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## Prebiotic, probiotic, and antibiotic growth promoters use in commercial broilers: A comparative study

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**ABSTRACT:** The objective of this study was to compare the effect of Mannan oligosaccharides (MOS), a prebiotic, probiotics (*Bacillus subtilis*), in the replacement of the antibiotics (Zinc Bacitracin 10%) in the broilers. For this purpose, two hundred broiler chicks were bought from a hatchery and allocated into five treatments having four replicates (10 birds in each). Birds were distributed into five groups: control, antibiotics (Zinc Bacitracin 10%), probiotics (*Bacillus subtilis*) and prebiotics (MOS), and a combination of probiotics and prebiotics groups. Five iso-nitrogenous and iso-caloric diets were prepared and offered to birds. Feed intake and body weight were recorded. At the end of the trial, birds were slaughtered to obtain carcass and gut health data. Data collected were examined by ANOVA under CRD and mean values were compared using Tukey's HSD (Honestly significant difference) test. Body weight gain was higher ( $p < 0.05$ ) in birds fed diet having probiotics and prebiotics in a combination form. Improved ( $p < 0.05$ ) FCR was recorded in birds fed diet having prebiotics alone and in combination with probiotics. Dressing percentage was higher ( $p < 0.05$ ) in birds fed diet having Probiotics + Prebiotics and control birds. Breast yield was higher ( $p < 0.05$ ) in birds fed having Probiotics + Prebiotics. In gut morphometric parameters, there was observed an increase in villus height, and a significant change of increase in villus surface area was seen. In conclusion, the addition of prebiotics in combination with probiotics, in feed, remarkably improved growth performance and carcass yield in commercial broilers.

**Keywords:** Mannan oligosaccharide, probiotic, broiler, performance, gut morphometry.

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## INTRODUCTION

Infectious diseases have always been a great threat in intensive animal production systems (Bosila et al., 2021; Guerrini et al., 2021; Saeed 2021; Tahir et al., 2021, Ul-Rahman et al., 2021; Abu et al., 2022). Use of antibiotics in broiler diet has been known to have an important role in reduction of the intestinal pathogens and diseases incidences (Elazab et al., 2021). However, now consumers are more concerned about the residue of antibiotics in poultry eggs and meat (Mathur and Singh, 2005). Antibiotic resistance became a global issue in humans as well as poultry and livestock species. Therefore, European Union banned the use of antibiotics in poultry diet in 2006 (European Commission, 2006). Now several researchers are trying to discover new alternative sources of antibiotics in poultry diet (Al-Sarraj 2021; Mohamed et al., 2021; Mohsin et al., 2021; Rani et al., 2021; Rafay et al., 2021; Rehman et al., 2021). Thus, acidifiers, probiotics, prebiotics and phenolic compounds are being studied to replace antibiotics in poultry diet (Baurhoo et al., 2009; Ismael *et al.*, 2022; Rashid et al., 2022).

Probiotics are dietary supplements consisting of live microbes that beneficially affect the host organism by their beneficial effects associated with the improvement in the microbial balance in intestine. Use of probiotics in poultry diet improves the performance in broilers. Birds fed on diet containing *Lactobacillus* Spp. or *Lactococcus lactis* showed increased livability (Brzóska et al., 2012). Prebiotics are non-digestible dietary supplements that selectively stimulate the growth or/and activity of one or a particular number of bacteria in the gastrointestinal tract and results in improved health status of host (Hajati and Rezaei, 2010). Prebiotics also considered as growth promoter in poultry diet. Kamran et al. (2013) noted that broiler fed diet having prebiotics had higher feed consumption and better FCR.

Commercial MOS is a well-known prebiotic/ feed additive, acquired from external layer of yeast (*Saccharomyces cerevisiae*) cell walls. Use of 0.05% MOS improved populations of lactobacilli and decreased *E. coli* in bird intestines (Kim et al., 2011). MOS is also produced by enzymatic hydrolysis of agricultural wastes like copra meal (Ariandi and Meryandini, 2015). Hitherto, no scientific studies have been done to elucidate the effect of prebiotic, probiotics and their combination on digestive function and growth in any species.

So, objective of this study was to examine the ef-

fect of MOS (a prebiotic), *Bacillus subtilis* (a probiotic) and their combination on growth performance, carcass parameters and gut health in broilers, thereby replacing traditionally used antibiotics.

## MATERIALS AND METHODS

**House preparation:** Experimental trial was carried out at the research center of Directorate of Farms, University of Agriculture Faisalabad. One week before the arrival of birds, the house was whitewashed and disinfected. House was fumigated before the onset of chicks. The litter material and wood-shaving were spread in all pens. To keep the litter material dry, it was raked on a daily basis. The experimental birds were raised under the same environmental conditions such as space, light, humidity, ventilation and temperature.

**Experimental design:** A total of two hundred (200) day-old broiler chicks (Arbor Acres) were procured from a local hatchery and randomly allocated into 5 treatments. Each group was allocated into 4 replicates containing 10 birds in each. The feed composition offered to the birds are given in the table 1. Five iso-nitrogenous and iso-caloric diets were prepared.

| Treatments  | Diet composition                                     | Addition (g)/ ton feed |
|-------------|--|------------------------|
| Treatment A | Basal diet (control)                                 | Nil                    |
| Treatment B | Basal diet + Antibiotic (Zinc Bacitracin 10%)        | 400 gm                 |
| Treatment C | Basal diet + Probiotics ( <i>Bacillus subtilis</i> ) | 50 gm                  |
| Treatment D | Basal diet + Prebiotics (Mannan oligosaccharide)     | 1000 gm                |
| Treatment E | Basal diet + Probiotics + Prebiotics                 | 1000 gm                |

**Growth performance:** Body weight was noted at the end of each subsequent week. Feed intake was considered as follow:

$$\text{Feed Intake} = \text{Feed consumption} - \text{Feed remaining}$$

FCR was calculated week-wise using the subsequent equation.

$$\text{FCR} = \text{Feed intake (g)} / \text{Body weight gain (g)}$$

**Carcass characteristics:** At the end of experiment, two broiler birds from each pen were slaughtered for carcass traits and data regarding carcass weight, breast and thigh weight and internal organs (liver, gizzard, and heart) weight were recorded.

**Table 1.** Feed ingredients and composition of starter and grower diets for broilers

| Feed ingredients (%)                               | Starter | Grower |
|--|---------|--------|
| Corn   | 40.15   | 57.57  |
| Rice broken  | 15.0    | ---    |
| Rice polish  | ---     | 4.00   |
| Wheat bran   | 1.34    | ---    |
| Soya meal  | 11.54   | 9.60   |
| Sunflower meal                                     | 12.00   | 13.00  |
| Canola meal  | 9.00    | 5.00   |
| Rapeseed meal                                      | 5.00    | 7.60   |
| Guar meal  | 1.00    | ---    |
| Molasses   | 2.00    | ---    |
| Dicalcium phosphate                                | 1.73    | 1.96   |
| Premix*  | 1.00    | 1.00   |
| Sodium chloride                                    | 0.21    | 0.21   |
| Sodium bicarbonate                                 | 0.03    | 0.065  |
| Proximate composition (%)                          |         |        |
| Crude protein                                      | 19.6    | 18.5   |
| Crude fibre  | 1.26    | 1.80   |
| Crude fat  | 2.16    | 2.35   |
| Total ash  | 5.77    | 5.40   |
| Calculated apparent metabolizable energy (Kcal/kg) | 2,750   | 2,850  |

\*Vitamin mineral premix (each kg contained): K, 70 g; Ca, 195 g; Mg, 6 g; Na, 18 g; Zn, 28 37mg; Cu, 400 mg; Fe, 2,000 mg; Se, 8 mg; I, 40 mg; Mn, 1,200 mg; Co, 20 mg; vitamin D3, 80,000 IU; vitamin A, 200,000 IU; vitamin K3, 34 mg; vitamin E, 1072 IU; Thiamine, 35 mg; Riboflavin, 135 mg; Ascorbic acid, 1,300 mg; vitamin B6, 100 mg; Niacin, 1,340 mg; vitamin B12, 670 µg; folic acid, 34 mg; and biotin, 3,350 µg.

**Gut Morphology:** After slaughtering of birds, duodenum and ileum specimens were collected and fixed, the process to preserve tissues from ongoing degradational changes, in 10% neutral buffered formalin solution for 72 hours, processed, embedded in paraffin, and sectioned at 4 µm with the help of a microtome. By using an image analysis software (ToupView 3.7) the following parameters were measured:

- (i) villus height (VH)
  - (ii) villus width (VW)
  - (iii) Depth of crypt (CD)
  - (iv) VH/VW ratio
  - (v) VH/CD ration
  - (vi) Villus surface area ( $\text{mm}^2$ ) =  $2\pi$  (VH) x (VW/2)
- (Sakamoto et al., 2000)

**Statistical Analysis:** Data was computed using Microsoft Excel® and analyzed with one-way of variance analysis (ANOVA), using statistical software Statistix 8.1. The means of parameters were compared using

Tukey's honestly significant difference (HSD) test (Steel et al., 1997). The level of significance was kept at 5 percent.

## RESULTS

Results of probiotics, prebiotics and combination of probiotics and prebiotics in replacement to antibiotics on growth performance are given in following.

### Growth performance

Mean values of body weight gain, feed intake and FCR during starter, finisher and overall phases are given in Table 2.

Statistical analysis of weight gain in starter revealed non-significant difference among different treatments ( $p > 0.05$ ). However, body weight gain was higher in birds fed diet having prebiotics in their diet. Although, in finisher and overall phases, significant change was observed in weight gain amongst all group but in finisher phase, the weight gain was higher in birds fed diet having probiotics and prebiotics in combination form. In all phase of weight gain was higher in birds fed diet having probiotics and prebiot-

ics in combination form.

Statistical analysis of feed intake in starter, finisher and overall phases revealed non-significant difference ( $p > 0.05$ ) among different treatments except the starter and finisher phases feed intake in which higher value was recorded in birds having prebiotics in their diet. However, in overall phase feed intake was higher in birds fed diet having prebiotics in their diet and control diet.

Statistical analysis of FCR in starter phase revealed non-significant difference ( $p > 0.05$ ) among different treatments (Table 2). However, better FCR was recorded in birds fed diet having probiotics and prebiotics. In finisher and overall phases, FCR revealed significant difference ( $p = 0.05$ ) among different treatments. Improved FCR was recorded in birds fed diet having prebiotics alone and in combination with probiotics.

Mortality: Statistical analysis of mortality revealed significant difference ( $p = 0.05$ ) among different treatments. Birds fed diet having prebiotics in their diet had lower mortality during starter and overall phase as compared to other treatments.

Carcass characteristics: Mean values of dressing percentage, thigh yield and breast yield are given in

table 3. Statistical analysis of dressing percentage, thigh yield and breast yield revealed significant difference ( $p = 0.05$ ) among different treatments. Dressing percentage was higher in birds fed diet having Probiotics + Prebiotics and control birds. Thigh yield was higher in birds fed control diet. Chest yield was higher in birds fed having Probiotics + Prebiotics. Statistical analysis of liver percentage, gizzard percentage and heart percentage revealed non-significant difference ( $p = 0.05$ ) among different treatments. However, liver percentage was higher in birds fed having Probiotics + Prebiotics. Gizzard percentage was higher in birds fed having antibiotics. Heart percentage was higher in birds fed having antibiotics.

### Gut Morphology

a. Duodenum: Mean values of different parameters are given in table 4. Villus height was higher in birds fed supplemented with prebiotics alone and in combination with probiotics while villus width was increased in birds fed supplemented with probiotics + prebiotics. Crypt depth was higher in antibiotic group and lower in prebiotic group. Ratio of villus height & crypt depth and villus height & villus width was higher in prebiotic group was higher in prebiotic group and lower in probiotic + prebiotic group, respectively. Interestingly, Ratio of villus height & crypt depth was decreased in the antibiotic treated group. The villus

**Table 2.** Effects of treatments on mean ( $\pm$ SEM) values of growth performance parameters and mortality (%) during starter, finisher and overall phases

| Treatments              | Starter Phase 1-21 days |                      |                 |                               | Finisher phase 22-35 days         |                      |                               |                 | Overall gain 1-35 days            |                      |                               |                               |
|-------------------------|-------------------------|----------------------|-----------------|-------------------------------|-----------------------------------|----------------------|-------------------------------|-----------------|-----------------------------------|----------------------|-------------------------------|-------------------------------|
|                         | Weight gain (g/bird)    | Feed intake (g/bird) | FCR             | Mortality (%)                 | Weight gain (g/bird)              | Feed intake (g/bird) | FCR                           | Mortality (%)   | Weight gain (g/bird)              | Feed intake (g/bird) | FCR                           | Mortality (%)                 |
| Control                 | 821.35 $\pm$ 19.83      | 1234.60 $\pm$ 83.91  | 1.50 $\pm$ 0.13 | 12.50 $\pm$ 4.81 <sup>a</sup> | 1091.92 $\pm$ 45.68 <sup>b</sup>  | 1986.51 $\pm$ 83.05  | 1.82 $\pm$ 0.01 <sup>a</sup>  | 0.00 $\pm$ 0.00 | 1913.27 $\pm$ 54.21 <sup>c</sup>  | 3221.11 $\pm$ 22.82  | 1.68 $\pm$ 0.05 <sup>a</sup>  | 12.50 $\pm$ 4.81 <sup>a</sup> |
| Antibiotics             | 797.96 $\pm$ 48.34      | 1241.99 $\pm$ 103.22 | 1.57 $\pm$ 0.25 | 20.83 $\pm$ 4.81 <sup>a</sup> | 1125.28 $\pm$ 39.59 <sup>ab</sup> | 1941.96 $\pm$ 98.85  | 1.73 $\pm$ 0.12 <sup>ab</sup> | 0.00 $\pm$ 0.00 | 1923.24 $\pm$ 11.47 <sup>bc</sup> | 3183.95 $\pm$ 89.23  | 1.66 $\pm$ 0.05 <sup>ab</sup> | 20.83 $\pm$ 4.81 <sup>a</sup> |
| Probiotics              | 831.20 $\pm$ 15.42      | 1184.96 $\pm$ 7.02   | 1.43 $\pm$ 0.45 | 12.50 $\pm$ 4.81 <sup>a</sup> | 1106.26 $\pm$ 37.34 <sup>b</sup>  | 1915.49 $\pm$ 42.62  | 1.73 $\pm$ 0.04 <sup>ab</sup> | 2.27 $\pm$ 4.55 | 1937.47 $\pm$ 37.34 <sup>bc</sup> | 3100.45 $\pm$ 66.42  | 1.60 $\pm$ 0.01 <sup>bc</sup> | 14.58 $\pm$ 4.17 <sup>a</sup> |
| Prebiotics              | 851.65 $\pm$ 18.04      | 1304.27 $\pm$ 47.44  | 1.53 $\pm$ 0.45 | 0.00 $\pm$ 0.00 <sup>b</sup>  | 1172.66 $\pm$ 59.73 <sup>ab</sup> | 1902.90 $\pm$ 119.00 | 1.62 $\pm$ 0.04 <sup>b</sup>  | 2.08 $\pm$ 4.17 | 2024.30 $\pm$ 77.10 <sup>ab</sup> | 3207.17 $\pm$ 123.35 | 1.58 $\pm$ 0.002 <sup>c</sup> | 2.08 $\pm$ 4.17 <sup>b</sup>  |
| Probiotics + Prebiotics | 836.44 $\pm$ 22.24      | 1178.35 $\pm$ 98.37  | 1.41 $\pm$ 0.06 | 12.50 $\pm$ 4.81 <sup>a</sup> | 1211.75 $\pm$ 50.97 <sup>a</sup>  | 2019.26 $\pm$ 106.24 | 1.67 $\pm$ 0.03 <sup>b</sup>  | 2.27 $\pm$ 4.55 | 2048.19 $\pm$ 37.39 <sup>a</sup>  | 3197.61 $\pm$ 53.25  | 1.56 $\pm$ 0.01 <sup>c</sup>  | 14.58 $\pm$ 4.17 <sup>a</sup> |
| <b>P-value</b>          | <b>0.132</b>            | <b>0.231</b>         | <b>0.392</b>    | <b>0.0001</b>                 | <b>0.015</b>                      | <b>0.398</b>         | <b>0.003</b>                  | <b>0.735</b>    | <b>0.003</b>                      | <b>0.262</b>         | <b>0.0001</b>                 | <b>0.001</b>                  |

$p < 0.05$  showed the significance difference. Different superscript letters show that these values are significantly different from one-another through columns.

**Table 3.** Effects of treatments on mean ( $\pm$ SEM) values of various carcass and internal organ parameters

| Treatments             | Dressing percentage            | Thigh yield percentage         | Breast yield percentage        | Liver percentage | Gizzard percentage | Heart percentage |
|------------------------|--------------------------------|--------------------------------|--------------------------------|------------------|--------------------|------------------|
| Control                | 57.65 $\pm$ 1.48 <sup>a</sup>  | 23.38 $\pm$ 1.33 <sup>a</sup>  | 34.26 $\pm$ 0.52 <sup>ab</sup> | 2.05 $\pm$ 0.21  | 1.00 $\pm$ 0.07    | 0.53 $\pm$ 0.08  |
| Antibiotics            | 53.97 $\pm$ 0.81 <sup>b</sup>  | 21.29 $\pm$ 0.83 <sup>b</sup>  | 32.68 $\pm$ 0.62 <sup>b</sup>  | 2.43 $\pm$ 0.34  | 1.14 $\pm$ 0.19    | 0.68 $\pm$ 0.15  |
| Probiotics             | 55.13 $\pm$ 1.58 <sup>ab</sup> | 21.49 $\pm$ 0.61 <sup>b</sup>  | 33.64 $\pm$ 1.18 <sup>b</sup>  | 2.36 $\pm$ 0.31  | 0.97 $\pm$ 0.12    | 0.55 $\pm$ 0.05  |
| Prebiotics             | 55.73 $\pm$ 1.46 <sup>ab</sup> | 21.81 $\pm$ 0.63 <sup>ab</sup> | 33.93 $\pm$ 1.21 <sup>ab</sup> | 2.25 $\pm$ 0.17  | 1.17 $\pm$ 0.13    | 0.62 $\pm$ 0.05  |
| Probiotics+ Prebiotics | 57.96 $\pm$ 1.71 <sup>a</sup>  | 21.55 $\pm$ 0.61 <sup>ab</sup> | 36.41 $\pm$ 1.95 <sup>a</sup>  | 2.55 $\pm$ 0.20  | 1.03 $\pm$ 0.12    | 0.56 $\pm$ 0.08  |
| <b>P-value</b>         | <b>0.006</b>                   | <b>0.021</b>                   | <b>0.008</b>                   | <b>0.116</b>     | <b>0.163</b>       | <b>0.190</b>     |

$P < 0.05$  showed the significance difference. Different superscript letters show that these values are significantly different from one-another through columns.

**Table 4.** Effects of treatments on mean values of duodenum and Ilium histology

| Treatments              | Villus height (µm)           | Villus width (µm)          | Crypt depth (µm)            | VH: CD                   | VH: VW                   | Villus Surface Area (mm <sup>2</sup> ) | Tunica muscularis (µm)     | Tunica muscularis (inner circular) (µm) | tunica muscularis (outer longitudinal) (µm) | Tunica serosa (µm)       | Tela submucosa (µm)      | Epithelium thickness (µm) |
|-------------------------|------------------------------|----------------------------|-----------------------------|--------------------------|--------------------------|--|----------------------------|---|---|--------------------------|--------------------------|---------------------------|
| <b>Duodenum</b>         |                              |                            |                             |                          |                          |  |                            |   |   |                          |                          |                           |
| Control                 | 1297.17±62.87 <sup>bc</sup>  | 148.42±42.53 <sup>b</sup>  | 153.82±38.67 <sup>bc</sup>  | 8.93±2.30 <sup>abc</sup> | 9.24±2.13 <sup>ab</sup>  | 0.61±0.20 <sup>b</sup>                 | 135.83±73.20 <sup>ab</sup> | 69.10±35.09 <sup>ab</sup>               | 66.73±38.21 <sup>b</sup>                    | 61.47±40.69              | 56.85±43.15              | 55.09±36.41 <sup>a</sup>  |
| Antibiotics             | 1276.90±68.72 <sup>a</sup>   | 198.65±37.26 <sup>a</sup>  | 224.61±49.88 <sup>a</sup>   | 6.04±1.86 <sup>c</sup>   | 6.64±1.26 <sup>bc</sup>  | 0.80±0.14 <sup>b</sup>                 | 149.57±30.67 <sup>ab</sup> | 71.95±9.14 <sup>ab</sup>                | 77.62±28.32 <sup>ab</sup>                   | 46.28±14.81              | 56.02±15.71              | 28.22±7.97 <sup>b</sup>   |
| Probiotics              | 1407.78±122.13 <sup>ab</sup> | 190.57±38.60 <sup>a</sup>  | 192.66±43.74 <sup>ab</sup>  | 7.63±1.73 <sup>bc</sup>  | 7.69±1.85 <sup>abc</sup> | 0.85±0.21 <sup>b</sup>                 | 99.08±40.76 <sup>b</sup>   | 44.95±15.95 <sup>b</sup>                | 54.13±24.90 <sup>b</sup>                    | 57.79±11.78              | 37.34±3.81               | 32.47±5.09 <sup>ab</sup>  |
| Prebiotics              | 1429.62±75.78 <sup>a</sup>   | 149.25±65.45 <sup>b</sup>  | 127.14±23.34 <sup>c</sup>   | 11.57±2.07 <sup>a</sup>  | 12.50±8.58 <sup>a</sup>  | 0.68±0.32 <sup>b</sup>                 | 103.16±32.43 <sup>b</sup>  | 50.01±15.03 <sup>b</sup>                | 53.15±18.24 <sup>b</sup>                    | 54.68±9.63               | 37.22±8.96               | 32.68±9.60 <sup>ab</sup>  |
| Probiotics + Prebiotics | 1464.68±105.63 <sup>a</sup>  | 312.27±43.24 <sup>a</sup>  | 170.58±48.73 <sup>abc</sup> | 9.27±2.80 <sup>ab</sup>  | 3.59±2.08 <sup>c</sup>   | 1.44±0.23 <sup>a</sup>                 | 207.68±72.65 <sup>a</sup>  | 92.92±24.25 <sup>a</sup>                | 114.76±51.42 <sup>a</sup>                   | 56.86±14.92              | 61.24±21.45              | 40.73±17.83 <sup>ab</sup> |
| <b>P-value</b>          | <b>0.0001</b>                | <b>0.0001</b>              | <b>0.0001</b>               | <b>0.0001</b>            | <b>0.001</b>             | <b>0.0001</b>                          | <b>0.01</b>                | <b>0.0001</b>                           | <b>0.003</b>                                | <b>0.651</b>             | <b>0.081</b>             | <b>0.039</b>              |
| <b>Ilium</b>            |                              |                            |                             |                          |                          |  |                            |   |   |                          |                          |                           |
| Control                 | 889.31±58.53 <sup>b</sup>    | 151.62±82.50 <sup>ab</sup> | 121.93±28.70 <sup>b</sup>   | 7.69±2.07                | 7.34±3.20 <sup>b</sup>   | 0.43±0.24 <sup>c</sup>                 | 127.95±76.03 <sup>a</sup>  | 64.31±43.21 <sup>bc</sup>               | 63.64±33.70 <sup>b</sup>                    | 43.91±30.40 <sup>a</sup> | 47.68±32.59 <sup>b</sup> | 16.15±8.61 <sup>b</sup>   |
| Antibiotics             | 986.26±37.16 <sup>b</sup>    | 135.54±51.52 <sup>ab</sup> | 156.07±37.31 <sup>ab</sup>  | 6.63±1.53                | 8.24±3.00 <sup>ab</sup>  | 0.42±0.15 <sup>c</sup>                 | 90.28±7.16 <sup>c</sup>    | 46.58±4.37 <sup>c</sup>                 | 43.70±5.64 <sup>b</sup>                     | 27.84±9.01 <sup>c</sup>  | 41.24±3.96 <sup>b</sup>  | 26.26±9.45 <sup>ab</sup>  |
| Probiotics              | 1212.37±112.85 <sup>a</sup>  | 207.31±40.45 <sup>a</sup>  | 201.63±42.43 <sup>a</sup>   | 6.22±1.28                | 6.03±1.16 <sup>b</sup>   | 0.79±0.17 <sup>a</sup>                 | 185.76±19.71 <sup>b</sup>  | 93.57±7.44 <sup>b</sup>                 | 92.19±17.19 <sup>a</sup>                    | 71.59±11.52 <sup>b</sup> | 81.33±17.95 <sup>a</sup> | 27.93±8.08 <sup>a</sup>   |
| Prebiotics              | 1233.86±169.79 <sup>a</sup>  | 179.50±43.29 <sup>ab</sup> | 163.60±66.64 <sup>ab</sup>  | 8.41±2.76                | 7.29±2.10 <sup>b</sup>   | 0.69±0.16 <sup>ab</sup>                | 244.88±36.16 <sup>a</sup>  | 131.27±30.49 <sup>a</sup>               | 113.61±7.40 <sup>a</sup>                    | 95.40±11.96 <sup>a</sup> | 76.06±13.82 <sup>a</sup> | 26.78±4.25 <sup>a</sup>   |
| Probiotics + Prebiotics | 1296.04±81.16 <sup>a</sup>   | 129.22±42.73 <sup>b</sup>  | 200.13±44.95 <sup>a</sup>   | 6.74±1.40                | 10.88±2.97 <sup>a</sup>  | 0.53±0.19 <sup>bc</sup>                | 126.97±38.76 <sup>c</sup>  | 64.78±24.18 <sup>bc</sup>               | 62.19±15.41 <sup>b</sup>                    | 38.22±9.46 <sup>c</sup>  | 44.97±6.52 <sup>b</sup>  | 21.54±7.48 <sup>ab</sup>  |
| <b>P-value</b>          | <b>0.0001</b>                | <b>0.022</b>               | <b>0.003</b>                | <b>0.112</b>             | <b>0.005</b>             | <b>0.0001</b>                          | <b>0.0001</b>              | <b>0.0001</b>                           | <b>0.0001</b>                               | <b>0.0001</b>            | <b>0.0001</b>            | <b>0.014</b>              |

$P < 0.05$  showed the significant difference. Different superscript letters show that these values are significantly different from one-another through columns.

surface area was higher in group treated with combine probiotic & prebiotic. Tunica muscularis was higher in probiotic + prebiotic group while lower tunica muscularis was noted in probiotic group. Tunica muscularis (inner circular) was higher in probiotic + prebiotic group. Tunica muscularis (outer longitudinal) was higher in probiotic + prebiotic group. Tunica serosa was higher in control group. Tela submucosa was higher in probiotic + prebiotic group. Epithelium thickness was higher in control group.

**b. Ilium:** Mean values of different parameters are given in table 4. Villus height was higher in probiotic, prebiotic and probiotic + prebiotic group. Villus width was higher in probiotic and lower in probiotic + prebiotic group. Crypt depth was higher in probiotic and probiotic + prebiotic group. Villus height: villus width was higher probiotic + prebiotic group. Villus surface area was higher probiotic group. Tunica muscularis was higher in prebiotic group. Tunica muscular is (inner circular) was higher in prebiotic group. Tunica muscular is (outer longitudinal) was higher in prebiotic group. Tunica serosa was higher in prebiotic group. Tela submucosa was higher in probiotic and prebiotic group. epithelium thickness was higher in probiotic and prebiotic group and lower in control group.

## DISCUSSION

In the recent decades, the uncontrolled use of growth promoting antibiotics has been increasing the risk of developing of antibiotic resistant. Due to

growing concerns about antibiotic resistance and the potential for a ban of using antibiotic growth promoters in many countries in the world, there is increasing interest in finding alternatives to antibiotics in poultry production (Royan, 2018). Therefore, this study was conducted primarily to investigate the effect of Mannan oligosaccharides (MOS), *Bacillus subtilis* and their combination in replacement to antibiotics on growth performance, carcass parameters and gut morphology of broilers. Improved weight gain and FCR recorded in birds fed diet having prebiotics alone and symbiotically with probiotics. Contrary to our findings, Murshed et al. (2015) tested different doses of MOS according to the cycle phase and obtained better performance. The nutritional requirement and digestive capacity in accordance with the digestive enzyme and intestinal mucosa are considerably change with age and phase of the animal which could influence the required dose of the animals (Omerovic et al., 2016). Besides, feeding strategies, farm management and pen hygiene can also affect the animal performance. These factors may modify the impact of MOS dietary inclusion (Amouei et al., 2021). Feed intake of broiler chickens was not increased by the supplementations of probiotic, prebiotics or symbiotic in the present study. Several other studies also showed that the addition of probiotics or prebiotics alone or in combinations as symbiotic in feeds had no effect on the feed intake of broiler chickens (Mookiah et al., 2012). Unlike our results, Leblebici and Aydoğan (2018) who examined the influence of Mannan oligosaccharide on

growth performance and conclude that MOS had no influence on body weight gain, feed intake and FCR. The difference could be due to the type of consumption, which Leblebici and Aydoğan was taken water soluble, while in the present study was mixed in feed.

Ritzi et al. (2014) found that use of probiotic reduced the *E. coli* population and improved the production performance of broiler. Kamran et al. (2013) explored the influence of prebiotic (mannan oligosaccharide) in replacement to antibiotics (enramycin, zinc bacitracin and furazolidone) on production performance in birds. It was observed that mannan oligosaccharide can replace antibiotics without any negative influence on broiler growth performance. Abudabos and Yehia (2013) examined the influence of dietary Mannan oligosaccharide on growth performance in broiler under *Clostridium perfringens* challenge and showed that Mannan oligosaccharide at 0.05% can be used in broiler diet in replacement to antimicrobial growth promoters without affecting growth performance of broiler. The exact mechanism(s) underlying the growth promoting effects of probiotic and prebiotic is unclear, but it is apparent that both probiotic and prebiotic function by modifying the intestinal microflora.

Dressing percentage in terms of breast yield percentage was recorded significantly higher in birds fed diet having probiotics + prebiotics while liver, heart, gizzard weight was not affected by different dietary treatment. These findings are in line with the results of Salehimanesh et al. (2015). However, Yakhkeshi et al. (2012) reported that non-significant changes in the carcass quality of broilers fed with probiotics and the outcome reported by Leblebici and Aydoğan (2018) who studied the influence of Mannan oligosaccharide on carcass parameter in broiler and observed that use of Mannan oligosaccharide had no influence on carcass yield. Dietary treatments did not affect ( $P>0.05$ ) the other carcass parameters like breast meat yield, thigh meat yield, liver, gizzard, spleen, abdominal fat, and heart weight. Similar results were reported that breast meat yield, thigh meat yield (Pelicia et al., 2004), liver, gizzard, heart (Mohamed et al., 2008). These discrepancies of results can be attributed to the differences between strains, hybrids, age, sex, plane of nutrition, nutrient composition of the diet, microbial population of gastrointestinal tract, inclusion levels of probiotics and prebiotics in the diet, duration of supplementation or other environmental conditions.

Alone MOS and in symbiotically with probiotics

caused significant increase in villus height, surface area and villus width of duodenum and ileum. However, villus height: crypt depth was higher in prebiotic group and lower in antibiotic group. Results are in line with the findings of Al-Baadani et al. (2016) stated that addition of prebiotic and probiotic increased villus length and surface area in comparison to placebo and antibiotic group. Kritdayopas et al. (2019) reported that addition of prebiotic and symbiotic increased villus height in duodenum, jejunum, and ileum part of intestine. Increased surface area reported in this trial as a result of probiotic, prebiotic and symbiotic supplementation may improve the absorption of nutrients (Khambualai et al., 2010). Samanya and Yamauchi (2002) reported that *B. subtilis* led to an increase in villus height in the small intestine. Oliveira et al. (2009) found that the addition of the antibiotic to broiler feed caused low villi height, which was explained by the suppressing effect of the antibiotic on beneficial bacteria in the gut, such as lactobacillus and bifidobacteria. This pertinent improvement in the gut histomorphometry can be considered a rationale to the improve FCR and weight gain through providing more surface area for nutrient absorbance. The exact mechanism behind this phenomenon is unclear hitherto, but it might involve some growth promotor(s) that could be activated through the MOS feeding consequently leads to improve intestinal mucosae.

## CONCLUSION

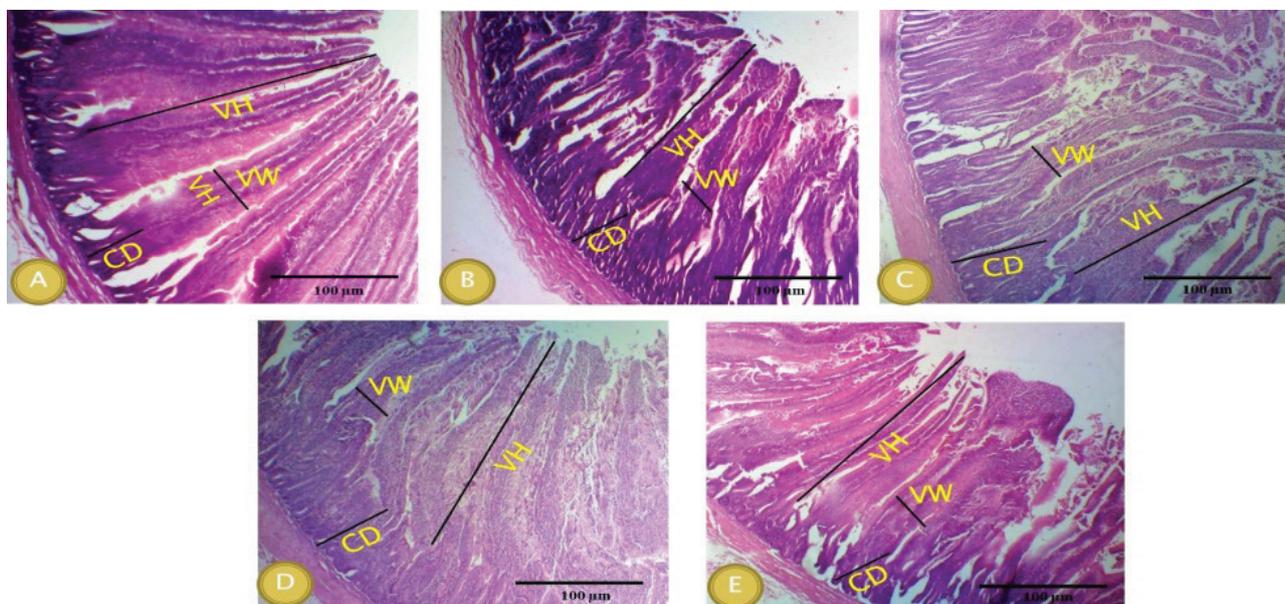
It can be concluded that, generally, the addition of prebiotics in symbiotically with probiotics had a more beneficial effect on growth performance, carcass yield with concomitant growth in the intestinal morphology in commercial broilers than that of antibiotics. Further studies should be planned to explore the molecular mechanisms of pre- and probiotics use in the poultry feed that ultimately leads to formulate a economical poultry feed for the farmers.

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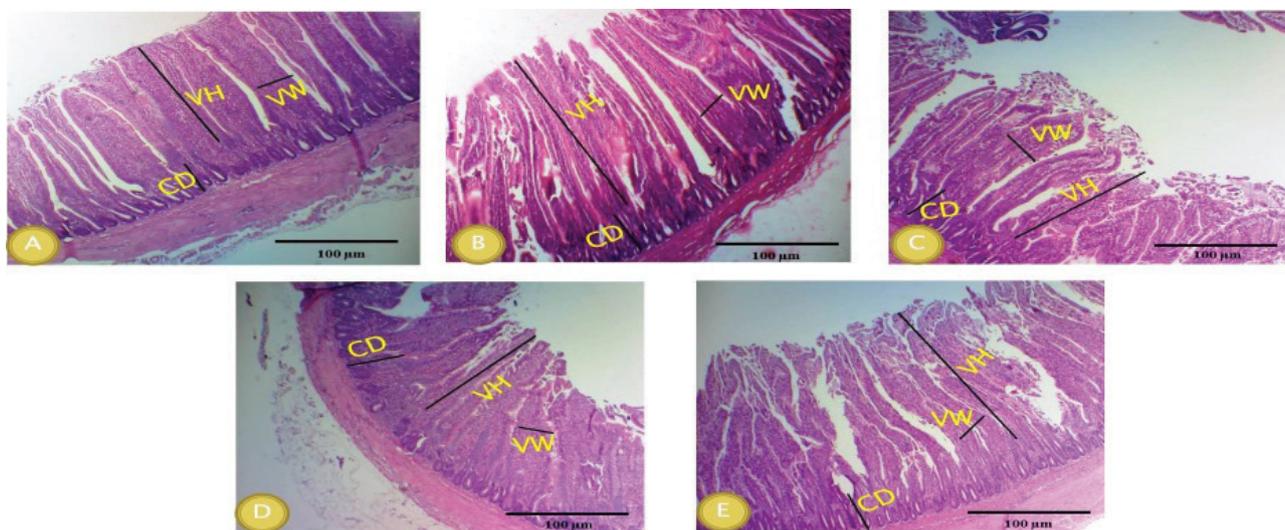
## AUTHOR'S CONTRIBUTION:

Anas Sarwar Qureshi conceived the idea, Aabid Ali and M Usman executed the research plan, Sarmad Rehan, Farah Deeba, M Usman and MU Atteq contributed in write up.



**Plate 1:** Histomicrograph of duodenum from different treatment groups (H&E; 100X)

A = Control, B = Antibiotic (ZB 10%) @ 400 g/ton, C = Probiotics (BS) 50 g/ton, D = Prebiotics (MOS) @1000 g/ton, E = Probiotics + Prebiotics (1000 g/ton). Histological parameters of villi including Villus height (VH) and crypt depth (CD) was recorded highest in Group E but villus width was seen widest in Group C.



**Plate 2:** Histomicrograph of Ilium from different group (H&E; 100X)

A = Control, B = Antibiotic (ZB 10%) @ 400 g/ton, C = Probiotics (BS) 50 g/ton, D = Prebiotics (MOS) @1000 g/ton, E = Probiotics + Prebiotics (1000 g/ton). Histological parameters of villi including villus height (VH) and crypt depth (CD) was recorded highest in Group E but villus width was seen widest in Group C.

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