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Original Article

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ABSTRACT

The present study aimed to evaluate the influence of various levels of synbiotics on growth performance and intestinal morphology of fast-growing broilers during different phases. A total of 720, oneday-old straight run broilers (Ross 308) were randomly divided into 12 treatment groups according to a completely randomized design having three supplemented diets of synbiotics (a combination of probiotics and prebiotics) (0.5, 1, and 1.5%) reared at four phases [starter (1-11 days), grower (12-22 days), finisher (23-35 days), whole life (1-35 days)]. Every treatment was replicated six times with ten birds each. The parameters were evaluated in growth performance (feed intake, body weight, feed conversion ratio, and mortality) and intestinal morphology (Villus height, Crypt depth and Villus to Crypt ratio). Results of the present study revealed that growth performance, feed intake (p=0.0029), body weight (p=0.0001) and feed conversion ratio (p=0.0001) were improved with 1 percent synbiotics diet supplementation, while 1.5 percent synbiotics level showed lower body weight. Crypt death (p=0.0529) has been marginally greater in the whole life. It can be inferred that a 1 % synbiotics supplemented diet has had a beneficial impact on growth and gut health throughout life.

INTRODUCTION

Poultry production, especially broiler farming is the fastest source to provide high-quality meat and nutrients like protein for the human body. Those birds fed on the commercial poultry feed can be exposed to extensive variation throughout the gastrointestinal tract (Manafi et al. 2017). Beneficial bacteria play an important role in the intestinal tract, which ultimately affects the performance of the birds because those bacteria enhance the nutrients absorption, gut morphology, and immune response (Lee et al., 2002). The beneficial synthetic bacteria are available in the form of probiotics and prebiotics and also synbiotics that are supplemented in broiler feed to increase feed efficiency and minimize the cost of production (Sarangi et al., 2016). These growth promoters like synbiotics are in practice for many decades to control disease threat (Akinleve et al., 2008). However, the long use of antibiotics in broilers rearing can impart residues in meat. Some scientists found various feed additives like prebiotic, probiotic, and synbiotics as alternate antibiotics in poultry nutrition (Patterson & Burkholder, 2003). The specific feed additive like *synbiotics* is used nowadays to improve gut activity, enhance the immune system (Hamasalim, 2016), reduce pH, maximize the digestibility of the nutrients, and also to increase the protective gut mucus (Nikpiran et al., 2014). Intestines play an important role in the digestion and absorption of nutrients to maintain proper body functions. The spreading of pathogens throughout the intestines



Effects of Different Feeding Regimes on Growth Performance and Intestinal Morphology of Commercial Broilers

often results in inflammation that is the source of production loss, a higher percentage of mortality, and also contaminates poultry products (Baurhoo *et al.*, 2009). The supplementation of *synbiotics* in feed minimizes the colonies of intestinal salmonella, E. coli, and other harmful bacteria (Baurhoo *et al.*, 2007a). It also increases the goblet cell number of the intestinal villi which increases the nutrient absorption in the small intestine (Baurhoo *et al.*, 2007b).

The addition of *synbiotics* or other growth promoters in the broiler diet improved villus height gradually at 35 days of age. The increase in the surface area of the villus in the bird's small intestine during the 1st, 3rd, and 5th weeks resulted in increased growth performance and carcass yield in broilers (Miles *et al.*, 2006). Although, the data regarding supplementation of *synbiotics* in poultry feed is abundant, information regarding its addition at different phases of broiler life is scarce. Keeping this in view, the present study was planned to evaluate the effect of different *synbiotics* levels on growth performance and histological parameters during different stages of life in broilers.

Pakistan is a developing country where poultry industry is the second largest growing enterprise providing employment opportunities to more than 1.5 million people. However, there is a big space for the poultry producers from Pakistan to capture the international market. To meet the demand of local market and to fulfil the criteria of European and other developed countries, producers must meet the antibiotic free production of poultry. Pakistan Poultry Industry is trying hard to boost the business by exporting the poultry products. However, the usage of antibiotics, little space provided to the birds during rearing and lack of knowledge about the dose rate of antibiotics' alternatives are the major hurdles in this regard.

Therefore, the present study was conducted to evaluate the effect of various levels of *synbiotics* and their effect on growth performance and intestinal morphology of commercial broilers reared under different life phases.

MATERIALS AND METHODS

This trial was conducted at the Poultry Research and Training Centre, the University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. A total of 720, one-day-old straight-run broilers (Ross-308) were obtained from a commercial hatchery and assigned to 12 treatment groups consisting of 3 levels

of synbiotics during 4 phases of life. A 3×4 factorial arrangement of treatments followed a Completely Randomized Design. Three different levels of synbiotics were offered during various growth phases including starter (1-10 days), grower (11-22 days), finisher (23-35 days), and the whole life period (1 to 35 days). The chicks were divided randomly into 12 treatment groups having 6 replicates of 10 chicks each. The supplemented feed was offered to the birds according to the phases during the whole study (Table 1). For ad libitum feeding and clean, fresh drinking water, each replicate $(1 \times 1 \text{ m}^2 \text{ floor density})$ was equipped with one round feeder and a nipple drinker. For the first week after hatching, brooding temperature and relative humidity (RH) washeld at 34 ± 1 °C and 62 \pm 3 percent, respectively. After that, the temperature was steadily decreased until it reached 24 °C on day 21 with RH 65%. A lighting program of 23L:1D was implemented throughout the study.

Table 1 – Calculated composition of the basal dietcomponents and nutrients.

Ingradiant	Inclusion level (%)					
Ingredient	Starter	Grower	Finisher			
Corn 12	58.62	60.29	60.88			
Soybean Meal 45	28.82	25.86	20.13			
Rice Polish 17	4.00	4.00	5.50			
Fish Meal 48	3.00	4.00	4.19			
Soy Oil	1.67	2.81	4.00			
Canola			3.00			
Calcium CO3	1.23	1.10	0.92			
Mono Calcium Phosphate	0.82	0.49	0.18			
L-Lysine SO4	0.62	0.47	0.37			
DL-Methionine	0.39	0.32	0.25			
Common Salt	0.29	0.27	0.22			
Threonine	0.22	0.15	0.15			
Vitamin Mineral Premix	0.15	0.15	0.15			
L-Argnine	0.14	0.07	0.04			
Winzyme HTR	0.01	0.01	0.01			
Total	100	100	100			

Parameters evaluated

Growth performance

The data were collected regarding the growth performance of the broilers including feed intake (g), body weight (g), and feed conversion ratio (FCR). Bodyweight and feed intake were recorded every week from each treatment group for the entire 35 days of the experiment. The body weight gain was calculated by subtracting the initial body weight from the final body weight. The feed conversion ratio was also recorded by dividing feed intake over body weight gain (Ghasemi *et al.*, 2016).



Intestinal morphology

On the 35th day, 3 birds per replicate were randomly picked up and slaughtered by the halal method (Altaf *et al.*, 2019). Ileum samples were obtained for intestinal morphology to determine the height of the villus (μ m), crypt depth (μ m), and the ratio of the villus to crypt (Giannenas *et al.*, 2012).

Statistical Analysis

The collected data were analyzed using PROC GLM in SAS software 9.1 over a factorial ANOVA. For comparison of significant treatment means, Duncan's Multiple Range (DMR) test (Duncan, 1955) was applied. The following mathematical model was used:

$$\label{eq:eq:approx_ijk} \begin{split} Y_{ijk} &= \mu + \alpha_i + \beta_j + \left(\alpha\beta\right)_{ij} + \epsilon_{ijk} \\ \text{Where,} \end{split}$$

 Y_{ijk} = Observation of dependent variables recorded on i^{th} and j^{th} treatment groups

 μ = Population mean

 α_i = Effect of ith synbiotics level (i = 1, 2, 3)

 β_i = Effect of jth phase feeding (j = 1, 2, 3, 4)

 $(\dot{\alpha}\beta)_{ij}$ = Interaction effects of i^{th} and j^{th} treatment group

 ϵ_{ijk} = Residual effect associated with i^{th} and j^{th} treatment group, NID ~ 0, σ^2

RESULTS AND DISCUSSION

Growth performance

Feed Intake

Means of both treatments (Phases and levels of *Synbiotics*) individually and in interaction demonstrated differences in feed consumption (Table 3). The highest feed consumption was observed fed at 1.5 percent *synbiotic* levels, while the lowest (p=0.0029) feed consumption at 0.5 percent level in the diet. The increase in feed intake in broilers could perhaps be due to the addition of *synbiotics* enhancing function of gastric juice thus increasing the utility of the nutrients in the intestine. Similarly, Samli *et al.* (2007) reported that feed intake was improved with the addition of *synbiotics* in the diet. Sarangi *et al.* (2016) have observed substantially higher feed consumption in birds when supplemented with a diet fed with *synbiotics*.

As far as the different phases are concerned, no major variations in feed consumption have been found between all treatment groups. The relationship between levels and phases of *synbiotics* showed differences in feed intake. During the growing phase, the birds fed

Effects of Different Feeding Regimes on Growth Performance and Intestinal Morphology of Commercial Broilers

at a 1.5 percent level of dietary *synbiotics* reported the highest feed consumption, while the lowest was found in birds fed 1 percent of *synbiotics* level.

Table 2 – Schedule of Vaccination for experimental birds.

		•
Days	Vaccines	Routes
1	ND+IB	Eye Drop
5	ND+IB	Eye Drop
12	IBD	Spray
19	ND+IB	Drinking water

ND = Newcastle Disease; IB = Infectious Bronchitis; IBD = Infectious Bursal Disease

Bodyweight

Different treatments have shown a pronounced effect on the bodyweight including different phases and levels of *synbiotics* and their interaction (Table 3). *Synbiotics* showed the highest body weight at a 1 percent inclusion level, while supplementation with 0.5 percent *synbiotics* showed the lowest body weight. The beneficial properties of *synbiotics*, which promote the growth of one or a small number of bacteria in the colon, raising the host's health and body weight (Ashayerizadeh *et al.*, 2009). Supplementation with *synbiotics* at a 1 percent diet stage greatly raises body weight in broilers (Erdoğan *et al.*, 2010).

As far as the different phases are concerned, no major differences were found between all treatment groups in body weight. There was a difference in body weight in the interaction between *synbiotics* levels and phases. The birds fed at a 1 percent level of *synbiotics* in the diet showed the highest body weight throughout the whole life cycle, whereas the lowest was observed in birds fed 0.5 percent *synbiotics* level in the grower phase. The supplemented diet of *synbiotics* found significantly higher body weight in broilers over the entire life cycle (Samli *et al.*, 2007).

Feed Conversion Ratio

There were major differences between the different treatment groups in the overall feed conversion ratio (FCR) (Table 3). The birds fed 1 percent of *synbiotics* showed improved FCR compared to the others. Feed conversion ratio may improve the intestinal environment as dietary *synbiotics* feeding reduces intestinal pH and increases the activity of digestive enzymes produced in the gut (Samli *et al.*, 2007). Abdul-Raheem and Abd-Allah. (2011) indicated improvement of the final body weight of *synbiotics* supplemented broilers compared to the prebiotic groups. In the present study, the better FCR in *synbiotics* supplemented birds might be due to the increased nutrient absorption which might have



Effects of Different Feeding Regimes on Growth Performance and Intestinal Morphology of Commercial Broilers

Table 3 – Effects of various levels of synbiotics on growth performance and intestinal morphology (mean ± standard errors) of broiler chickens raised during different phases.

Treatment		FI	BW	FCR	MOR	VH	CD	V:C
Phases	Starter	3671.78	2209.26	1.67	9.67	827.24	209.99ª	4.05
	Grower	3705.33	2216.39	1.68	10.22	713.79	151.75 ^b	4.7
	Finisher	3749.44	2225.57	1.69	7.8	634.86	167.16 ^{ab}	3.75
	Whole life	3799.44	2261.27	1.69	7.22	699.93	150.89 ^b	4.61
<i>p</i> -value		0.261	0.6593	0.9771	0.98	0.5485	0.0524	0.3911
Synbiotic levels (%)	0.5	3730.08 ^{ab}	2106.97 ^b	1.77ª	5.01	737.19	173.67	4.26
	1	3621.83 ^b	2384.44ª	1.52 ^b	7.33	689.82	179.01	3.73
	1.5	3842.58ª	2192.97 ^b	1.76ª	4.85	729.86	157.16	4.84
<i>p</i> -value		0.0029	0.0001	0.0001	0.5201	0.9061	0.5402	0.1488
SEM		27.8	24.27	0.03	0.85	52.03	8.62	0.27

Superscripts on different means within column differ significantly at $p \le 0.05$.

FI = feed intake (g); BW = body weight (g); FCR = feed conversion ratio; MOR = mortality (%); VH = villus height (µm); CD = crypt death (µm); V:C = villus height to crypt depth ratio.

influenced the overall efficiency of the diet and bird's weight. Khalaji *et al.* (2010) investigated the effect of iso-Malto oligosaccharide (IMO) in concentrations of 1, 2, and 4%, but found no differences in feed efficiency and FCR. Feed conversion rate (FCR) was observed to be significantly better in birds when supplementation with *synbiotics* was offered (Awad *et al.*, 2009). Similarly, Mountzouris *et al.* (2007) reported that FCR was improved with the addition of *synbiotics* in the diet.

As far as the different phases are concerned, there were no major differences in FCR between all treatment groups. The interaction between *synbiotics* levels and phases showed a variation in feed conversion ratio. The birds fed at a 1% level of *synbiotics* in the diet during the whole life phase showed the best FCR, whereas, the lowest was observed in the birds fed *synbiotics* level of 0.5 % in the finisher phase. Dizaji *et al.* (2012) found significantly better FCR during the whole life phase when fed with *synbiotics* in the diet.

Mortality

Means of mortality percentage did not differ in the phases, various levels of *synbiotics*, and their interaction among all treatment groups (*p*>0.05).

Intestinal morphology

Villus height

Means of various phases and levels of *synbiotics* were reported non-significant in villus height (Table 4). In 1 percent of *synbiotics* supplemented groups raised during the entire life period, the contact between different treatment groups showed higher villus height, while the lowest was found in 0.5% of the *synbiotics* supplemented group during the whole life.

Crypt Ratio

Histological parameters differed among different levels of synbiotics, phases, and their interaction (Table 4). Crypt depth was better during the entire life phases compared to others. Significant interactions were observed regarding the villus height, crypt depth, and villus to crypt ratio. The addition of synbiotics in the diet has been reported to influence epithelial cells thereby increasing the number of cells present in the villi. Therefore, the surface area of the villus is increased which also maximizes nutrients digestion (Awad et al.,2008). The reduction in the height of the villus in the surface region and the rise in the thickness of the crypt depth leads to a decrease in the absorption of nutrients in the small intestine that eventually affects the production of the birds (Xu et al., 2003). The shortening of the crypt depth is beneficial for nutrient absorption in the gut. Synbiotics can influence deeper crypt tissues which are the source to inhibit the crypt cell renewal and also the regeneration of the villus cells if needed (Giannenas et al., 2012).

Villus to Crypt Ratio

No major variations were found between the total means of the phases and *synbiotics* levels (Table 3). The ratio between villus and crypt was raised during the finisher phase in the interaction 1 percent level of *synbiotics* supplemented group, while 0.5 percent *synbiotics* level showed the lowest growing phase values. The addition of *synbiotics* in the broiler diet was found to significantly improve villus to crypt ratio; these improvements indicate better nutrient absorption throughout the small intestine of the birds (Shokri *et al.*, 2017). Hassanpour *et al.* (2013) also found the maximum values of intestinal morphology with added diet in*synbiotics* at 1g/kg. The increasedratio of the



Table 4 – Interaction effects of phases and levels of synbiotics on growth performance and intestinal morphology (means ± standard errors) of broilers.

Treatment		FI	BW	FCR	MOR	VH	CD	V:C
Starter	0.5	3625.6	2095.14 ^{ab}	1.73 ^{ab}	6.67	851.61 ^{ab}	239.45ª	3.47 ^{bc}
	1	3605.6	2378.71ª	1.52°	3.33	772.66 ^{ab}	210.42 ^{ab}	3.71 ^{bc}
	1.5	3784.00	2153.94 ^{ab}	1.76ª	10.01	857.46 ^{ab}	180.12 ^{ab}	4.98 ^{abc}
Grower	0.5	3670.67	2044.07 ^c	1.79ª	10.01	1000.50ª	153.64 ^{ab}	6.58ª
	1	3565.33	2376.62ª	1.51 ^{bc}	6.67	627.86 ^{ab}	174.28 ^{ab}	3.42 ^{bc}
	1.5	3928.00	2228.47 ^{ab}	1.77ª	10.01	502.99 ^{ab}	127.34 ^b	4.10 ^{abc}
Finisher	0.5	3767.00	2145.38ab	1.76ª	13.33	729.97 ^{ab}	176.66 ^{ab}	4.13 ^{abc}
	1	3645.67	2387.27ª	1.53 ^{bc}	10.01	358.38 ^b	135.89 ^b	2.68 ^c
	1.5	3835.67	2144.07 ^{ab}	1.79ª	13.33	816.24 ^{ab}	188.92 ^{ab}	4.46 ^{abc}
Whole life	0.5	3905.00	2143.29 ^{ab}	1.83ª	20.01	356.64 ^b	124.94 ^b	2.86 ^{bc}
	1	3622.6	2395.14ª	1.50 ^c	13.33	1010.40ª	195.45 ^{ab}	5.12 ^{abc}
	1.5	3822.67	2245.38ab	1.71 ^{abc}	10.06	742.75 ^{ab}	132.29 ^b	5.84 ^{ab}
SEM		27.8	24.27	0.03	0.85	52.03	8.62	0.27
<i>p</i> -value		0.0675	0.0453	0.0186	0.3300	0.0405	0.0497	0.0312

Superscripts on different means within column differ significantly at $p \le 0.05$.

FI = feed intake (g); BW = body weight (g); FCR = feed conversion ratio; MOR = mortality (%); VH = villus height (µm); CD = crypt death (µm); V:C = villus height to crypt depth ratio.

villus to crypt ratio by *synbiotics*-fed birds may be attributed to the increase of epithelial cells in the gut (Deng *et al.,* 2012).

CONCLUSIONS

In conclusion, supplementing various levels of dietary *synbiotics* and different phases of commercial broiler may boost the efficiency of growth and intestinal morphology that enhances intestinal immersion. Besides, feed at a 1 % level of dietary *synbiotics* in birds had a positive impact on the growth efficiency and crypt depth.

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