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Effect of Dietary Supplementation of a Non-antibiotic Growth Promoter on Growth Performance and Intestinal Histomorphology in Broilers

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ABSTRACT

The current research trial was conducted to evaluate the effects of a non-antibiotic growth promoter on growth performance and histomorphology in broiler chickens. One hundred and twenty day-old broiler chicks were randomly distributed into four treatment groups with three replicates per treatment (10 chicks per replicate) under Completely Randomized Design. Four iso-nitrogenous and iso-caloric diets *i.e.* A, B, C and D were supplemented with a natural growth promoter (NGP) at the rate of 0 (control), 1.5, 3 and 4.5g/kg of feed, respectively. Feed consumption (FC) and weight gain (WG) were recorded to check the feed conversion ratio (FCR) on weekly basis. At the end of trial, two chicks from each replicate were randomly slaughtered to determine carcass traits. Samples of intestine (duodenum, jejunum, ileum) were collected to perform histomorphology. Results indicated that FC, WG and FCR were linearly improved (P<0.05) in B, C and D groups as compared with control (A group). Carcass characteristics and relative organs weight showed non-significant results (P>0.05). However, data obtained on live body weight and abdominal fat pad were significantly different (P<0.05) among all dietary treatments. Histomorphology of small intestine showed variations in intestinal wall width (IW), lumen area (LA), villi height (VH) and villi width (VW) with respect to supplementation of NGP. Maximum IW and VW was observed in duodenum and jejunum respectively, whereas greater LA and VH was noticed in ileum. In conclusion, supplementation of non-antibiotic NGP improved the performance and histomorphology of broiler chickens.

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Authors' Contribution
MS and FA designed the study. JI executed the experimental work while MNS helped him. MS and AAK helped in statistical analysis and manuscript writing. TA and MA proofread the article.

Key words
Broiler, Histomorphology, Non-antibiotic growth promoter, Performance

INTRODUCTION

Antibiotic growth promoters are biological and chemical substances which are commonly used in poultry feed to improve the growth performance of chickens. They are used in farm animals as facilitator of digestion and improving health of animals. Different previous studies have found good results on the performance by the supplementation of amino acids in place of antibiotic growth promoters in chickens (Saeed *et al*., 2018a, 2018b). They improve intake, appetite, weight gain and reduced feed conversion ratio (Broom, 2018; Rehman *et al*., 2019). Antibiotics change the composition and activities of intestinal microflora and convert them into 'antibiotic resistant' organisms (Brüssow, 2015). The consequences of antibiotic growth promoters led them to withdrawal from animal feeds since 1986 in Sweden, 1998 in Denmark (WHO, 2002), 1999 in Switzerland, and 2006 in European Union (Castanon, 2007).

After the ban of antibiotic growth promoters (AGPs) in several countries, there is a great initiative on the search of alternative feed additives with similar effects on production in farm animals. Many organic/natural substances have been found to replace AGPs like probiotics, prebiotics, synbiotics, organic acids, enzymes, oligosaccharides, natural herbs, different forms of clay, yeast extracts and essential oils (Nehru *et al*., 2017; Deraz, 2018; Abdel-Rahman *et al*., 2019; Malik *et al*.,

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2019). Lyses of yeast cells by exogenous enzymes or acids provides yeast extracts which are rich source of nutrients and contain vitamins, minerals, peptides and amino acids that contributes to form nutritionally balanced diets (Shurson, 2018).

Enzymes are very specific catalysts that catalyse the rate of a reaction and interestingly their reaction increases with increasing substrate concentration (Khattak *et al*., 2006). It decreases the quantity of non-degraded organic matter which is commonly used by gram negative bacteria to grow in a basic environment (pH>7). If those bacteria do not grow, the acidophilic bacteria will grow, the gastric tract becomes more acidic (pH 4-6) which is a better condition for the optimum chemical process of the digestion and increasing the beneficial gut organisms viz., lactic acid bacteria and bifidobacterium (Quigley, 2019). Prebiotic molecules positively modulate the gut ecosystem by producing the antimicrobial compounds as well as improve the health, immunity and production in farm animals (Brink *et al*., 2006; Orayaga *et al*., 2016). They refine the process of digestion and balance the gut microflora. Economic performance is improved by increasing the nutrient availability of the feed through the activated digestion process (Guerreiro *et al*., 2018).

A lot of work has been done regarding the use of individual growth promoters (like enzymes, prebiotics, probiotics etc.) in the diet of broilers but combined use of enzymes and prebiotics as a natural growth promoter (NGP) is not been reported yet. Therefore, this study was planned to check the effects of varying levels of NGP that was formulated by combination of exogenous enzymes and prebiotics on growth performance and intestinal histomorphology in broiler birds.

MATERIALS AND METHODS

Experiment birds, diets and management

The trial got approval from Local scrutiny committee of the department keeping in view the International Animal Ethics consideration then it was conducted during winter season at Raja Muhammad Akram Animal Nutrition Research Center, University of Agriculture, Faisalabad. One hundred and twenty day-old broiler chicks having initial body weight 43-44 grams were procured from a local hatchery. Chicks were randomly divided into four groups (A, B, C and D) each having 3 replicates and each replicate consist of ten chicks. Antibiotic free experimental diets were formulated for 1-21 days (starter) and 22-35 days (finisher) periods according to (Leeson and Summers, 2005) standard. Starter diets had 21% crude protein and 2950 kcal/kg metabolizable energy while finisher diets had 19% crude protein and 3100 kcal/kg metabolizable energy (Table I). Group A was without NGP (control), whereas the diets of group B, C and D were supplemented with NGP (MFeed®) at the rate of 1.5, 3 and 4.5 g/kg of feed respectively. NGP is consist of enzymes (phytase 500 FTU/kg, xylanase 2000 U/kg and amylase 200U/kg) with other active prebiotic ingredients (alumino-silicates 0.2%, yeast extracts 35 mg/kg and seaweed extract 1%). These diets were fed *ad libitum* to the birds. Rearing practices *i.e.* temperature, ventilation and humidity were adopted according to standard requirements of birds (NRC, 1994). Broiler performance was measured in terms of feed consumption (FC), weight gain (WG) and feed conversion ratio (FCR) at the end of trial (35 d).

Table I. Ingredient and nutrient composition of basal starter and finisher diets.

Ingredient	Inclusion %	
	Starter	Finisher
Maize grains	61.97	66.27
Canola meal	6.55	3.24
Soybean meal	24.54	22.44
Corn gluten 60 %	2.23	2.5
Soya oil	1	2
Dicalcium phosphate	1.57	1.52
Limestone	1.33	1.16
Vitamin mineral premix*	0.5	0.5
L-Lysine HCl	0.15	0.15
DL-Methionine	0.1	0.1
L-Threonine	0.06	0.12
Nutrient composition (%)		
Crude protein	21.00	19.30
M.E	2950	3100
Crude fiber	3.13	2.72
Ether extract	3.80	4.88
Dig-Lysine	1	0.9
Dig-Methionine	0.4	0.38
Calcium	1	0.9
Available P	0.4	0.38
CP : ME Kcal	1:140	1:160

*Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 μg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; Fe, 80 mg; Zn, 40 mg; Mn, 60 mg; I, 0.18 mg; Cu, 8 mg; Se,0.15 mg.

Sampling and analysis

At 35 day of age, six birds from each group (2 per replicate) were randomly selected, weighed and slaughtered humanly and dressing percentage was calculated after evisceration (Kamboh and Zhu, 2013). Weight of various organs including liver, heart, gizzard, intestine, breast, wing and abdominal fat pad were measured using digital weight balance.

For intestinal histomorphological analysis, samples from distal portion of duodenum, jejunum and ileum were collected from slaughtered birds and fixed in 10% PBS (phosphate buffered saline). These intestinal segments were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene and embedded in paraffin wax. A microtome was used to make cuts of 5μm which were mounted on glass slides and stained with hematoxylin-eosin (Sigma Co, USA). Three slides per intestinal segment were prepared for microscopy and three values per measurement (intestinal wall width (IW), lumen area (LA), villi height (VH), villi width (VW)) were obtained to take an average value. The values were measured using a light microscope coupled with a digital imaging analysis system as described previously (Kamboh and Zhu, 2014).

Statistical analysis

The data obtained during the experiment was subjected to statistical analysis using Analysis of Variance Technique under Completely Randomized Design by using JMP software (version 5.0.1a; SAS Institute, 2000). The differences among the treatment means were compared by using Duncan's Multiple Range Test (Steel *et al.*, 1996).

RESULTS

Performance of broilers

Statistical analysis revealed that performance was improved ($P<0.05$) by the supplementation of NGP (Table II). The trend was linearly associated with the supplementation. Compared with control (2518.28 g), maximum FC was noticed in chicks under utmost supplementation (2777.96 g) (group D). As compared to control (1292.90 g), groups B (1369.09 g), C (1453.56 g) and D (1556.52 g) exhibited significantly ($P<0.05$) higher weight gain. Chicks supplemented 1.5 and 3g (B and C group respectively) showed similar but improved feed conversion ratio when compared with the control; whereas, best ($P<0.05$) FCR was observed by supplementation of 4.5g/kg of NGP in feed (group D). There was no difference in mortality percentages between treated and control groups.

Organs weight

The effect of supplementation of NGP on various organs weight of broilers has been summarized in Table III. Non-significant effects ($P>0.05$) of dietary NGP were obtained in relative weights of liver, heart, gizzard, intestine, breast and wing. However, weight of abdominal fat pad was improved ($P<0.05$) 62.6% and 70.1% in group C and D respectively as compared to group A (Control).

Table II. Performance of boiler chickens supplemented with natural growth promoter.

Parameters	Diets[1]				SEM[2]
	A	B	C	D	
Feed intake	2518.28^{b}	2595.70^{b}	2761.42^{a}	2777.96^{a}	7.34
Weight gain	1292.90^{c}	1369.09^{b}	1453.56^{ab}	1556.52^{a}	5.74
FCR	1.95^{a}	1.89^{ab}	1.89^{ab}	1.79^{b}	.03

Mean with different superscripts within the same row reflect significant differences ($p<0.05$). [1]Group A, Basal diet without any supplementation (Control) group B, C and D supplemented with NGP at the rate of 1.5, 3 and 4.5g/kg of feed; [2]SEM, Standard error mean.

Table III. Effect of natural growth promoter on different organs weight of boiler chickens.

Parameters	Diets[1]				SEM[2]
	A	B	C	D	
Dressing percentage (%)	55.59	56.65	55.58	53.45	8.54
Liver weight (g)	3.52	4.29	3.31	4.13	1.13
Heart weight (g)	1.09	1.32	1.13	1.23	0.5
Gizzard weight (g)	2.95	2.84	2.54	2.93	1.17
Intestinal weight (g)	14.82	13.34	14.53	14.58	6.7
Breast weight (g)	34.85	34.69	35.77	35.71	4.8
Wing weight (g)	5.00	4.75	2.85	5.40	2.0
Abdominal fat pad (g)	2.81^{c}	3.52^{bc}	4.57^{ab}	4.78^{a}	2.3

Mean with different superscripts within the same row reflect significant differences ($p<0.05$). [1]Group A, Basal diet without any supplementation (Control) group B, C and D supplemented with NGP at the rate of 1.5, 3 and 4.5g/kg of feed; [2]SEM = Standard error mean.

Histomorphology of small intestine

The data subjected to statistical analysis showed that supplementation of NGP showed improved ($P<0.05$) histomorphology of small intestine (Table IV). Increasing trend of IW, LA, VH and VW was observed ($P<0.05$) in all three compartments of small intestine (duodenum, jejunum and ileum) with supplementation of NGP. Maximum IW and VW was observed in duodenum and jejunum respectively; whereas greater LA and VH was noticed in ileum.

DISCUSSION

Prebiotics are non-digestible food ingredients like plant parts, plant extracts, essential oils etc., that help promote the health status of individuals. In recent literature, exogenous enzymes (Cowieson and Kluenter, 2019) and prebiotics (Solis-Cruz *et al*., 2019) have been indicated as potential candidates for replacement of antibiotic growth promoters. These choices are ecofriendly and have no any deleterious effects on product (meat or egg) quality as well as human health. These natural alternatives not only improving the feed efficiency but also increasing the overall well-being of farm animals by modulating their immune and health status (Kamboh and Zhu, 2014; Deraz, 2018). In current experiment, the effects of a natural growth promoter formulated by prebiotics and enzymes were investigated in broilers for growth performance, organ weight and histomorphology of intestine.

Table IV. Intestinal histomorphology of boiler chickens supplemented with natural growth promoter.

Parameters (µm)	Diets[1]				SEM[2]
	A	B	C	D	
Duodenum					
Intestinal wall width	1428.70^{c}	1265.42^{d}	1591.98^{b}	2245.10^{a}	6.90
Lumen	1.47^{c}	1.50^{c}	1.79^{b}	2.29^{a}	0.09
Villi length	346.97^{b}	346.97^{b}	415.00^{ab}	449.00^{a}	5.79
Villi width	122.48^{c}	142.87^{b}	163.29^{a}	163.28^{a}	3.20
Jejunum					
Intestinal wall width	1081.73^{b}	1224.60^{b}	1408.29^{a}	1142.96^{b}	8.7
Lumen	1.78^{c}	2.22^{b}	2.39^{b}	2.94^{a}	0.10
Villi length	380.98^{c}	489.84^{bc}	632.71^{ab}	693.94^{a}	4.79
Villi width	129.26^{c}	170.07^{bc}	224.51^{ab}	265.33^{a}	2.76
Ileum					
Intestinal wall width	1122.55^{c}	1326.65^{b}	1714.44^{a}	1836.90^{a}	7.15
Lumen	1.58^{c}	1.66^{c}	2.61^{b}	2.92^{a}	0.20
Villi length	510.25^{b}	551.07^{b}	714.35^{ab}	775.56^{a}	4.98
Villi width	163.28^{d}	183.69^{c}	224.51^{b}	244.92^{a}	3.11

Mean with different superscripts within the same row reflect significant differences ($p<0.05$). [1]Group A, Basal diet without any supplementation (Control) group B, C and D supplemented with NGP at the rate of 1.5, 3 and 4.5g/kg of feed; [2]SEM, Standard error mean.

Results of this study showed that average values of FC among different treatment groups were significantly higher than control birds. These findings are in agreement with the results of Owen *et al*. (2012), Damiri *et al*. (2012) and Duan *et al*. (2013) who supplemented different levels of kaolin clay, sodium bentonite and alumino-silicates respectively to broilers and reported significant improvement in feed intake. Likewise, in consistence with our study, Damiri *et al*. (2012) and Bailey *et al*. (2006) reported improved FCR and WG in broilers by supplementation of sodium bentonite and alumino-silicates respectively. Alumino-silicate is a well-known sorbent and an important constituent of kaolin and other clay minerals. It is very famous to absorb aflatoxins from the feed (Miazzo *et al*., 2005). Hence the improved performance in our experiment by supplementation of NGP (containing alumino-silicate as an ingredient) and researches of aforementioned workers by inclusion of mineral compounds could be credited to aflatoxin reducing effects, as chronic sub-clinical levels of aflatoxicosis have significant effects on production of farm animals (Gilani *et al*., 2016). Galarza-Seeber *et al*. (2016) reported that stressors like chronic dietary toxins cause intestinal inflammation that may lead to ‘leaky gut’, a condition that seriously drop the nutrients absorption, weight gain and intestinal development as well.

Supplementation of NGP in broiler diets showed non-significant effects in organ visceral weight except live body weight and abdominal fat pad. These results are in accordance with Duan *et al*. (2013), who reported non-significant results ($P>0.05$) in relative weights of heart, liver, spleen, lung and kidney with the addition of graded levels of montmarillonite in broiler chicks. High fat pad yield probably because of improved weight gain in NGP supplemented groups as compared to control group.

Results of this study indicated that sections of the small intestine (duodenum, jejunum and ileum) showed an improved IW, LA, VH and VW in NGP supplemented groups. These findings are supported by Hu *et al*. (2013), who reported that supplementation of different levels of zinc-oxide montmarillonite in broiler diets increased the VH of duodenal sections of small intestine. Similar results were obtained by Wan *et al*. (2013), who reported that supplementation of clay in ducklings diet increased the VH and crypt depth of small intestine. Similarly, some other studies have reported the positive effects on intestinal morphometric structures by the dietary inclusion of prebiotics like flavonoids (Kamboh and Zhu, 2014), mannanoligosaccharide (Rahimi *et al*., 2019), sea weed (Michiels *et al*., 2012) and blend essential oils (Reisinger *et al*., 2011). Improved intestinal observations indicated that NGP have a positive effect on the epithelial cells mitosis, because improved VH and/or VW indicates the activated cell mitosis (Saeed *et al*., 2017). The improved villi (in terms of length or thickness) indicates the digestive tract maintenance that could be credited to significant amount of antioxidants found in various constituents (like sea weed) of our dietary growth promoter. Because a large body of evidences suggested that bioflavonoids and polyphenols found in botanicals, have significant antioxidant effects. Antioxidant effects at cellular level exerted by plant antioxidants may lead to improved production, immunity, health, gut function and meat quality in poultry (Kamboh and Zhu, 2013; Kamboh *et al*., 2016).

CONCLUSIONS

Our results suggested that natural growth promoters having combination of organic enzymes and prebiotics could be replace with synthetic antibiotics in broiler production. Supplementation of these growth promoters have beneficial impact on production performance and intestinal histomorphology that may provide a wider area for absorption and utilization of nutrients for body.

Statement of conflict of interest

The authors declare no conflict of interest

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