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Effect of synbiotic supplementation on growth, immunity and intestinal morphometry of broiler chickens in summer season

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ABSTRACT

To ascertain the effect of synbiotic supplementation on growth, immunity and intestinal morphometry in broiler chicken during summer season, one hundred sixty-day old Vencobb broiler chicks were randomly allocated into five treatments. Each treatment had 32 birds consisting of 4 replicates of 8 birds each. T_0: Basal diet; T_1: Basal diet + AGP (*Salinomycin*); T_2: Basal diet + prebiotic [Mannan oligosaccharides (MOS)]; T_3: Basal diet + probiotic (Lactoplus) and T_4: Basal diet+ synbiotic (MOS+ Lactoplus). The birds were reared in three phases: pre starter phase (0-1 wk), starter phase (2-3 wks) and finisher phase (4-5 wks) and fed as per BIS (2007). The experiment was continued for the period of 35 days. Gain in body weight, FCR, immunity, morphometry of intestine and histology of liver and kidney were studied. Supplementation of synbiotic had significant effect on body weight and FCR. The antibody titre against Ranikhet disease vaccine and the CBH reaction against PHA-P were much greater ($p<0.05$) in birds fed diet supplemented with synbiotic compared to control group. Significant improvement ($p<0.05$) in intestinal villi length, villi width and crypt depth was observed in synbiotic supplemented birds. Histology of liver and kidney showed normal architecture. It may be concluded that synbiotic supplementation in broiler chickens improved the production performance, immunity and intestinal morphometry without any adverse effect on vital organs.

Key words: Chickens, Growth, Histopathology, Immunity, Synbiotic

In summer season, broiler chicken frequently suffer from reduced growth, feed intake, immunity and improper intestinal development leading to high mortality (Syafwan *et al.* 2011). In such cases, feed is frequently modified, incorporating various supplements and additives to achieve desired performance. A range of substances including antibiotics, prebiotics, probiotics, enzymes, hormones, and yeast cultures are supplemented in the diet to augment birds performance. While antibiotics have historically been employed as growth promoters, concerns have arisen due to their residual effects on human health and the development of cross resistance with human medicines. Moreover, public apprehensions regarding food safety, environmental pollution, and general health issues associated with residual antibiotics in poultry products have prompted exploration of alternatives to antibiotic growth promoters. Synbiotic is a combination of probiotic and prebiotic (Ashraf *et al*. 2013) which improves survival of probiotics by providing suitable substrate through prebiotics. Synbiotic creates a unique opportunity to optimize the survival and activity of live microorganisms in the gut, enhancing animal welfare and overall performance. The addition of prebiotics, such as mannan oligosaccharides (MOS), in the feed has demonstrated promising potential in diminishing pathogens such as *E.coli* and *Salmonella*, while concurrently fostering the proliferation of *Lactobacilli* and *Bifidobacteria* (Hanamanta *et al*. 2011). These microorganisms facilitate the establishment of a harmonious bacterial environment within the gastrointestinal tract, nurturing gut integrity and maturation (Hassanein and Soliman 2010). These microorganisms aid in the digestion of food by increasing the action of digestive enzymes and reducing the level of harmful bacterial enzymes (Alagawany *et al.* 2018), leading to better feed consumption, digestion, and reduced ammonia production. It also neutralizes toxins, stimulate immune functions, and improve overall health (Patterson and Burkholder 2003). Probiotics transform the gut ecosystem by introducing essential enzymes, lowering pH levels, and enhancing enzyme activity (Abd El-Hack *et al.* 2020). While there exists a good number of literature concerning the supplementation of synbiotic in the diet of broiler birds, comprehensive research comparing synbiotic

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with traditional interventions such as probiotics, prebiotics, and antibiotics, particularly, in summer season remains limited. Therefore, the present experiment was planned to examine the effects of synbiotic supplementation on growth, FCR, immunity, intestinal morphometry and histopathology of liver and kidney in broiler chickens.

MATERIALS AND METHODS

Experimental period: The experiment was conducted in the month of April and May (Summer season) at Livestock farm complex, OUAT, Bhubaneswar. The maximum and minimum temperature were 40.70°C (105.26°F) and 27.4°C (81.4°F), respectively during the experimental period.

Dietary treatments: The approval of Institutional Animal Ethics Committee (433/GO/Re/ S/01CCSEA) was accorded for the study. One hundred and sixty-day old Vencobb chicks were randomly allocated into five treatments *i.e.* T_0 group (regular basal diet, control) ,T_1 (basal diet + antibiotic growth promoter, AGP (*Salinomycin*@500g/ton of feed),T_2 (basal diet + prebiotic [mannan oligosaccharides (MOS@500g/ton of feed), T_3 (basal diet + probiotic [Lactoplus containing *Lactobacillus acidophilus (MTCC 10307)]* @200g/ton) and T_4 (basal diet + synbiotic (MOS + Lactoplus)). Each treatment has 32 birds consisting 4 replicates of 8 birds each. The basal diet was prepared as per the recommendation of BIS, 2007. Ingredient and proximate composition of the basal diet is presented in Table 1. The chicks were reared as per the standard managemental practices in a deep litter system. Before arrival of chicks, the shed and equipment's like feeder and drinker were disinfected with Germex (Vetneeds lab). The chicks were wing banded, weighed and allotted to different treatments before the start of the experiment. Adequate light and ventilation were provided during brooding period. The chicks were inoculated with RD (lasota) vaccine on day 7, IBD (intermediate) vaccine on day 14 and a booster of RD on day 21 of the experiment. The feeding program was divided into three phases, pre-starter (day 1 to day 7), starter (day 8 to day 21) and finisher (day 22 to day 35). The proximate principles and phosphorus content of the experimental diet were analyzed as per AOAC (1995) and calcium was estimated as per Talapatra *et al.* (1940). *Preparation of synbiotic:* Probiotic *Lactobacillus acidophilus (MTCC 10307)* @ 30 X 10^7 per g and prebiotics MOS (14-18%) was procured from department of Microbiology, OUAT, Bhubaneswar and premixed in the feed as per the recommendation of the department. MOS was prepared from the cell wall of *Saccharomyces cerevisiae* after centrifugation at 6000 rpm for 15 minutes followed by extraction with 1% NaOH (Maru *et al.* 2015).

Growth and feed efficiency: Calculations of different production parameters such as average daily gain (ADG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) were done at different phase of growth of broiler chicken.

Determination of cellular immunity: As according Edelman *et al.* (1986) cellular immunity was assessed using the cutaneous basophillc hyper sensitivity (CBH) test. At the 35th day of the experiment, six birds from each group were injected intra-dermally with 100 micrograms of phytohaemaglutinin-P (PHA-P) into the foot web. A digital slide calliper was used to measure the thickness of the foot web before and post 24 h of injection.

$$\text{CBH response} = \frac{\text{Skin thickness post injection}}{\text{Skin thickness pre- injection}} \times 100$$

Determination of antibody titre: Humoral immunity was evaluated according to Abdallah *et al.* (2009) following hemagglutination test. The primary response was measured by immunizing the birds with 0.1ml of R_2B vaccine on the 35^{th}day. The antibody titre of collected blood was measured using a U-shaped microtiter plate having 96 wells. The $\log_2$ of the reciprocal of the highest serum dilution producing agglutination of sheep RBC was used to calculate all HA antibody titres.

Intestinal morphometry study: Representative portion of the duodenum and ileum from 04 birds of each group

Table 1. Ingredients and chemical composition of the basal diet

Sl. No.	Particulars	Prestarter (%) (day 1 to 7)	Starter (%) (day15 to 21)	Finisher (%) (day 22 to 35)
1.	Maize	51.30	53.30	56.60
2.	Soyabean	43.20	39.20	34.90
3.	Edible oil	2.0	4.0	5.0
4.	DCP	1.80	1.80	1.80
5.	Limestone	0.96	0.96	0.96
6.	Methionine (DL)	0.05	0.05	0.05
7.	Lysine (L)	0.03	0.03	0.03
8.	Vitamin and mineral premix	0.16	0.16	0.16
9.	Salt	0.50	0.50	0.50
	Chemical composition (% DM)			
1.	CP	22.50	21.20	19.60
2.	EE	2.90	5.20	5.80
3.	CF	4.80	4.60	5.10
4.	Total Ash	9.20	8.30	8.90
5.	NFE*	60.60	60.70	60.60
6.	Ca	1.30	1.20	1.02
7.	Total P	0.55	0.62	0.50
8.	Metabolizable energy (Kcal/ kg) *	2985	3105	3210

*Calculated value

Table 2. Effect of synbiotic on production performance of birds

Attributes	Treatment						p-Value
	T0	T1	T2	T3	T4	SEM	
				Prestarter (1^{st} to7^{th} day)			
Final BW on Day 7 (g)	157.80	160.45	160.95	160.75	165.55	8.20	0.603
ADG (g)	16.71	16.99	17.10	17.02	17.67	0.72	0.155
ADFI (g)	18.72	19.19	19.32	18.89	19.79	0.47	0.370
FCR	1.12	1.13	1.13	1.11	1.12	0.03	0.182
				Starter (8^{th} to 21^{st} day)			
Final BW on Day 21 (g)	709.60^{a}	752.35^{b}	745.20^{b}	811.00^{c}	823.25^{c}	16.33	0.290
ADG (g)	39.41^{a}	42.28^{b}	41.73^{b}	46.45^{c}	46.98^{c}	1.70	0.162
ADFI (g)	60.30	61.73	62.60	66.42	65.77	2.30	0.220
FCR	1.53^{c}	1.46^{b}	1.50^{ab}	1.43^{a}	1.40^{a}	0.03	0.088
				Finisher (22^{nd} to35^{th} day)			
Final BW on Day 35 (g)	1635.30^{a}	1826.40^{b}	1803.60^{b}	1855.40^{bc}	1937.40^{c}	40.45	0.020
ADG (g)	66.12^{a}	76.72^{b}	75.60^{b}	74.60^{b}	79.58^{b}	3.28	0.023
ADFI (g)	128.94	135.02	137.59	130.55	131.31	3.80	0.060
FCR	1.95^{d}	1.76^{c}	1.82^{c}	1.75^{b}	1.65^{a}	0.02	0.028
				Whole phase (1^{st} to 35^{th} day)			
ADG (g)	45.56^{a}	51.00^{b}	50.35^{b}	51.82^{b}	54.16^{c}	1.27	0.025
ADFI (g)	81.55	82.10	84.59	84.99	82.32	2.80	0.041
FCR	1.79^{c}	1.61^{b}	1.68^{b}	1.64^{b}	1.52^{a}	0.02	0.033

^{abc}Values bearing different superscripts in a row differed significantly (p<0.05)
Items: Final BW (body weight), ADG (average daily weight gain); ADFI (average daily feed intake); FCR (feed conversion ratio)

(Total 20 birds) were collected in 10% formalin solution at 35^{th} day of the experiment. The formalin fixed sample was washed in running tap water overnight followed by dehydrated with ascending grades of alcohol. Then the sample was cleared by xylene followed by preparation of paraffin block and cutting of section in the size of 5-micron thickness. The required slide was prepared and stained by routine hematoxylin and eosin method (H&E) and observed under the light microscope (Leica DM500) for the histological interpretations.

Histopathological studies of vital organ: To assess the effect of synbiotic on liver and kidney, tissue samples were collected from 04 birds of T4 group and fixed in 10% formalin followed by cutting in 5µm thick paraffin sections and stained with routine Haematoxylin and Eosin staining technique. The slides were then stained and observed with a Leica DM500 light microscope and representative photographs of the histological interpretations were taken.

Statistical analysis: Data collected by the above methods were analyzed using a close analysis of variance with Statistical Packages for the social science (SPSS, version 16.0) and Tukey Least Significant Difference test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Chemical composition of basal feed: The proximate and composition of the basal diet are outlined in the Table 1. The pre-starter, starter, and finisher broiler rations contained crude protein percentage of 22.50, 21.20 and 19.60, respectively. The basal feed met the stipulated requirements by the Bureau of Indian Standards (2007) with respect to the crude protein and metabolizable energy values.

Effect on production performance: Results showed a statistically significant (p< 0.05) difference in the body weight of broiler chicks at different weeks of life between control groups and supplemented groups during the summer season (Table 2). The initial live body weight of the day-old experimental broiler chicks was nearly uniform across all groups, indicating proper randomization in the distribution of chicks among the experimental treatments. At prestarter phase, no significant difference was observed with respect to various production performance of birds. At starter and finisher phases, there was a significant (p<0.05) difference between control group and salinomycin, prebiotic, probiotic and synbiotic supplemented groups with respect to body weight gain and FCR of birds. It was also observed that supplementation of synbiotic showed a better response compared to antibiotics and pre or probiotics supplemented birds in summer season.

The present findings of supplementation of pre and probiotics, either individually or combined (synbiotic), on body weight gain of birds in summer season was in agreement with the findings of Ali *et al.* (2019), who observed higher body weight gain (p<0.05) in commercial broiler birds supplemented with prebiotic and probiotic combination than non-supplemented group. Ashayerizadeh *et al.* (2011) observed that daily body weight gain and feed efficiency were enhanced (p<0.05) by synbiotic (prebiotics (900 g/tonne) and probiotics (2000 g/tonne) in comparison to control. Abdel-Raheem *et al.* (2012) observed probiotic and synbiotic supplemented broilers had significantly (p<0.05) greater BW, and better FCR compared to control and prebiotics supplemented birds. The growth-promoting effects of probiotic and synbiotic could be related to better FCR due to more efficient nutrient utilization (energy, protein, minerals and vitamins) from feed. These supplements improved the feed efficiency by improving gut health in terms of factors such as increased intestinal microbial population, improved integrity of intestines, improved appetite and stimulating the immune system (Al-Sultan *et al*. 2016). The increased growth rate may be attributed to enhanced nutrient absorption and intestinal microbial ecology in synbiotic, pre and probiotics supplemented birds. The increase in body weight due to improved feed consumption and its digestion by increasing digestive enzyme activity in supplemented birds have earlier been reported (Rehman *et al.*2020).

Effect on immunity of birds: Supplementation of pre and probiotics either individually or combined (synbiotic) in the diet of Vencobb broilers enhanced the humoral and cellular immunity. Antibody titre against Ranikhet disease vaccine and the CBH reaction against PHA-P was much greater (p<0.05) in birds fed diet with synbiotic supplementation compared to the control group. (Table 3). Supplementation of synbiotic got maximum immune enhancement in the experimental birds. Similar to present observation, Song *et al.* (2022) found that synbiotic and antibiotic-supplemented broilers had higher Immunoglobulin A, Immunoglobulin G, and Interleukin-10 levels and lower TNF, Interleukin-2, and Interleukin-6 (p<0.05) than the control broilers. Hassanpour *et al.* (2013) reported higher antibody levels in synbiotic supplemented (0.1 and 0.2%) broilers compared to control birds. Study conducted by Salehimanesh *et al.* (2016) further revealed that prebiotic and synbiotic supplementation increased (p<0.05) antibody titre against ND vaccine and anti-SRBC titres on days 28 and 42 of the experiment. The increased immunity might be due to neutralization of enterotoxins, and stimulation of immune function in synbiotic supplemented birds than control (Rehman *et al.* 2020). Increased immunity might also be attributed to phagocytes or macrophage stimulation, a higher release of cytokines (Interferon α, Interferon γ, Interferon α) and better production of antibodies. (Hassanpour *et al.* 2013).

Intestinal morphometry studies: Transfer of nutrients through intestine is required for high availability of nutrients for maintenance, growth and production of animals and birds, which, in turn, depends on surface area provided by the villi and crypt. The villi length (μm) and villi width at middle (μm) of duodenum and ileum in the small intestine of broilers of different treated groups differed significantly (p<0.05) compared to the control birds. The crypt depth was significantly higher (p<0.05) in treated birds than control in duodenal part of the intestine (Table 4).

Similar to the results of our study, Bogucka *et al.* (2019) reported enhanced villus width and surface area in the jejunum and ileum of broilers fed prebiotic and symbiotic. Similarly, Ghasemi and Taherpour (2013) supplemented the broiler diet with probiotics, prebiotics and synbiotic for six weeks and observed that villus height and villus height: crypt depth ratio in the jejunum were significantly (p<0.05) improved by synbiotic supplementation. Awad *et al.* (2009) reported that supplementation of either probiotics

Table 3. Immunity of birds

Attributes	Treatment					SEM	p -Value
	T0	T1	T2	T3	T4		
CBH Response (%)	140.00^{a}	157.80^{ab}	173.40^{b}	202.00^{c}	204.20^{c}	9.09	<0.01
Log_2 value HA titre	5.33^{a}	6.03^{b}	6.37^{bc}	6.66^{c}	7.33^{d}	0.08	<0.01

^{abcd}Values bearing different superscripts within a row differed significantly ($p < 0.05$).

Table 4. Intestinal morphological parameters of experimental birds at the end of experiment

Parameter	Organ	Treatment					SEM	p -Value
		T_0	T_1	T_2	T_3	T_4		
Villi length (μm)	Duodenum	485.20^{a}	504.12^{a}	548.28^{b}	563.10^{bc}	600.25^{c}	19.05	<0.01
	Ileum	371.00^{a}	374.30^{a}	432.20^{b}	465.10^{b}	491.50^{c}	14.30	0.010
Villi width at middle (μm)	Duodenum	96.50^{a}	98.05^{a}	138.00^{b}	145.30^{b}	170.45^{c}	11.55	0.016
	Ileum	57.15^{a}	70.50^{ab}	83.55^{c}	88.15^{c}	103.10^{d}	16.50	0.027
Crypt depth (μm)	Duodenum	232.05^{a}	228.10^{a}	267.00^{b}	282.00^{bc}	289.10^{bc}	21.50	0.033
	Ileum	173.10	171.25	179.00	183.05	187.10	11.00	0.120

^{abcd}Values bearing different superscripts within a row differed significantly ($p < 0.05$).

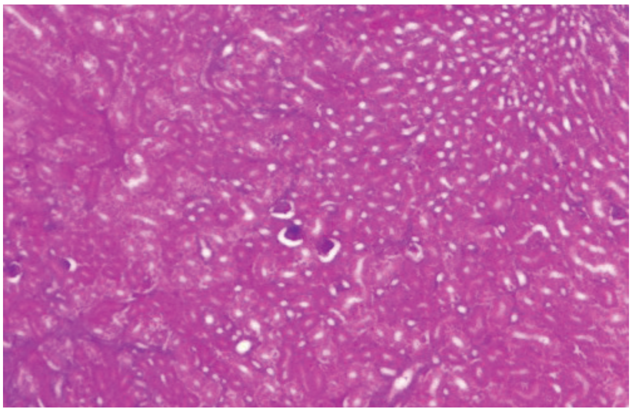

Fig. 1. Normal architecture was found in liver parenchyma of synbiotic supplemented birds (T_4) (H&E ×100)

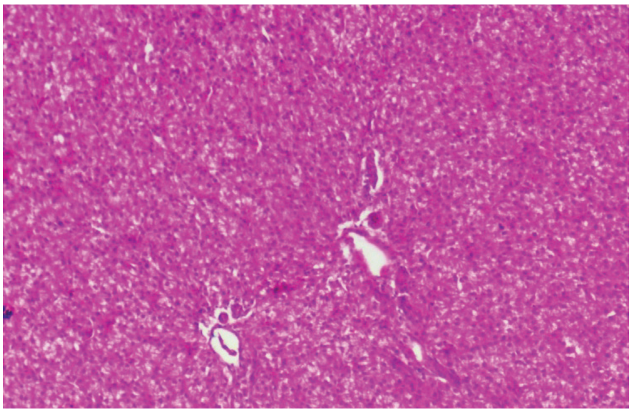

Fig. 2. Normal architecture was found in tubular epithelium of kidney in synbiotic supplemented birds (T_4) (H&E ×100)

or synbiotic in the broiler diet for five weeks improved ($p<0.05$) both the villus height: crypt depth ratio and villus height. In another study, synbiotic supplementation significantly increased ($p<0.05$) villus height in broilers at 6 weeks of age (Abdel-Raheem *et al.* 2012). The improved intestinal morphometry might be due to production of short chain fatty acids in synbiotic supplemented birds, which stimulates the growth of intestinal cells (Salvi and Cowles 2021).

Histopathology study of liver and kidney: Histopathology of H&E-stained tissue sections in synbiotic supplemented birds (T4) revealed no alterations in liver (Fig.1) and kidney (Fig 2). The hepatocytes were quite abundant in number. Fair numbers of blood vessels were noted in the capsule and parenchyma of the liver. Similar to our observation, Oi *et al.* (2021) observed no significant changes in the normal histology of kidney supplemented with synbiotic in broiler chickens.

From this experiment, it may be concluded that the synbiotic can be used as an alternate to the antibiotics as a growth promoter for improved immunity and intestinal morphometry without any harm to the vital organs.

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