Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.011

RESEARCH ARTICLE

# Effect of *Lactobacillus gallinarum* PL 53 Supplementation on Xylose Absorption and Intestinal Morphology in Broilers Challenged with *Campylobacter jejuni*

Madiha Khan[1,4], Aftab Ahmad Anjum[1]*, Muhammad Nawaz[1], Ali Raza Awan[2], Muhammad Asad Ali[1] and Faseeh ur Rehman[3]

[1]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Department of Anatomy & Histology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Microbiology, Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan
*Corresponding author: aftab.anjum@uvas.edu.pk

ARTICLE HISTORY (19-336)

Received: July 30, 2019
Revised: October 08, 2019
Accepted: October 16, 2019
Published online: January 25, 2020

**Key words:**
D-xylose
Histomorphology
Intestinal absorption
Intestinal morphology
*Lactobacillus*

**ABSTRACT**

A feeding trial of indigenous probiotic lactobacilli strains including *Lactobacillus gallinarum* PL 53, *L. paracasei* PL 120 and *L. gallinarum* PL 149 was conducted to monitor the effect on intestinal absorption capacity and histological changes in broiler chicks challenged with *Campylobacter jejuni*. A total of 45, day old chicks were randomly assigned to nine experimental groups (5 birds/ group). Group A was negative and B positive control while group C, D and E prevention model, F, G and H treatment model and group I antibiotic control. Groups other than negative control received *Campylobacter jejuni* ($10^6$ CFUs/bird) challenge on day 14 by oral gavage. The groups of prevention model received lactobacilli (~$10^8$ CFUs/bird) strains from day 1-35 and treatment model received lactobacilli from day 15-35. The absorption capacity of intestine was monitored by concentration of D-xylose in plasma (0.5 and 1 hour post administration). The probiotic group *L. gallinarum* PL 53 (group C) significantly enhanced D- xylose absorption capacity as compared to control groups (59.69±2.07mg/dL) in both the prevention (72.83±1.20mg/dL) and treatment (71.11±2.27mg/dL) models. Intestinal morphology was observed by measuring villi length, width, crypt depth and surface area of the small intestine. PL 53 increased the villi length of ileum (930±4.00µm), jejunum (890±8.00µm) and duodenum (1350±4.00µm). The group which received *L. gallinarum* PL 53 strain had maximum surface area of the intestinal segments with 0.41±0.03mm$^2$, 0.45±0.03mm$^2$ and 0.72±0.02mm$^2$ of ileum, jejunum and duodenum, respectively. In conclusion, the PL 53 *L. gallinarum* may improve the gut performance and absorption capacity of broilers.



**To Cite This Article:** Khan M, Anjum AA, Nawaz M, Awan AR, Ali MA and Rehman FU, 2020. Effect of *Lactobacillus gallinarum* PL 53 supplementation on xylose absorption and intestinal morphology in broilers challenged with *Campylobacter jejuni*. Pak Vet J, 40(2): 163-168. http://dx.doi.org/10.29261/pakvetj/2020.011

## INTRODUCTION

D-xylose test is used to determine mal-absorption syndrome in chicks which may occur due to many diseases (Yu *et al.*, 2015). D-xylose is used as indicative component as it is poorly metabolizable pentose sugar and readily absorbed from small intestine similar to glucose absorption in mammals (Mansoori, 2010). The probiotics can alter the absorption capacity of intestines for D-xylose indicating positive impact of probiotics on intestinal health and performance in broilers (Wealleans *et al.*, 2017). The elevation in D-xylose concentration in plasma with time is a good indicative of the absorption capacity of intestinal tract (Mansoori, 2010). The D-xylose feed in diet increases the plasma concentration of xylose which can be used as an indicator for nutrient digestibility capacity which in turns indicate the feed conversion efficiency of broilers and their growth performance status (Yu *et al.*, 2015).

Probiotics may also alter the gut morphometry, decrease the mortality ratio, increase body weight and improve the efficiency in production of commercial poultry (Dersjant-Li *et al.*, 2013). The chicks fed with probiotics positively influence the intestinal morphometric measurements increasing the villi height as well as height: crypt ratio and villus surface area of the duodenum,

jejunum and ileum of small intestines indicating the impact on intestinal health in poultry birds and ultimately on growth performance (Al-Baadani *et al.,* 2016). The increase in villus height affect beneficially the digestive and absorptive functioning of intestines mainly because of increased surface area for absorption of nutrients (Hassan *et al.,* 2014). Probiotics are used as growth promoters in broiler diet and provide an cost effective alternative to low level of growth promoting antibiotics (Mehdi *et al.,* 2018).

The probiotics were isolated and characterized previously by the authors and their anti-*Campylobacter jejuni* activity had been evaluated (unpublished data). But the data on indigenous probiotics having anti-campylobacter potential is limited in Pakistan. Also, the selected probiotics including *L. gallinarum* PL 53, *L. paracasei* PL 120 and *L. gallinarum* PL 149 effect on weight gain of broiler chicks as well as their effect on gut microbiota and immunomodulation had been evaluated in another study (Khan *et al.,* 2019). The present study was designed to evaluate the effect of previously characterized indigenous strains of probiotics *L. gallinarum* PL 53, *L. paracasei* PL 120 and *L. gallinarum* PL 149 on intestinal absorption and morphology of broiler chicks challenged with *C. jejuni*. The *C. jejuni* could have an impact on the absorption capacity and histomorphology significantly deteriorating the villi length and surface area of intestinal segments.

## MATERIALS AND METHODS

**Microbes and growth conditions:** Previously characterized lactobacilli strains *Lactobacillus gallinarum* PL 53, *L. paracasei* PL 120 and *L. gallinarum* PL 149 (unpublished data) were grown in anaerobic conditions on MRS agar at 37ºC for 48 hours. *Campylobacter jejuni* (ATCC 33291) were grown on *Campylobacter* Cefex agar supplemented with sheep blood at 42ºC for 48 hours.

**Experimental animals and housing:** A total of 45 broiler chicks were procured from a commercial hatchery (Punjab chicks, Pakistan poultry breeder hatchery) at the day of hatch and reared for 35 days following Khan *et al.* (2019). Experimental design is presented in Table 1. The group A, B and C were control groups (negative, positive and antibiotic). Groups C, D and E were prevention model which were given probiotics from day 1 while groups F, G and H were treatment model which were administered with probiotics after challenging with *C. jejuni* (day 15-35).

**D-Xylose test:** The D-xylose test was carried out according to the method of Mansoori *et al.* (2015). After inoculation of D-xylose solution (5%), the blood was drawn on 0, 0.5 and 1 hour using sterile syringes and plasma was separated. To observe the concentration of D-xylose, phloroglucinol color reagent was used followed by measuring absorbance at 554nm using spectrophotometer (Doerfler *et al.*, 2000; Regassa *et al.*, 2016). The D-xylose standard curve was also prepared (0-70 mg/2mL) (Mansoori, 2010).

**Histo-morphometric parameters of small intestine:** Sections from small intestine (ileum, jejunum and duodenum) were embedded using molten paraffin (Khan *et al.*, 2017). The staining was done with hematoxylin and eosin (H&E) and the slides were observed light microscope (Olympus CX31, Olympus USA) (Ashraf *et al.*, 2013). The histomorphometric parameters of small intestines including villus height, width, surface area, crypt depth, villus height, crypt depth ratio and lamina propria thickness were measured using Labomed Pixel Pro software. The data were categorized into grading scores using Mankin Histopathological index (Histological- Histochemical Grading System (HHGS)) (Gibson-Corley *et al.,* 2013). The score 0 was given to the negative control group while any deviation from control values was given score accordingly (0-4).

## RESULTS

The D-xylose concentration in plasma of broiler birds at 0, 30 and 60 minutes on day 35 of age is presented in Table 2. The negative control A showed 59.69±2.07mg/dL concentration while, *C. jejuni* control B and antibiotic group I exhibited 49.81±3.28mg/dL and 49.32±2.14 mg/dL concentrations of xylose in plasma after 60 minutes. D- xylose concentration in control groups were significantly ($P<0.05$) lower than of probiotic groups measured concentrations (C: 72.83, D: 59.66, E: 58.85, F: 71.11, G: 55.29, H: 59.40mg/dL). Group C showed significantly higher concentration ($P<0.05$) of D-xylose (65.17±1.18 mg/dL) at 30 minutes xylose administration while, 72.83± 1.20mg/dL after 60 minutes interval. Group F also showed higher concentration of D-xylose after 60 minutes post inoculation (71.11±2.27mg/dL) among all the treatment probiotic groups ($P<0.05$). It can therefore be stated that the *Lactobacillus gallinarum* PL 53 (administered to group C and F) had the best absorption capacity among all the probiotic and control groups used in this study.

**Table 1:** Experimental groups of broiler birds

| Groups | Experimental plan | Treatment |
|---|---|---|
| A | Control groups | No treatment |
| B | | *Campylobacter jejuni* |
| C | Prevention model | PL 53 + challenged (day 14) |
| D | | PL 120 + challenged (day 14) |
| E | | PL 149 + challenged (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 | | Enrofloxacin + challenged (day 14) |

PL 53: *Lactobacillus gallinarum*, PL 120: *Lactobacillus paracasei,* PL 149: *Lactobacillus gallinarum, Campylobacter jejuni* ATCC 33291.

**Table 2:** The D-xylose concentration in plasma of broiler birds at 0, 30 and 60 minutes on day 35 of age challenged with *Campylobacter jejuni*

| Groups | 0 min | 30 min | 60 min |
|---|---|---|---|
| | Concentration (mg/dL) | Concentration (mg/dL) | Concentration (mg/dL) |
| A | $20.09\pm0.13^{bc}$ | $52.80\pm1.14^{b}$ | $59.69\pm2.07^{c}$ |
| B | $17.87\pm0.15^{ab}$ | $45.35\pm2.17^{a}$ | $49.81\pm3.28^{a}$ |
| C | $25.23\pm0.17^{d}$ | $65.17\pm1.18^{d}$ | $72.83\pm1.20^{d}$ |
| D | $25.37\pm0.15^{d}$ | $56.39\pm1.12^{c}$ | $59.66\pm2.19^{c}$ |
| E | $21.19\pm0.10^{c}$ | $46.39\pm1.15^{a}$ | $58.85\pm2.25^{c}$ |
| F | $19.71\pm0.11^{abc}$ | $56.41\pm1.23^{c}$ | $71.11\pm2.27^{d}$ |
| G | $20.77\pm0.25^{c}$ | $54.77\pm2.07^{bc}$ | $55.29\pm2.17^{b}$ |
| H | $16.92\pm0.15^{a}$ | $46.15\pm1.18^{a}$ | $59.40\pm3.27^{c}$ |
| I | $21.21\pm0.25^{c}$ | $52.33\pm1.12^{b}$ | $49.32\pm2.14^{a}$ |

A: Negative control; B: Positive control; C, D & E: Prevention model groups supplemented with PL 53, PL 120 and PL 149 strains, respectively from day of hatch; F, G & H: Treatment model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively after challenge (day 15-35); I: Antibiotic; $^{a,b,c,d}$: Values having different superscripts differ significantly while comparing different rows among same column ($P<0.05$).

**Table 3:** Effect of lactobacilli on morphometric parameters of small intestine in chicks on day 35 of age challenged with *C. jejuni*

| | Parameters | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| ILEUM | Villus height (µm) | 690±3.00$^{c}$ | 510±12.0$^{b}$ | 930±4.00$^{i}$ | 730±4.00$^{d}$ | 850±6.00$^{h}$ | 840±5.00$^{g}$ | 480±2.00$^{a}$ | 810±10.0$^{f}$ | 750±5.00$^{e}$ |
| | Villus width (µm) | 90.0±3.00$^{b}$ | 110±2.00$^{d}$ | 140±3.00$^{f}$ | 100±1.00$^{c}$ | 90.0±3.00$^{b}$ | 110±1.00$^{d}$ | 170±4.00$^{g}$ | 120±3.00$^{e}$ | 80.0±3.00$^{a}$ |
| | Crypt depth (µm) | 50.0±2.00$^{a}$ | 90.0±3.00$^{d}$ | 50.0±5.00$^{a}$ | 50.0±1.00$^{a}$ | 60.0±2.00$^{b}$ | 60.0±1.00$^{b}$ | 130±2.00$^{e}$ | 70.0±3.00$^{c}$ | 50.0±3.00$^{a}$ |
| | Villus height: crypt depth | 13.80±1.01$^{d}$ | 5.66±0.98$^{b}$ | 18.60±0.88$^{i}$ | 14.60±1.02$^{g}$ | 14.17±1.05$^{f}$ | 14.00±1.25$^{e}$ | 3.69±0.99$^{a}$ | 11.57±1.05$^{c}$ | 15.00±0.88$^{h}$ |
| | Lamina propria thickness (µm) | 90.0±1.00$^{b}$ | 90.0±2.00$^{b}$ | 140±2.00$^{c}$ | 90.0±1.00$^{b}$ | 90.0±1.00$^{b}$ | 90.0±1.00$^{b}$ | 70.0±1.00$^{a}$ | 90.0±2.00$^{b}$ | 90.0±3.00$^{b}$ |
| | Epithelium width (µm) | 20.0±1.00$^{a}$ | 30.0±2.00$^{b}$ | 30.0±1.00$^{b}$ | 30.0±1.00$^{b}$ | 20.0±0.4$^{a}$ | 20.0±1.00$^{a}$ | 30.0±1.00$^{b}$ | 30.0±1.00$^{b}$ | 20.0±4.00$^{a}$ |
| | Villus surface area (mm$^{2)}$ | 0.20±0.01$^{a}$ | 0.18±0.01$^{a}$ | 0.41±0.03$^{e}$ | 0.23±0.02$^{b}$ | 0.24±0.01$^{bc}$ | 0.29±0.02$^{d}$ | 0.26±0.01$^{c}$ | 0.31±0.02$^{d}$ | 0.19±0.02$^{a}$ |
| JEJUNUM | Villus height (µm) | 950±11.0$^{e}$ | 720±6.00$^{b}$ | 890±8.00$^{d}$ | 950±3.00$^{e}$ | 880±9.00$^{c}$ | 1180±20.0$^{h}$ | 1110±5.00$^{g}$ | 710±10.0$^{a}$ | 980±11.0$^{f}$ |
| | Villus width (µm) | 110±3.00$^{a}$ | 120±2.00$^{b}$ | 160±4.00$^{e}$ | 110±1.00$^{a}$ | 140±3.00$^{d}$ | 110±4.00$^{a}$ | 130±3.00$^{c}$ | 190±3.00$^{f}$ | 130±3.00$^{c}$ |
| | Crypt depth (µm) | 90.0±3.00$^{d}$ | 80.0±2.00$^{c}$ | 50.0±2.00$^{a}$ | 100±1.00$^{e}$ | 80.0±2.00$^{c}$ | 60.0±4.00$^{b}$ | 110±4.00$^{f}$ | 150±3.00$^{g}$ | 90.0±3.00$^{d}$ |
| | Villus height: crypt depth | 10.55±1.01$^{b}$ | 9.00±0.92$^{b}$ | 17.80±0.98$^{c}$ | 9.50±0.77$^{b}$ | 11.00±0.95$^{b}$ | 19.67±1.05$^{c}$ | 10.09±0.65$^{b}$ | 4.73±1.01$^{a}$ | 10.88±1.01$^{b}$ |
| | Lamina propria thickness (µm) | 90.0±2.00$^{a}$ | 90.0±2.00$^{a}$ | 160±4.00$^{d}$ | 120±1.00$^{c}$ | 90.0±3.00$^{a}$ | 90.0±2.00$^{a}$ | 120±2.00$^{c}$ | 110±2.00$^{b}$ | 90.0±2.00$^{a}$ |
| | Epithelium width (µm) | 20.0±1.00$^{a}$ | 20.0±1.00$^{a}$ | 30.0±1.00$^{b}$ | 30.0±0.4$^{b}$ | 40.0±1.00$^{c}$ | 20.0±1.00$^{a}$ | 30.0±1.00$^{b}$ | 40.0±1.00$^{c}$ | 20.0±1.00$^{a}$ |
| | Villus surface area (mm$^{2)}$ | 0.32±0.04$^{b}$ | 0.27±0.05$^{a}$ | 0.45±0.03$^{e}$ | 0.33±0.02$^{b}$ | 0.39±0.01$^{c}$ | 0.41±0.0$^{cd}$ | 0.45±0.05$^{e}$ | 0.42±0.02$^{d}$ | 0.40±0.01$^{cd}$ |
| DUODENUM | Villus height (µm) | 1260±14.0$^{c}$ | 1290±42.0$^{d}$ | 1350±4.00$^{f}$ | 970±4.00$^{a}$ | 1290±8.00$^{d}$ | 1110±21.0$^{b}$ | 1320±9.00$^{e}$ | 1390±10.0$^{g}$ | 1390±12.0$^{g}$ |
| | Villus width (µm) | 120±1.00$^{d}$ | 80.0±2.00$^{a}$ | 170±2.00$^{g}$ | 130±3.00$^{e}$ | 120±1.00$^{d}$ | 150±6.00$^{f}$ | 90.0±3.00$^{b}$ | 110±2.00$^{c}$ | 120±2.00$^{d}$ |
| | Crypt depth (µm) | 70.0±1.00$^{a}$ | 70.0±1.00$^{a}$ | 70.0±2.00$^{a}$ | 70.0±4.00$^{a}$ | 90.0±1.00$^{b}$ | 90.0±4.00$^{b}$ | 70.0±3.00$^{a}$ | 90.0±1.00$^{b}$ | 110±2.00$^{c}$ |
| | Villus height: crypt depth | 18.00±0.92$^{cd}$ | 18.42±1.22$^{d}$ | 19.29±0.77$^{d}$ | 13.86±0.77$^{ab}$ | 14.33±0.88$^{ab}$ | 12.33±1.05$^{a}$ | 18.85±0.97$^{d}$ | 15.44±0.98$^{bc}$ | 12.63±1.02$^{ab}$ |
| | Lamina propria thickness (µm) | 120±1.00$^{e}$ | 110±2.00$^{d}$ | 110±8.00$^{d}$ | 170±1.00$^{h}$ | 150±4.00$^{g}$ | 90.0±2.00$^{b}$ | 130±1.00$^{f}$ | 70.0±1.00$^{a}$ | 100±1.00$^{c}$ |
| | Epithelium width (µm) | 30.0±1.00$^{b}$ | 20.0±1.00$^{a}$ | 70.0±4.00$^{c}$ | 30.0±1.00$^{b}$ | 20.0±0.4$^{a}$ | 30.0±2.00$^{b}$ | 20.0±1.00$^{a}$ | 20.0±1.00$^{a}$ | 30.0±1.00$^{b}$ |
| | Villus surface area (mm$^{2}$) | 0.47±0.05$^{d}$ | 0.32±0.02$^{a}$ | 0.72±0.02$^{f}$ | 0.40±0.01$^{c}$ | 0.49±0.03$^{d}$ | 0.52±0.01$^{e}$ | 0.37±0.01$^{b}$ | 0.48±0.01$^{d}$ | 0.52±0.01$^{e}$ |

$^{a,b,c,d,e,f,g,h,i}$: Values having different superscripts differ significantly among same row in different column (P<0.05). A: Negative control; B: Positive control; C, D & E: Prevention model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively from day of hatch; F, G & H: Treatment model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively after challenge (day 15-35); I: Antibiotic.

**Table 4:** Histological scores on morphometric parameters of small intestine in chicks on day 35 of age challenged with *Campylobacter jejuni*

| | Parameters | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| ILEUM | Villus length | 0 | 0 | 3 | 1 | 2 | 2 | 0 | 2 | 1 |
| | Villus width | 0 | 1 | 2 | 0 | 0 | 1 | 4 | 1 | 0 |
| | Crypt depth | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| JEJUNUM | Villus length | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 |
| | Villus width | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 3 | 1 |
| | Crypt depth | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 0 |
| DUODENUM | Villus length | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| | Villus width | 0 | 0 | 5 | 1 | 0 | 3 | 0 | 0 | 0 |
| | Crypt depth | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 4 |

Ileum: Villus length: 0:400-600, 1:700, 2:800, 3:900, Villus width: 0:90-100, 1: 110-120, 2:130-140, 3:150-160 4:170-180, Crypt depth: 0:50-70, 1:80-100, 2:110-130, 3:140-160; Jejunum: Villus length: 0:700-900, 1:1000, 2:1100, 3:1100-1200, Villus width: 0:110-120, 1: 130-150, 2:160-180, 3:190-210, Crypt depth: 0:50-90, 1:100-110, 2:120-130, 3:140-160; Duodenum: Villus length: 0:1000-1300, 1:1301-1600, Villus width: 0:80-120, 1: 130, 2:140, 3:150, Crypt depth: 0:70, 1:80, 2:90, 3:100, 4:110.

The effect of lactobacilli on histomorphometric parameters is presented in table 3. Villi height in ileum was increased in probiotic groups (C: 930, D: 730, E: 850, F: 840, H: 810µm) as compared to negative control (690µm) and *C. jejuni* control groups (510µm). Similarly, the villus surface area of ileum was also increased in probiotic groups (C: 0.41, D: 0.23, E: 0.24, F: 0.29, G: 0.26 and H: 0.31mm$^2$) as compared to negative control group (0.20mm$^2$). The villi height and crypt depth ratio of ileum in probiotic groups were comparatively higher (C: 18.60, D: 14.60, E: 14.17, F: 14.00, H: 11.57) as compared to *C. jejuni* control group (5.66). *Lactobacillus gallinarum* PL 53 isolate in group C showed significant increase in villus surface area (0.41±0.03mm$^2$) and villus height and crypt depth ratio (18.60±0.88) in ileum (P<0.05).

While comparing the effect of lactobacilli on morphometric measurements of jejunum, group C and G had maximum villus surface area (0.45mm$^2$) as compared to all other experimental groups (A: 0.32, B: 0.27, D: 0.33, E: 0.39, F: 0.41, H: 0.42mm$^2$). While comparing the results of duodenum, group C had maximum villi height (1350±4.00µm) and villi width (170±2.00µm). PL 53 group C had a significant effect on duodenum villi surface area (0.72±0.02mm$^2$) and ratio of villus height and crypt depth (19.29±0.77). The histological scores also showed statistically significant increase in villus height, villus width and crypt depth of probiotics supplemented group when compared with control group (P<0.05) (Table 4). Histomicrograph of ileum, jejunum and duodenum of different groups on day 35 of age of chicks challenged with *Campylobacter jejuni* is presented in figure 1, 2 and 3, respectively.

## DISCUSSION

Probiotic supplementation has a significant effect on overall health and intestinal performance of the broilers (Applegate *et al.*, 2010; Mountzouris *et al.*, 2010). This study was designed to evaluate the effect of previously characterized probiotic strain *Lactobacillus gallinarum* PL 53, *L. paracasei* PL 120 and *L. gallinarum* PL 149 (Khan *et al.*, 2019) on intestinal absorption capacity and morphology in broiler chicks challenged with *C. jejuni*. *C. jejuni* ATCC 33291 was used as a challenged organism and although it is generally prevalent in broiler without causing an apparent disease symptoms, it could have a negative impact on the absorption capacity and histomorphology significantly deterioting the villi length and surface area of intestinal segments affecting the overall gut function (Awad *et al.*, 2015).

The D-xylose test is an indicator for the measurement of the absorption capacity by the small intestine and to evaluate the growth performance in broilers (Doerfler *et al.*, 2000; Semrad, 2005; Mansoori, 2010; Yu *et al.*, 2015). It indicates the variation in intestinal absorption capacity due to different dietary and physiological conditions of the birds (Mansoori *et al.*, 2012). The mechanism of D-xylose absorption test includes the diffusion and passage of xylose from the intestinal membrane in healthy animals through the trans-cellular pathway (Chang *et al.*, 2004; Chang and Karasov, 2004).

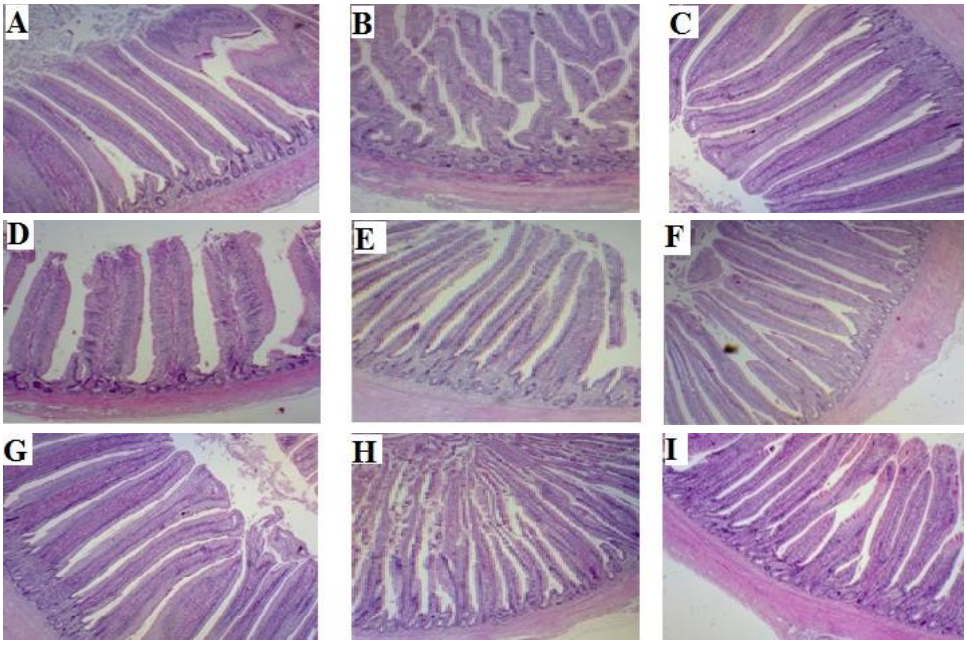


**Fig. 1:** Histomicrograph of ileum of different groups on day 35 of age of chicks challenged with *Campylobacter jejuni.* A: Negative control; B: Positive control; C, D & E: Prevention model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively from day of hatch; F, G & H: Treatment model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively after challenge (day 15-35); I: Antibiotic.

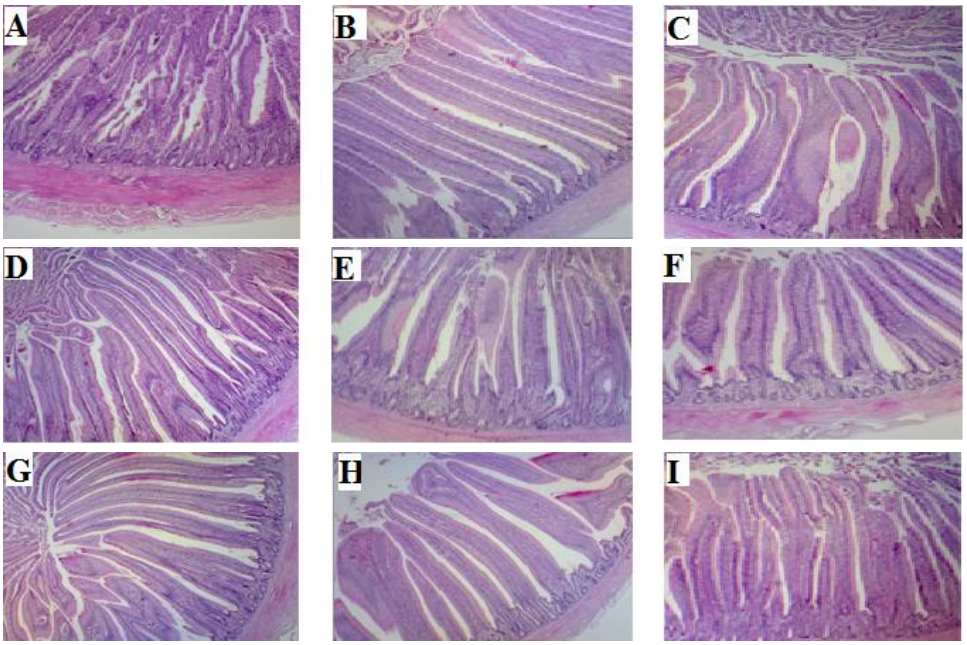


**Fig. 2:** Histomicrograph of jejunum of different groups on day 35 of age of chicks challenged with *Campylobacter jejuni*. A: Negative control; B: Positive control; C, D & E: Prevention model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively from day of hatch; F, G & H: Treatment model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively after challenge (day 15-35); I: Antibiotic.

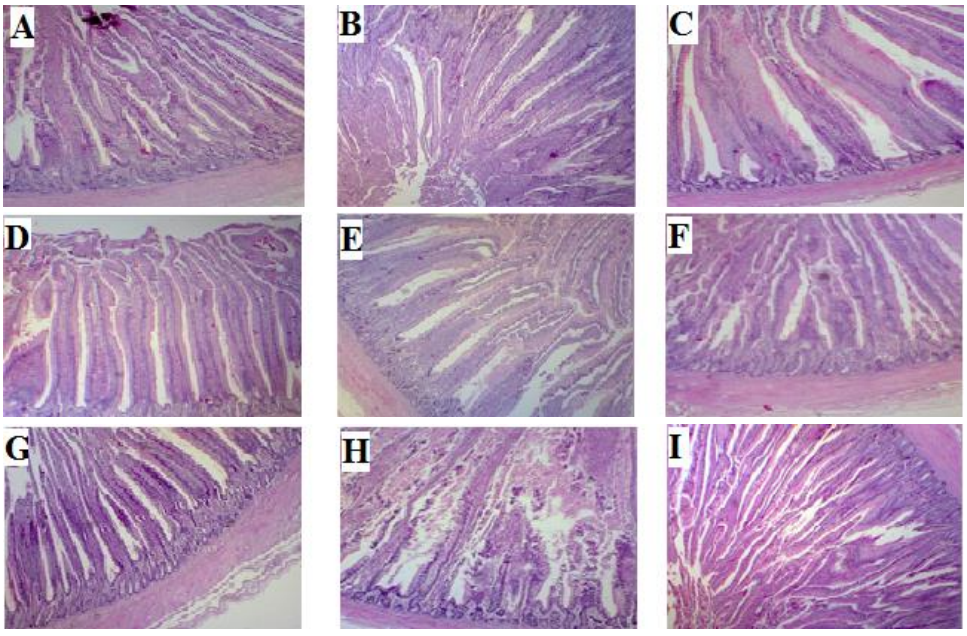


**Fig. 3:** Histomicrograph of duodenum of different groups on day 35 of age of chicks challenged with *Campylobacter jejuni.* A: Negative control; B: Positive control; C, D & E: Prevention model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively from day of hatch; F, G & H: Treatment model groups supplemented with PL 53, PL 120 and PL 149 lactobacilli, respectively after challenge (day 15-35); I: Antibiotic.

The xylose is then absorbed by the small intestine and the concentration of xylose in plasma particularly within three hours post inoculation indicates the adsorption capacity of the chicks (Doerfler *et al.*, 2000). In another study, organic acids also increased the digestibility as well as absorption capacity of small intestine enhancing available nutrients by increasing the beneficial microflora (Ziaie *et al.*, 2011; Olnood *et al.*, 2015; Khan and Iqbal, 2016).

In this study, the concentration in plasma samples collected from birds supplemented with probiotic *L. gallinarum* PL 53 were comparatively higher (C: 72.83mg/dL and F: 71.11mg/dL) as compared to control groups after 60 minutes post inoculation. Therefore, the significantly high concentration of D-xylose was monitored in *L. gallinarum* PL 53 (group C) 65.17±1.18 mg/dL after 30 minutes and 72.83±1.20mg/dL was monitored after 60 minutes of inoculation in preventive model groups. The *L. gallinarum* PL 53 group (F) also demonstrated highest concentration of D-xylose (71.11±2.27mg/dL) in treatment model group.

The histo-morphological measurements of segments of small intestine play an important role in determining the absorption capacity and growth performance in birds (Yu *et al.*, 2007; Mehdi *et al.*, 2018). It has been reported that the use of probiotics particularly *Lactobacillus* spp. enhanced the absorptive capacity by the small intestine with an increase in villus height and crypt depth and better overall gut health and growth performance by balancing microflora (Wang *et al.*, 2017; Li *et al.*, 2018). Our results were in accordance to previous studies in which, probiotic supplementation in drinking water significantly increased villus height and in turn also enhanced the villus surface area as compared to control groups. The most effective probiotic supplementation according to our data was *L. gallinarum* PL 53 which increased significantly villus surface area as: 0.41, 0.45 and 0.72mm$^2$ in ileum, duodenum and jejunum, respectively. The results obtained in this study also demonstrated the increase in villus height and crypt ratio in probiotics supplemented groups as compared to positive control group (5.66±0.98mm). The ratio in the ileum segment was 18.60, 13.60 and 14.17mm in PL53, PL 120 and PL 149 probiotic groups, respectively in birds supplemented from day 1 to 35. In jejunum segment of small intestine, the highest ratio was obtained in PL 53 supplemented group (17.80±0.98mm) in prevention model as compared to positive control group (9.63±0.92mm). In duodenum segment, the highest ratio was also obtained in PL 53 supplemented group (19.29±0.77mm) as compared to all the experimental groups (A: 18.00, B: 18.42, D: 13.86, E: 14.33, F:12.33, G:18.85, H: 15.44 and I: 12.63), respectively.

In a previous study, significantly higher ratio between villus height and crypt depth in the ileum segment of small intestine was recorded in broiler fed with probiotic *L. johnsonii* (6.29) as compared to negative control group (5.72) indicating the enhanced absorption capacity (Olnood *et al.*, 2015). In contrast, if the decrease in villus length or the fusion of villi was observed then there would be considerable loss of digestion and absorption of nutrients by the small intestine (Van Dijk *et al.*, 2002). The results obtained in previous experiments showed a similar increase in gut health and morphology by increase in villi height of ileum (592µm), jejunum (1012µm) and duodenum (1486µm) with the supplementation of *L. plantarum* strains (RS5, RI11, RG11 and RG14) (Thanh *et al.*, 2009). In another study, villus height was recorded 1016µm when supplemented with *L. reuteri* as compared to groups supplemented with *L. reuteri* (1016µm), *Bacillus subtilis* and *Saccharomyces cerevisiae* (1167µm) and control group (774.16µm). Similar increase was observed in villus width in probiotic supplemented groups when compared to control groups (Salim *et al.*, 2013). Rodríguez-Lecompte *et al.* (2012) observed an increase in histomorphometric measurements in segments of small intestine by administration of probiotics and organic acids in young chicks demonstrating nutrient digestibility. Overall, growth performance as well as meat yield of broilers were increased (Allahdo *et al.*, 2018). The organic acids and probiotics stimulate the normal crypt cells proliferation increasing the cell turnover rate of healthy tissues and prevent the colonization of pathogens (Paul *et al.*, 2007; Khan, 2013). These results clearly indicated the effect of probiotics on enhancement of villus height.

**Conclusions:** It was concluded that *Lactobacillus gallinarum* PL 53 may improve the gut performance and absorption capacity of broilers.

**Acknowledgments:** This work was partially supported by Higher Education Commission (HEC) project # 4333/ NRPU/R&D/HEC/14/278. The work was also supported by project # 7058/Punjab/NRPU/R&D/HEC/ 2017.

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

## REFERENCES

Al-Baadani H, Abudabos A, Al-Mufarrej S, *et al.,* 2016. Effects of dietary inclusion of probiotics, prebiotics and synbiotics on intestinal histological changes in challenged broiler chickens. S Afr J Anim Sci 46:157-65.

Allahdo P, Ghodraty J, Zarghi H, *et al.,* 2018. Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens. Ital J Anim Sci 17:675-85.

Applegate T, Klose V, Steiner T, *et al.,* 2010. Probiotics and phytogenics for poultry: myth or reality? J Appl Poult Res 19:194-210.

Ashraf S, Zaneb H, Yousaf M, *et al.,* 2013. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J Anim Physiol Anim Nutr 97:68-73.

Awad WA, Molnár A, Aschenbach JR, *et al.*, 2015. *Campylobacter* infection in chickens modulates the intestinal epithelial barrier function. Innate Immun 21:151-60.

Chang MH, Chediack J, Caviedes-Vidal E, *et al.,* 2004. L-glucose absorption in house sparrows (*Passer domesticus*) is nonmediated. J Comp Physiol B 174:181-8.

Chang MH and Karasov WH, 2004. How the house sparrow *Passer domesticus* absorbs glucose. J Exp Biol 207:3109-21.

Dersjant-Li Y, Awati A, Kromm C, *et al.,* 2013. A direct fed microbial containing a combination of three-strain *Bacillus sp*. can be used as an alternative to feed antibiotic growth promoters in broiler production. J Appl Anim Nutr 2:1-6.

Doerfler R, Cain L, Edens F, *et al.,* 2000. D-Xylose absorption as a measurement of malabsorption in poult enteritis and mortality syndrome. Poult Sci 79:656-60.

Gibson-Corley KN, Olivier AK and Meyerholz DK, 2013. Principles for valid histopathologic scoring in research. Vet Pathol 50:1007-15.

Hassan H, Youssef AW, El-Daly EF, *et al.,* 2014. Performance, caecum bacterial count and ileum histology of broilers fed different direct-fed microbials. Asian J Poult Sci 8:106-14.

Khan I, Zaneb H, Masood S, *et al.,* 2017. Effect of Moringa oleifera leaf powder supplementation on growth performance and intestinal morphology in broiler chickens. J Anim Physiol Anim Nutr 101:114-21.

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 39:473-8.

Khan S, 2013. Probiotic microorganisms-identification, metabolic and physiological impact on poultry. World's Poult Sci J 69:601-12.

Khan SH and Iqbal J, 2016. Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res 44:359-69.

Li CL, Wang J, Zhang HJ, *et al.,* 2018. Intestinal morphologic and microbiota responses to dietary *Bacillus spp*. in a broiler chicken model. Front Physiol 9:1-18.

Mansoori B, 2010. D-Xylose absorption capacity of broiler intestine in response to phytic acid. Br Poult Sci 51:158-61.

Mansoori B, Nodeh H and Modirsanei M, 2012. Differences in intestinal absorptive capacity of chickens for D-Xylose. Iran J Vet Med 6:273-8.

Mansoori B, Rogiewicz A and Slominski B, 2015. The effect of canola meal tannins on the intestinal absorption capacity of broilers using a D- xylose test. J Anim Physiol Anim Nutr 99:1084-93.

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, *et al.,* 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Anim Nutr 4:170-8.

Mountzouris K, Tsitrsikos P, Palamidi I, *et al.,* 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult Sci 89:58-67.

Olnood CG, Beski SS, Choct M, *et al.,* 2015. Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim Nutr 1:184-91.

Paul SK, Halder G, Mondal MK, *et al.,* 2007. Effect of organic acid salt on the performance and gut health of broiler chicken. J Poult Sci 44:389-95.

Regassa A, Kiarie E, Sands J, *et al.,* 2016. Nutritional and metabolic implications of replacing cornstarch with D-xylose in broiler chickens fed corn and soybean meal-based diet. Poult Sci 96:388-96.

Rodríguez-Lecompte J, Yitbarek A, Brady J, *et al.,* 2012. The effect of microbial-nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration. J Anim Sci 90:2246-54.

Salim H, Kang H, Akter N, *et al.,* 2013. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poult Sci 92:2084-90.

Semrad S, 2005. Malassimilation syndromes in large animals. Merck veterinary manual, ninth ed.(Ed. CM Kahn). Merck Co. Inc., Whitehouse Station, NJ, USA pp:301-6.

Thanh N, Loh T, Foo H, *et al.,* 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br Poult Sci 50:298-306.

Van Dijk J, Huisman J and Koninkx J, 2002. Structural and functional aspects of a healthy gastrointestinal tract. Nutr Health Gastrointestinal tract pp:71-96.

Wang H, Ni X, Qing X, *et al.,* 2017. Live probiotic *Lactobacillus johnsonii* BS15 promotes growth performance and lowers fat deposition by improving lipid metabolism, intestinal development, and gut microflora in broilers. Front Microbiol 8:1073.

Wealleans AL, Walsh MC, Romero LF, *et al.,* 2017. Comparative effects of two multi-enzyme combinations and a *Bacillus* probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides and gut microflora in broiler chickens. Poult Sci 96:4287-97.

Yu B, Liu J, Chiou M, *et al.,* 2007. The effects of probiotic *Lactobacillus reuteri* Pg4 strain on intestinal characteristics and performance in broilers. Asian-australas J Anim Sci 20:1243-51.

Yu W, Zhang X, Hussain A, *et al.,* 2015. Intestinal absorption function of broiler chicks supplemented with Ginkgo leaves fermented with *Bacillus species*. Pak J Zool 47: 479-90.

Ziaie H, Bashtani M, Torshizi MK, *et al.,* 2011. Effect of antibiotic and its alternatives on morphometric characteristics, mineral content and bone strength of tibia in Ross broiler chickens. Glob Vet 7:315-3.