



Note

Comparative study on antibiotic and synbiotic as growth promoters using duodenal tissue profiling in broilers

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The poultry sector plays a considerable role in the development and spread of Antimicrobial resistance (AMR) because antibiotics are widely used, frequently for non-therapeutic purposes such as growth enhancement. To mitigate AMR in poultry production, several approaches have been recommended, including minimizing antibiotic usage and encouraging alternative interventions like prebiotics, probiotics, and their combined synergistic applications. In this experiment we step out a new approach to combine both prebiotics and probiotics, known as synbiotics. The present study was conducted on ninety (n = 90) day-old Cobb 400 broiler chicks. The chicks were randomly allocated into three dietary treatment groups viz; Group I (control group), Group II (AGP feed group) and Group III (synbiotic feed group). The aim of this experiment was to explore the duodenal tissue of broiler chicken after supplementation of Antibiotic growth promoter (AGP) and synbiotic with a logical and sequential approach. In synbiotic feed group the height of villous was longest while that of AGP group was similar to control group. Among connective tissue fibers the distribution of reticular fibers were more followed by collagen and elastic fibers. The histochemical reactions were more intense in synbiotic feed group followed by AGP and control group. From this study it can be concluded that the growth of chickens is directly correlated with anatomical structures of small intestine. The synbiotic has beneficial effects on the growth performance, intestinal health and immune function of broiler chickens as compare to Antibiotic growth promoter (AGP) and basal diet group.

Keywords: Antibiotic growth promotor, Goblet cells, Intestine, Villi

The poultry industry is one of the fastest-growing sectors in global livestock production¹. In India, the poultry population has shown a significant increase, with broiler chickens (*Gallus gallus domesticus*)

being raised primarily for meat production². The rising demand for poultry meat has led to widespread use of antibiotic growth promoters (AGPs) in feed to enhance growth and performance. However, continuous sub-therapeutic use of antibiotics can lead to the emergence of antibiotic-resistant bacteria^{3,4} which poses serious risks to animal and human health⁵. Consequently, their use has been banned or restricted in many countries, including the European Union⁶ and the United States⁷. This has prompted the exploration of safer alternatives such as prebiotics, probiotics, and their synergistic combinations, known as synbiotics. Prebiotics are non-digestible carbohydrates that support the growth of beneficial gut bacteria⁸, while probiotics are live microbial supplements that promote gut health and immunity⁹. Synbiotics enhance the colonization and activity of probiotics by providing them with a suitable substrate^{10,11}. Several studies have reported synbiotics to improve growth performance, gut health, immune response, and disease resistance in broilers¹²⁻¹⁵. Although many researchers have evaluated the effects of AGPs and synbiotics on performance, immunity, and gut microbiota, limited attention has been given to their effects on the histology and histochemistry of the intestinal tract. The intestine, especially the duodenum, plays a vital role in nutrient absorption and immune function^{16,17} and its structural integrity is crucial for maintaining gut health.

Material & Methods

Experimental design and management

The present study was conducted on ninety (n = 90) day-old Cobb 400 broiler chicks, which were procured and reared under standard managerial and biosecurity conditions at the Poultry Farm, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura. The chicks were randomly allocated into three dietary treatment groups, each comprising thirty birds, further divided into three replicates of ten birds each. Group I (control) was provided with a basal diet alone, Group II (AGP group) received the basal diet supplemented with Antibiotic, Virginiamycin at the rate of 1 gram per 50 kilograms of feed, sourced from MAARK Pharma and Group III (synbiotic group) was

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administered the basal diet supplemented with symbiotic, ADDON® Poultry Max, a synbiotic formulation, at the rate of 10 grams per 50 kilograms of feed, procured from VIRBAC. Birds in all groups were offered feed and water *ad libitum* and maintained under uniform environmental conditions throughout the experimental period. At 2, 4, and 6 weeks of age, two birds from each replicate (six birds per group per time point) were randomly selected and humanely sacrificed following a 12-hour fasting period. Duodenal samples were collected immediately post-mortem for further processing. All procedures were carried out in accordance with the protocol approved by the Institutional Animal Ethics Committee (IAEC) of DUVASU, Mathura (India) (Approval no. IAEC/24/ 1/45 dated 5-03-2024).

Histological and Histochemical procedures

The collected duodenal tissues were fixed in 10% neutral buffered formalin and processed using standard paraffin embedding techniques. Tissue sections of 5 µm thickness were prepared and subjected to various histological and histochemical staining protocols to evaluate the structural and biochemical characteristics of the intestinal wall. These tissue sections were stained with Haematoxylin and Eosin⁶³ for general histoarchitecture, Masson's trichrome stain for collagen fibers⁶³, Gordon and Sweet's method for reticular fibers⁶⁴ and Verhoeff's stain for elastic fibers⁶³. Micrometrical measurements were recorded with the help of Leica DM 750 computerized image analyser. Each observation was recorded at six different places and the data was put for statistical analysis. The tissue sections were also subjected to Periodic Acid Schiff's stain/ PAS stain⁶³ for detection of neutral Polysaccharides, Muller's colloidal (hydrous) ferric oxide⁶³ for Acid mucopolysaccharides (AMPs).

Results and Discussion

Histological observation

The present study revealed that the wall of all segments of the small intestine in broiler chickens, across all groups, consisted of four tunics from the lumen outward: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. These observations were similar to previous findings in avian species^{18,19}, cattle²⁰, and black-winged kite²¹. The tunica mucosa was evaginated into various shapes of projections, the villi. The villi were finger-shaped in the duodenum in all groups, consistent with

observations in poultry²². The tunica mucosa consisted of lining columnar epithelium and lamina propria in all age groups across every treatment. These findings are in agreement with observations in fowl and domestic birds²³⁻²⁵. The lamina epithelialis consisted of simple columnar epithelium that was tall and showed an ovoid nucleus at the base and prominent microvilli at the free border across all age groups and treatments. This observation was consistent with previous report^{24,26}. However, goblet cells and enterochromaffin cells were also present in all treatments, in agreement with earlier studies in fowl and Japanese quail²⁷⁻²⁹, birds³⁰, and broiler strains³¹. Inter- as well as intraepithelial lymphocytes were discernible, similar to previous reports³²⁻³⁴. The lamina propria consisted of loose connective tissue as reported earlier in various species, including fowl^{28,34}, chicken³⁵, broiler and layer birds³⁶, African pied crow²⁶, and Japanese quail³⁷. The lamina muscularis consisted of circular muscle fibers placed between the lamina propria and tunica submucosa, similar to previous observations in fowl²⁴ and goats³⁸. The tunica submucosa was comprised of loose connective tissue along with the submucosal nerve plexus, blood vessels, and lymph vessels, consistent with earlier studies in birds³⁰, fowl³⁴, chicken³⁵, broiler and layer birds³⁶, African pied crow²⁶, and Japanese quail³⁷. In the present study, Brunner's glands or duodenal glands were not observed in the duodenum, which aligns with reports in birds^{19,39-43}. The tunica muscularis consisted of a thick inner circular layer and a thin outer longitudinal layer. Between these two layers, myenteric nerve plexuses and blood vessels were present across all age groups and treatments. These observations were in agreement with previous findings in fowl^{18,24,44-47}, African pied crow²⁶, *Elanus caeruleus* (avian)²¹, and broiler strains³¹. The tunica serosa consisted of loose connective tissue and mesothelium in all age groups and treatments. These observations were consistent with earlier reports in Japanese quail²⁹, fowl^{24,34}, African pied crow²⁶, and *Elanus caeruleus*²¹.

(A) Histological observations at 2 weeks of age

The histological observations of the duodenum in the present study revealed that the intervillous distance was greater in the control group, followed by the AGP group, and lowest in the synbiotic group. In the synbiotic group, the villous height was the greatest, while in the AGP group it was similar to the control group (Table 1). The occurrence of glands was

higher in the synbiotic group, followed by AGP and control groups (Fig. 1a). The infiltration of phagocytic cells was frequently observed in the control group (Fig. 1b). Nerve plexuses and blood vessels were more frequently noticed in the synbiotic group compared to the AGP and control groups (Fig. 1c).

(B) Histological observations at 4 weeks of age

At 4 weeks of age, the intervillous distance was greater in the control group, followed by the AGP group and then the synbiotic group (Fig. 1d). In the

synbiotic group, the villi were tallest, while in the AGP group they were similar to the control group (Table 2). Duodenal patches were observed only in the AGP group (Fig. 1e). Intestinal glands were well developed and occurred more frequently in the synbiotic group (Fig. 1f) than in the control and AGP groups. Micrometry revealed that the tunica muscularis was better developed in the synbiotic group compared to the control and AGP groups (Table 2). The above observations were similar to Awad *et al.* (2008)⁵⁴ who reported improved

Table 1 — Showing micrometerical analysis of Duodenum at 2 week of age in broiler chickens.

Parameter	Range	Group-I	Range	Group-II	Range	Group-III	F-Value
Villous Height	650.32-1001.36	891.59 ^a ±55.55	750.25-1099.57	922.88 ^a ±52.05	900.32-1248.99	1036.8 ^a ±61.14	1.83
Crypt Depth	90.8-147.32	121.42 ^a ±9.00	8.3-145.82	84.87 ^{ab} ±17.95	60.32-105.25	81.85 ^b ±6.81	3.23
VH:CD	4.41-11.02	7.63 ^a ±0.87	9.09-15.41	12.95 ^a ±0.91	5.15-96.41	24.43 ^a ±14.43	1.05
Villous width	89.64-112.62	104.52 ^b ±3.42	64.83-115.36	92.10 ^b ±8.21	90.26-145.36	128.44 ^a ±8.1	7.02**
Intervillous Distance	90-105	98 ^a ±2.12	85-120	103.3 ^a ±5.2	68.50-101.20	82.26 ^c ±5.63	5.68*
Thickness of epithelium	15.6-35.62	25.68 ^b ±2.67	20.7-28.6	24.58 ^b ±1.06	28.38-45.85	35.84 ^a ±2.56	7.78*
Thickness of lamina muscularis	14.09-25	19.56 ^b ±1.77	16.36-27.58	20.45 ^b ±1.628	19.76-30.32	26.74 ^a ±1.54	5.59**
Thickness of tunica submucosa	4.85-9.75	7.28 ^a ±0.88	3.42-7.25	5.14 ^b ±0.61	8.3-10.42	9.25 ^a ±0.33	9.89**
Thickness of inner circular muscle layer	83.5-98.13	90.98 ^b ±1.96	104.38-113.05	108.23 ^a ±1.25	77.09-140.65	118.27 ^a ±9.61	5.85**
Thickness of outer longitudinal muscle layer	9.45-13.79	10.79 ^a ±0.68	8.49-18.95	12.41 ^a ±1.71	8.65-26.05	15.97 ^a ±3.21	1.53
Thickness of tunica serosa	10.46-15.96	13.06 ^a ±0.77	4.65-13.76	9.73 ^a ±1.24	5.92-22.97	15.21 ^a ±2.71	2.39
Diameter of gland	35.62-55.65	45.82 ^b ±2.65	36.67-49.25	41.5 ^b ±2.04	40.29-71.1	58.89 ^a ±4.96	6.86**

n= 90, Fig in parenthesis, a, b, c, superscript showed the significant difference between groups. in superscript representing significant difference between the groups. **in superscript representing highly significant difference between the groups.

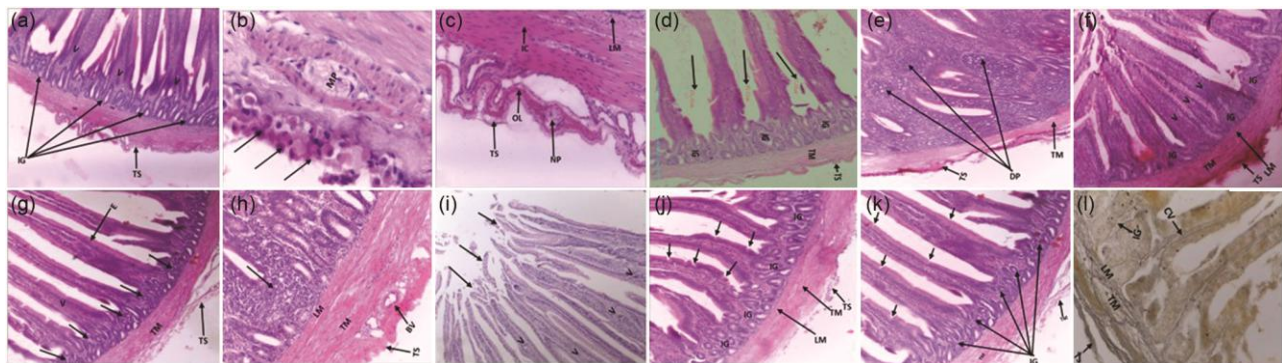


Fig 1 — Photomicrographs of duodenum at 2 weeks: (a) synbiotic group (H&EX100) , (b) control group (H&EX1000), (c) synbiotic group (H&EX400), At 4 weeks: (d) control group (H&EX100), (e) AGP group (H & EX40), (f-g) synbiotic group (F: H & EX100, G: H & EX40), At 6 weeks: (h, j) synbiotic group (H: H & EX40, J: H & EX100), (i, k) control group (I: H & EX200, K: H & EX100), (l) control group (Gordon and Sweet's stainx200). Figures show villi (V), intestinal glands (IG), lamina muscularis (LM), tunica muscularis (TM), tunica serosa (TS), myenteric plexus (MP), inflammatory cells (arrow), inner circular muscle layer (IC), neural plexus (NP), outer longitudinal muscle layer (OL), intervillous distance (arrow, D), duodenal patches (DP), epithelium (E), blood vessel (BV), degeneration of epithelium (arrow, I), secondary folding of villi (arrow, J), and reticular fibers in the core of villi (CV).

Table 2 — Showing micrometrical analysis of Duodenum at 4 week of age in broiler chickens.

Parameter	Range	Group-I	Range	Group-II	Range	Group-III	F value
Villous Height	1142.37-1337.43	1245.75 ^b ±31.68	1000.32-1411	1209.31 ^b ±60.26	757.47-2300.56	1917.61 ^a ±235.59	7.935**
Crypt Depth	172.16-226.37	202.24 ^a ±8.31	170.22-250.65	212.80 ^a ±13.71	128.89-225.63	195.20 ^a ±14.95	0.49
VH:CD	5.70-6.63	6.18 ^b ±0.12	5.88-11.09	9.60 ^a ±0.78	3.99-8.29	5.86 ^b ±0.62	12.65**
Villous width	144.93-220.67	178.38 ^a ±10.60	100.65-197.41	144.20 ^a ±15.31	122.31-180.75	158.25 ^a ±9.36	2.036
Intervillous Distance	67.68-201.61	119.94 ^a ±19.20	102.50-107.20	105.22 ^a ±0.84	84.50-95.30	89.83 ^a ±1.57	1.8
Thickness of epithelium	26.23-38.96	34.10 ^a ±1.93	28.75-39.73	34.12 ^a ±1.68	25.85-51.74	41.30 ^a ±3.46	2.818
Thickness of lamina muscularis	22.12-37.31	31.97 ^b ±2.35	22.34-31.29	27.66 ^b ±1.40	30.95-52.8	44.10 ^a ±3.05	12.921 **
Thickness of tunica submucosa	10.95-15.19	13.04 ^a ±0.74	6.58-12.36	9.46 ^b ±0.96	12.88-14.56	13.62 ^a ±0.26	9.758 **
Thickness of inner circular muscle layer	74.25-117.17	92.48 ^a ±6.25	70-194.55	117.10 ^a ±18.91	99.78-118.79	107.37 ^a ±3.23	1.133
Thickness of outer longitudinal muscle layer	11.37-14.52	13.00 ^a ±0.44	9.1-15.89	13.23 ^a ±0.95	5.19-15.16	11.36 ^a ±1.60	0.858
Thickness of tunica serosa	9.52-18.56	12.61 ^b ±1.42	13.1-21.6	17.93 ^a ±1.31	17.28-23.52	21.26 ^a ±0.91	12.425 **
Diameter intestine of gland	44.96-53.44	49.53 ^a ±1.43	43.62-53.67	48.90 ^a ±1.36	31.25-70.45	50.70 ^a ±6.37	0.056

n= 90, Figures in parenthesis, a, b, c, superscript showed the significant difference between groups in superscript representing significant difference between the groups. **in superscript representing highly significant difference between the groups.

performance with synbiotic supplementation and Bogusławska-Tryk *et al.* (2021)⁵² who confirmed positive effects of synbiotics on intestinal morphology.

(C) Histological observations at 6 weeks of age

At 6 weeks of age, the intervillous distance followed the same pattern: control group > AGP group > synbiotic group. The villous height was the greatest in the synbiotic group and similar in the AGP and control groups (Table 3). Intestinal glands were more frequent in the synbiotic group, followed by AGP and control groups (Fig. 1g). Duodenal patches in the lamina propria were most commonly observed in the control group (Fig. 1h). Nerve plexuses and blood vessels were more frequent in the synbiotic group than in AGP and control groups. Degeneration of the epithelium was commonly observed in the AGP group compared to other groups (Fig. 1i). Secondary foldings of villi were observed only in the synbiotic (Fig. 1j) and control (Fig. 1k) groups.

The abundance of reticular fibers was higher than other fibers in all groups, consistent with findings in goats³⁸. Reticular fibers were seen in the villous core, basement membranes, lamina propria, lamina muscularis, tunica submucosa, tunica muscularis, and tunica serosa (Fig. 1l), and (Fig. 2a). These fibers were thin at 2 weeks but became thicker

and more anastomosing with age, consistent with reports in avians⁵⁵. Collagen fibers were arranged obliquely through the inner circular muscle layer up to the outer longitudinal muscle layer (Fig. 2b), as described by Hodges²³. They were abundant in lamina muscularis, blood vessels (Fig. 2c), and nerves of the tunica serosa (Fig. 2d). The pattern matches earlier reports in fowl⁵⁶, Kadaknath fowl⁵⁷, and avians⁵⁸.

Elastic fibers were mainly found in the internal elastic lamina of blood vessels as reported by Sinha⁵⁹. They were thin at 2 weeks but became thicker and more wavy with age (Fig. 2e), similar to earlier reports^{44,56,57}. The density and coarseness of all three fiber types followed the order: synbiotic > AGP > control group.

Micrometrical analysis

Villous Height (VH)

At 2 weeks (Table 1, Fig. 3), there was no significant difference ($P>0.05$) between ages and treatment groups. At 4 weeks (Table 2, Fig. 4) and 6 weeks (Table 3, Fig. 5), villous height showed a highly significant F-value ($P<0.01$).

These findings are consistent with previous reports where synbiotic supplementation significantly increased VH ($P<0.05$) in broiler chickens at

Table 3 — Showing micrometrical analysis of Duodenum at 6 week of age in broiler chickens.

Parameter	Range	Group-I	Range	Group-II	Range	Group-III	F Value
Villous Height	1478.65-1800.45	1651.90 ^b ±55.30	1256.36-1500.69	1337 ^c ±38.68	1739.48-1898.36	1839.20 ^a ±27.31	36.44**
Crypt Depth	115.36-265.14	212.55 ^a ±20.84	146.7-261.26	193.31 ^a ±16.85	146.91-243.71	191 ^a ±12.93	0.47
VH:CD	5.58-15.61	8.47 ^a ±1.46	7.7-11.84	9.81 ^a ±0.56	4.94-8.68	7.13 ^a ±0.52	1.95
Villous width	235.26-248.32	244.56 ^a ±1.96	131.56-218.94	167.75 ^b ±12.23	156.36-225.63	182.62 ^b ±10.42	18.99**
Intervillous Distance	80-172	125.0 ^a ±16.02	98-105	101.0 ^{ab} ±0.96	72-95	85 ^b ±3.72	4.47
Thickness of epithelium	75.36-80.76	78.35 ^a ±0.94	33.21-74.65	59.31 ^b ±5.72	52.64-66.35	60.45 ^b ±2.29	8.78**
Thickness of lamina muscularis	25.63-28.8	26.96 ^b ±0.46	35.63-76.22	47.56 ^b ±6.12	19.36-26.65	24.21 ^b ±1.16	12.48**
Thickness of tunica submucosa	3.57-6.74	5.41 ^b ±0.46	3.36-8.69	5.67 ^b ±0.82	6.36-9.95	9.02 ^a ±0.58	9.85**
Thickness of inner circular muscle layer	155.94-168.75	161.67 ^b ±1.91	220.31-419.35	290.19 ^a ±30.79	95.19-135.63	110.75 ^b ±6.51	25.80**
Thickness of outer longitudinal muscle layer	14.5-16.25	15.29 ^b ±0.31	34.08-90.54	58.32 ^a ±9.27	22.92-26.94	24.90 ^b ±0.62	17.68**
Thickness of tunica serosa	14.82-17.62	16.10 ^b ±0.37	10.33-18.56	15.12 ^b ±1.33	18.49-30.03	27.43 ^a ±1.82	26.7**
Diameter of gland	45.9-70.36	61.77 ^a ±3.57	45.85-52.55	49.20 ^b ±0.92	47.75-101.27	72.89 ^a ±7.19	6.44**

n= 90, Figures in parenthesis, a, b, c, superscript showed the significant difference between groups in superscript representing significant difference between the groups. **in superscript representing highly significant difference between the groups.

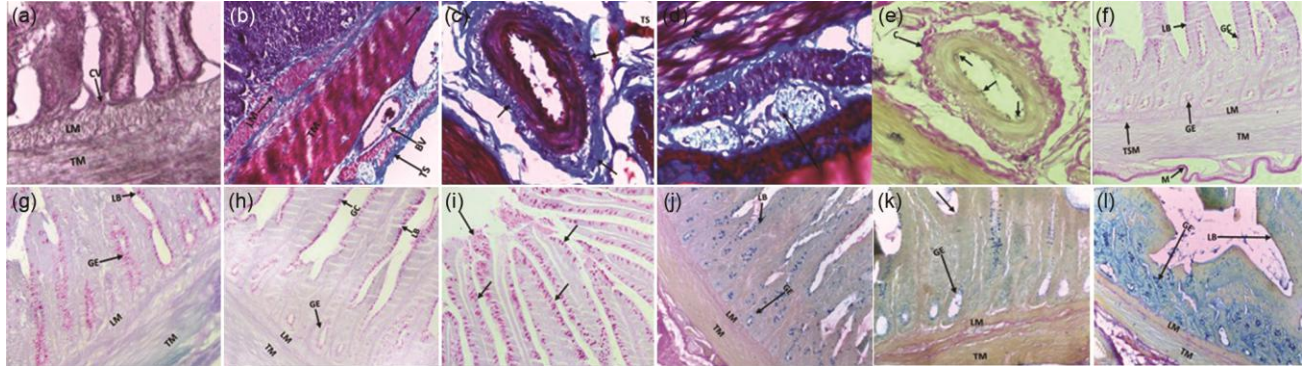


Fig 2 — Photomicrographs of duodenum at 2 weeks: (a) synbiotic group (Gordon and Sweet's stain ×20), (b) synbiotic group (Masson's Trichrome stain ×400), (c-d) control group (C: Masson's Trichrome stain ×1000; D: Masson's Trichrome stain ×1000), (e) synbiotic group (Verhoeff's stain ×1000), (f) synbiotic group (Periodic Acid Schiff's stain ×100), (g) Control group (Periodic Acid Schiff's stain ×100), (h) AGP group (Periodic Acid Schiff's stain ×100), (i) synbiotic group (Periodic Acid Schiff's stain ×100), (j) control group (Muller's Colloidal (hydrous) ferric oxide stain ×200), (k) AGP group (Muller's Colloidal (hydrous) ferric oxide stain ×200), and (l) synbiotic group (Muller's Colloidal (hydrous) ferric oxide stain ×200). At 6 weeks: Figures show reticular fibers in villous core (CV), collagen (Lamina muscularis (LM), Tunica muscularis (TM), Blood vessel (BV), arrows (Fig.2c) and elastic fibers (Lamina muscularis (LM), Tunica muscularis (TM), Blood vessel (BV), arrows (Fig.2e), and histochemical reactions (PAS and AMPs) in luminal border (LB), goblet cells (GC), glandular epithelium (GE) and arrowed (Fig.2i)..

5 weeks⁴⁸. Similar results were observed by Rodjan *et al.* (2017)⁴⁹, Bogucka *et al.* (2018)⁵⁰, and Pelicano *et al.* (2005)⁵¹.

Crypt Depth (CD)

At 2 weeks (Table 1, Fig. 3), 4 weeks (Table 2, Fig. 6), and 6 weeks (Table 3, Fig. 7), no significant differences ($P>0.05$) were observed. Awad *et al.*

(2009)⁴⁸ also reported no significant effect on CD with synbiotic supplementation. However, Bogusławska-Tryk *et al.* (2021)⁵² and Rodjan *et al.* (2017)⁴⁹ found significant increases ($P<0.05$) at 6 and 5 weeks, respectively. Pelicano *et al.* (2005)⁵¹ reported greater CD ($P<0.01$) in male Cobb broilers at 3 weeks with probiotic supplementation.

Villous Width (VW)

VW at 2 weeks (Table 1, Fig. 3) and 6 weeks (Table 3, Fig. 5) showed highly significant differences ($P<0.01$), whereas at 4 weeks (Table 2, Fig. 4), the difference was non-significant ($P>0.05$). Boguśławska-Tryk *et al.* (2021)⁵² reported no significant differences ($P<0.05$) across treatments at day 42.

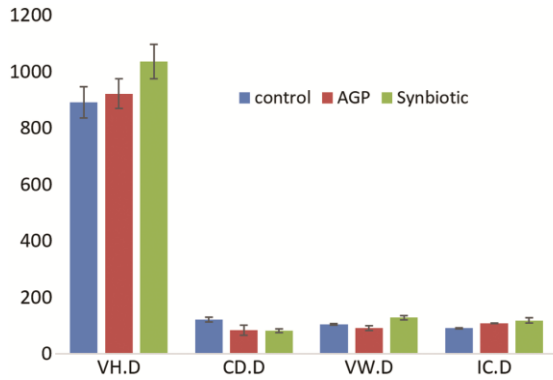


Fig. 3 — Bar diagram showing micrometrical analysis of duodenum in 2nd week old Chicken (VH.D- villous height, CD.D- Crypt depth, VW.D-villous width, IC.D- Inner circular muscle layer).

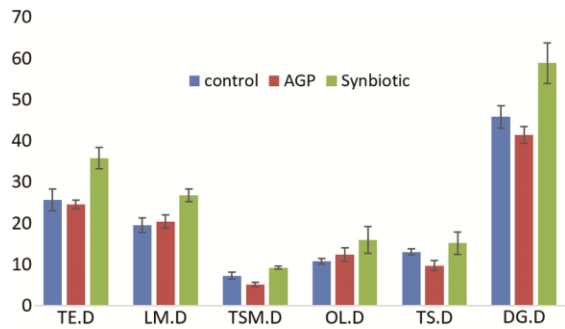


Fig. 4 — Bar diagram showing micrometrical analysis of duodenum in 4th week old Chicken (VH.D- villous height, CD.D- Crypt depth, VW. D-villous width, IC. D- Inner circular muscle layer).

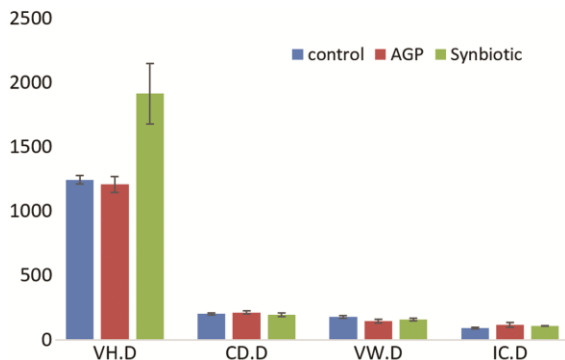


Fig. 5 — Bar diagram showing micrometrical analysis of duodenum in 6th week old Chicken (VH.D- villous height, CD.D- Crypt depth, VW.D-villous width, IC.D- Inner circular muscle layer).

Thickness of Various Tunics

In present study the thickness of epithelium at 2 weeks (Table 1, Fig. 6) showed significant ‘F’ value difference ($P<0.05$), at 4 weeks (Table 2, Fig. 7) showed non-significant ‘F’ value difference ($P>0.05$) and at 6 weeks (Table 3, Fig. 8) of age showed highly significant ‘F’ value difference ($P<0.01$) between age and treatment group.

The lamina muscularis at 2 weeks (Table 1, Fig. 6), 4 weeks (Table 2, Fig. 7) and 6 weeks (Table 3 , Fig. 8) of age showed highly significant ($P<0.01$) between age and treatment group. Fernandes *et al.* (2014)⁵³ in broiler at day 42 or 6th week observed significant ($P<0.01$) variation across tunica mucosa thickness in symbiotic treatment group.

The tunica submucosa at 2 weeks (Table 1, Fig. 6), 4 weeks (Table 2, Fig. 7) and 6 weeks (Table 3, Fig. 8) of age showed highly significant ($P<0.01$) between age and treatment group.

The inner circular muscle layer at 2 weeks (Table 1, Fig. 6) and 6 weeks (Table 3, Fig. 8) of age

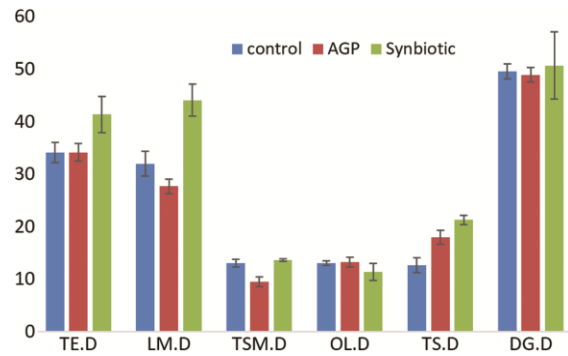


Fig. 6 — Bar diagram showing micrometrical analysis of various tunics in 2nd week. (TE.D- Thickness of epithelium, LM.D- Lamina muscularis, TSM.D-Tunica submucosa, OL.D- Outer longitudinal muscle layer, TS.D- Tunica serosa, DG.D- Diameter of intestinal gland).

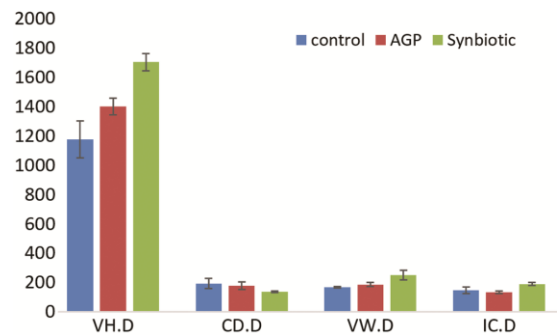


Fig. 7 — Bar diagram showing micrometrical analysis of various tunics in 4th week. (TE.D- Thickness of epithelium, LM.D- Lamina muscularis, TSM.D-Tunica submucosa, OL.D- Outer longitudinal muscle layer, TS.D- Tunica serosa, DG.D- Diameter of intestinal gland).

showed highly significant ($P<0.01$) and at 4 weeks (Table 3, Fig. 7) showed non-significance ($P>0.05$) between age and treatment group. In present study, outer longitudinal muscle layer at 6 weeks (Table 3, Fig. 8) of age showed highly significant ($P<0.01$) and at 2 weeks (Table 1, Fig. 6) and 4 weeks (Table 2, Fig. 7) respectively showed non-significant difference ($P>0.05$) between age and treatment group. Fernandes *et al.* (2014)⁵³ observed significant ($P<0.01$) increase in tunica muscularis thickness in symbiotic treatment group in broilers in contrast to antimicrobial agents. Bogusławska-Tryk *et al.* (2021)⁵² observed significant increase ($P<0.05$) in tunica muscularis thickness in symbiotic treatment than prebiotic and probiotics in broiler at 6th week.

The tunica serosa at 2 weeks and 4 weeks of age showed highly significant ($P<0.01$) and at 6 weeks respectively (Table 3 and Fig. 8) showed significant difference ($P<0.05$) between age and treatment group.

Gland Diameter

At 2 and 6 weeks, highly significant ($P<0.01$) differences were observed, whereas at 4 weeks, the differences were non-significant ($P>0.05$) (Table 1, 2 & 3). Awad *et al.* (2008)⁵⁴ reported improved performance ($P<0.05$) with synbiotic supplementation. Bogusławska-Tryk *et al.* (2021)⁵² confirmed effects of pre-, pro-, and synbiotics on intestinal morphology.

(B) Histochemical Observations

The luminal border of epithelial cells showed mild to intense PAS reaction, with goblet cells showing intense staining. Similar findings were reported in broilers and layer birds³⁶ and in *Elanus caeruleus*²¹. Lamina propria, tunica submucosa, and tunica muscularis showed mild reaction, as reported by Kachave³⁶ and Dang⁶⁰. Mesothelium of tunica serosa exhibited intense reaction (Fig. 2F), consistent with Mutsaers *et al.*⁶¹ Reactions were most intense in the synbiotic group (Fig. 2I), followed by AGP (Fig. 2H) and control group (Fig. 2G) (Table 4).

Table 4 — Showing histochemical reactions in duodenum at 2, 4, and 6 weeks of age after supplementation of Basal diet, AGP and Synbiotic (- Negative, + Mild, 2+ Moderate, 3+ Intense)

Group	Characters	2 week		4 week		6 week		
		PAS	AMP	PAS	AMP	PAS	AMP	
Group-I	Epithelium	Luminal border	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
		Basement membrane	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
	Propria-submucosa	Connective tissue	- To +	- To +	+	+	- To+	- To +
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Tunica muscularis	Inner muscle layer	+	+	+	+	+ To 2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Outer muscle layer	+	+	+	+	+ To 2+	+To2+
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Serosa	Mesothelium	+	+	+	+	-+To2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
Group-II	Epithelium	Luminal border	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
		Basement membrane	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
	Propria-submucosa	Connective tissue	- To +	- To +	+	+	- To+	- To +
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Tunica muscularis	Inner muscle layer	+	+	+	+	+ To 2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Outer muscle layer	+	+	+	+	+ To 2+	+To2+
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Serosa	Mesothelium	+	+	+	+	-+To2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+

(Contd.)

Table 4 — Showing histochemical reactions in duodenum at 2, 4, and 6 weeks of age after supplementation of Basal diet, AGP and Synbiotic (- Negative, + Mild, 2+ Moderate, 3+ Intense) (Contd.)

Group-III	Epithelium	Luminal border	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
		Basement membrane	- To +	- To +	+ To 2+	- To 2+	2+ To 3+	2+ To 3+
	Propria-submucosa	Connective tissue	- To +	- To +	+	+	- To+	- To+
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Tunica muscularis	Inner muscle layer	+	+	+	+	+ To 2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Outer muscle layer	+	+	+	+	+ To 2+	+To2+
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+
	Serosa	Mesothelium	+	+	+	+	+To2+	+To2+
		Connective tissue	- To +	- To +	+	+	- To+	- To +
		Blood vessels	-	-	+	+	- To+	- To+
		Nerve cells	+	-	-	+	- To+	- To+

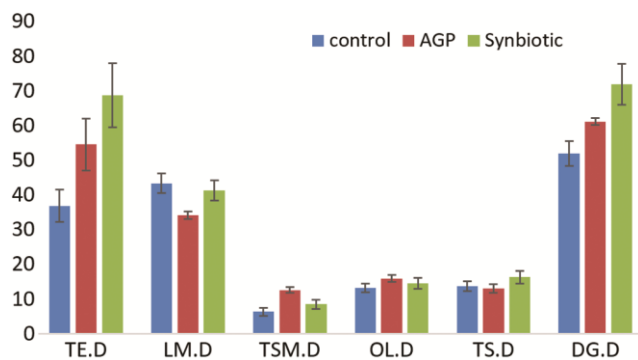


Fig. 8 — Bar diagram showing micrometrical analysis of various tunics in 6th week old Chicken (TE.D- Thickness of epithelium, LM.D- Lamina muscularis, TSM.D-Tunica submucosa, OL.D- Outer longitudinal muscle layer, TS.D- Tunica serosa, DG.D- Diameter of intestinal gland).

The luminal border of epithelial cells showed mild to intense AMP reaction, with intensity increasing with age. Goblet cells and intestinal glands showed moderate to intense staining (Fig. 2J), consistent with Neeraj *et al.*⁶² Reactions were most intense in the synbiotic group (Fig. 2L), followed by AGP (Fig. 2K) and control group (Fig. 2J).

Conclusion

From this study it can be concluded that the growth of chickens is directly correlated with anatomical structures of duodenum. The synbiotic has beneficial effects on the intestinal health of broiler chickens as compare to Antibiotic growth promotor (AGP) and basal diet group. The synbiotic can be successfully replace the Antibiotic growth promotor (AGP) in poultry feed.

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