L. crispatus* JCM 5810 while *L. gallinarum* ATCC33199, *L. helveticus* CNRZ32 and *L. acidophilus* NCFM reduced colonization of *C. jejuni* to some extent (Neal-McKinney *et al.*, 2012). Also in previous studies, the birds were orally gavaged on day 14 of age and count of cecal content was done on 21, 35 and 42 day of age (Guyard-Nicodeme *et al.*, 2015; Gracia *et. al.*, 2016). The number of *Campylobacter* counts was reduced to 0.5 log reduction in cecal counts in 14 day old chickens and 1.9 log reduction at day 35 of age in chickens (Neal-McKinney *et al.*, 2012; Arsi *et al.*, 2015; Saint-Cyr *et al.*, 2017).

Group C showed maximum log reduction of *C. jejuni* count, total plate count and coliform count suggests promising probiotic potential to prevent colonization of *C. jejuni* as well as other pathogens and maintenance of healthy microflora in the colon. This oral administration of probiotic bacteria could be a cost effective, simple and effective way to prevent the colonization of *C. jejuni* in poultry at a primary production level and can also persist in animals as a beneficiary gut microbiome for longer period of time. The further study on mechanism of action of probiotics on *C. jejuni* could give a clearer picture for the possible mechanisms resulting in prevention of pathogen to attach to intestinal epithelial lining. In a study conducted by Stern *et al*. (2008) exhibited the role of anti- *C. jejuni* bacteriocins produced by *L. salivarius* strain as an inhibitory component. The experiment conducted previously reported maximum $\log_{10}$ reduction in groups administered with *Bifidobacterium longum* PCB 133 when compared to the results of *L. plantarum* PCS 20 in an in vivo trial against *C. jejuni* exhibiting 1 log reduction (Stern *et al.*, 2008; Santini *et al.*, 2010). Another study revealed 0.82 log reduction in *C. jejuni* count on day 14 while 2.81 log reduction on day 35 when compared with control group using *Lactobacillus salivarius* as a probiotic treatment group indicating lactobacilli as an effective probiotic supplement to prevent the colonization of *C. jejuni* in an in vivo trials (Saint-Cyr *et al.*, 2017).

Lactobacilli strains were also evaluated for their effect on weight gain in birds as probiotics are reported to have positive impact on broiler growth and its improved feed conversion ratio (Asghar *et al.*, 2016). In our study, lactobacilli significantly increased the weight gain of broiler birds as compared to control groups. In previous studies, the probiotic bacteria *L. gasseri* fed birds had a remarkable improvement in weight gain and feed conversion ratio of poultry birds (Askelson *et al*., 2014). In previous studies it is evident that probiotics have an immune boosting effect on poultry stimulating its immune system and enhances immune response of the host against vaccines and pathogens (Asghar *et al.*, 2016). The probiotics interact effectively with intestinal epithelial cells enhancing intestinal immunologic barrier activating the immune system by regulating the gene expression of cytokines (Li *et al.*, 2014). In the present study, lactobacilli isolate (*L. gallinarum* PL 53) had the highest effect on geometric mean titer against NDV on day 28 of serum samples collected from broiler birds. Previously, reported lactobacilli administered through feed or water have significant effects on IgG response (Maassen *et al.*, 2000). In another study, *L. crispatus* SMP70 achieved higher antibody titer at day 21 against NDV indicating modulation of immune response in broiler chickens (Asghar *et al.*, 2016).

Conclusions: It was concluded that *Lactobacillus gallinarum* PL 53 is an effective probiotic exhibiting competitive exclusion of *C. jejuni* lowering microbial load significantly in an *in vivo* trial experiment as well as maintain the overall health of gut microbiota by preventing number of potential foodborne pathogens. *Lactobacillus gallinarum* PL 53 prevented *C. jejuni* colonization at primary production level.

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Authors contribution: AAA, MN and ARA conceived and designed study. MK executed the experiments. MK and MN analyzed the data. MK, MN and MAA prepared the manuscript. All authors critically revised the manuscript for important intellectual contents and approved the final version.

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