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Original Article

Histomorphometrical Study of the Prebiotic Effects on Intestine Morphology and Growth Performance of Broiler Chickens

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Abstract

This experiment was conducted to compare the effects of prebiotic as alternative feed additive to an antibiotic growth promoter (bacitracin methylene disalicyate) on the growth performance and morphometrical parameters of the small intestine of broiler chickens. One hundred and forty four day old broiler chicks were randomly assigned to one of three dietary treatments for 6 wk and each treatment contained four replicates (12 birds each). Dietary treatments were as follow: 1- Control (basal diet), 2- basal diet + antibiotic growth promoter and 3- basal diet + prebiotic. During the feeding experimental period, body weight, weight gain, feed intake and feed conversion ratio were measured. At the end of the experiment, small intestine segments were sampled and routine histological laboratory methods containing fixation, dehydration, clearing and paraffin embedding were used. Sections stained with haematoxylin and eosin for light microscopy evaluation and the height and width of villi and depth of crypts were measured. The results showed that body weight, weight gain and feed conversion ratio were not affected by dietary treatments. Prebiotic and antibiotic had significant ($P < 0.05$) effect on improvement of feed intake in 22 - 42 days and total period compared with the control. The addition of prebiotic or antibiotic increased the villus height in duodenum ($P < 0.05$) and prebiotic increased villus width of duodenum and ileum compared with other treatments. The duodenal crypt depth was increased by antibiotic compared with the prebiotic and control group. In conclusion, prebiotic can be used as a suitable alternative to antibiotic growth promoter.

Key words: Prebiotic, Antibiotic, Morphometry, Performance, Small intestine, Broiler chicken

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Introduction

For the past several decades antibiotic growth promoters have been included in poultry feeds worldwide at sub-therapeutic concentrations as a standard practice because of their positive effects on growth performance.[1,2] But usage of antibiotics has several set-backs such as residues problem in tissues, long withdrawal period, and development of resistance in microorganisms, allergies and genotoxicity.[3-6] Therefore, over the past few years, a great deal of interest has been generated on the evaluation of alternative means for manipulation of gastrointestinal microflora in livestock. The motivation for examining these alternatives comes from increased public scrutiny about the use of antibiotics in the animal feed industry as well as the need for a safe food supply.

An alternative approach to sub-therapeutic antibiotics in livestock is the use of prebiotics. Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth or metabolic activity of a limited number of intestinal microorganisms.[7] Prebiotics increase population of useful bacteria like lactobacilli and bifidobacteria, compete with pathogens for attaching to intestinal cells, increase production of volatile fatty acids, decrease intestinal pH, produce antimicrobial compounds, improve immune system, provide digestive enzymes and improve morphologic indices.[7-11]

Several studies have shown that addition of prebiotics to the diet of broilers have improved performance[10,12] and morphology of intestine.[10,13-15] In contrary, there are some reports that prebiotics did not influence poultry performance.[13,16]

In the present study comparison of effects of antibiotic growth promoter and prebiotic on growth performance and histomorphometery of small intestine in broilers has been evaluated.

Materials and Methods

One hundred and forty four day old chicks (Ross 308) were divided randomly into 3 groups with four replicates. In each replicate 12 chicks were studied for 42 days. Feeding of the chicks was based on starter, grower and finisher rations. The ration was the same for all groups and was based on corn and soybean. The experimental groups were: 1- control (basal diet), 2- basal diet + antibiotic growth promoter and 3- basal diet + prebiotic.

The prebiotic (ECOCELL®, Impextraco, Belgium) used in this study was derived from cell wall of *Saccharomyces cerevisiae* and its components were mannanoligosaccharide and β-glucan. We added the prebiotic in 0.1 % ratio into the ration based on the producer's instructions. Bacitracin (bacitracin methylene disalicyate) was used as an antibiotic to promote the growth. For starter 50 g ton^{-1} and for grower and finisher diets 25 g ton^{-1} were added to the ration.

All chicks were weighed upon arrival to the farm from hatchery and were weighed weekly afterwards. Feed intake and feed conversion ratio were determined for each pen. At the end of the experiment, from the middle-length of duodenum, jejunum and ileum a 2-cm long segment were transected; ingesta washed away using normal saline and fixated in 10 % neutral buffered formalin. Following histological fixation, the tissues were processed through a standard alcohol dehydration-xylen sequence and embedded in paraffin. From each segment 5 sections of 6-7 µm thickness were made and they were stained with haematoxylin and eosin (H & E). Morphometric analyses of digital photos of light microscopy were performed by means of an image analysis program (ImageJ software). In each, the villi height and width and crypts depth were determined by examining randomly 6 villi and 6 crypts. Later, an average of 30 values was obtained for each chick.

Statistical analysis. The SPSS program version 18 was used for data analysis. The results were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. Statistical significance was established at $P < 0.05$.

Results

Performance. The effects of dietary addition of prebiotic and antibiotic growth promoter on broiler performance are shown in Tables 1 and 2. At 21 and 42 days of age, there were no significant differences ($P > 0.05$) among experimental groups in weight and weight gain. However, at the end of the study period in the groups fed with prebiotic and antibiotic weight and weight gain were insignificantly more than the control group. Feed intake from 1 to 21 days was not affected in experimental groups. From 22 to 42 days and total period birds fed the control diet consumed more feed than did those in the other treatment groups ($P < 0.05$). Although in 22-42 days and total period feed conversion ratio in treatment groups was less than control group, the feed conversion ratio did not show any significant difference among experimental groups.

Histomorphometry. The results of histomorphometry of small intestine are shown in Tables 3 to 5. In duodenum, addition of prebiotic or antibiotic to the diet significantly increased ($P < 0.05$) the height of the villi in comparison with the control group and there was no significant difference between antibiotic and prebiotic groups regarding the villi height. Prebiotic significantly increased ($P < 0.05$) the villi's width of duodenum and ileum compared with other treatments and there was no significant difference among other groups. Antibiotic increased ($P < 0.05$) the crypts' depth of duodenum compared to prebiotic and the control group and there was no significant difference between prebiotic and the control group.

Table 1. Effects of feed additives on body weight and weight gain

Parameter	Live weight (g)			Weight gain (g)		
	0 d	**21 d**	**42 d**	**0-21 d**	**22-42 d**	**0-42 d**
Control	48 ± 0.6	590 ± 13	2267 ± 97	542 ± 13	1677 ± 87	2219 ± 97
Antibiotic	47 ± 0.2	569 ± 19	2274 ± 95	521 ± 19	1705 ± 95	2227 ± 95
Prebiotic	48 ± 0.17	581 ± 21	2352 ± 55	533 ± 21	1771 ± 66	2304 ± 55
***P*-value**	NS	NS	NS	NS	NS	NS

Means within a column with no common superscript differ significantly ($P < 0.05$, ANOVA, Duncan's test). NS: Not significant ($P > 0.05$). Results are reported as means ± S.E.

Table 2. Effects of feed additives on feed intake and feed conversion ratio

Parameter	feed intake (g)			feed conversion ratio (g:g)		
	0-21 d	**22-42 d**	**0-42 d**	**0-21 d**	**22-42 d**	**0-42 d**
Control	837 ± 6	3360^a ± 55	4197^a ± 55	1.6 ± 0.03	2 ± 0.12	1.9± 0.09
Antibiotic	816 ± 9	3063^b ± 91	3878^b ± 83	1.6 ± 0.06	1.8 ± 0.06	1.8 ± 0.04
Prebiotic	847 ± 14	3107^b ± 52	3954^b ± 63	1.6 ± 0.04	1.8 ± 0.09	1.7 ± 0.06
***P*-value**	NS	0.027	0.022	NS	NS	NS

Means within a column with no common superscript differ significantly ($P < 0.05$, ANOVA, Duncan's test). NS: Not significant ($P > 0.05$). Results are reported as means ± S.E.

Table 3. Effects of feed additives on villi's height in different segments of the small intestine (μm)

Parameter	Duodenum	Jejunum	Ileum
Control	$1562^{b} \pm 66$	1262 ± 34	929 ±25
Antibiotic	$1906^{a} \pm 60$	1299 ± 54	864 ± 21
Prebiotic	$1803^{a} \pm 49$	1344 ± 63	992 ± 51
***P*-value**	0.001	NS	NS

Means within a column with no common superscript differ significantly ($P < 0.05$, ANOVA, Duncan's test). NS: Not significant ($P > 0.05$). Results are reported as means ± S.E.

Table 4. Effects of feed additives on villi's width in different segments of the small intestine (μm)

Parameter	Duodenum	Jejunum	Ileum
Control	$174^{b} \pm 3$	112 ± 5	$118^{b} \pm 5$
Antibiotic	$174^{b} \pm 9$	110 ± 2	$128^{b} \pm 6$
Prebiotic	$205^{a} \pm 10$	117 ± 4	$146^{a} \pm 2$
***P*-value**	0.013	NS	0.002

Means within a column with no common superscript differ significantly ($P < 0.05$, ANOVA, Duncan's test). NS: Not significant ($P > 0.05$). Results are reported as means ± S.E.

Table 5. Effects of feed additives on crypts' depth in different segments of the small intestine (μm)

Parameter	Duodenum	Jejunum	Ileum
Control	$167^{b} \pm 2$	166 ± 6	149 ± 3
Antibiotic	$203^{a} \pm 12$	152 ± 7	146 ± 7
Prebiotic	$179^{b} \pm 2$	160 ± 4	156 ± 6
***P*-value**	0.005	NS	NS

Means within a column with no common superscript differ significantly ($P < 0.05$, ANOVA, Duncan's test). NS: Not significant ($P > 0.05$). Results are reported as means ± S.E.

Discussion

In the present study, addition of growth stimulants did not have significant effect on weight and weight gain. However, at the end of the study, in the groups fed with prebiotic and antibiotic weight and weight gain were insignificantly more than the control group and such a difference was considerable when prebiotic was used. In our study, the best feed conversion ratio was recorded for prebiotic and antibiotic, respectively. The best performance was achieved when prebiotic was used.

Review of the literature revealed that results in case of growth promoter usages in diets of broilers were different. Oliveira *et al.*, showed that addition of prebiotic to the diet of the broilers was ineffective.[16] Baurhoo *et al.*, reported that when prebiotic or antibiotic was added to the diet of broilers the weight and consumed feed decreased significantly compared to those of the control group. They also concluded that feed conversion ratio remained unchanged when prebiotic or antibiotic was used.[13] Mohamed *et al.*, demonstrated that prebiotic or antibiotic increased weight gain and decreased feed conversion ratio, however, the values were not significant.[17]

Hooge reviewing 44 reports conducted on broilers concluded that addition of prebiotic ended up improvement in growth performance.[12] Markovic *et al.*, having studied effects of prebiotic and antibiotic on performance of broilers showed that addition of these growth promoters increased the body weight and daily weight gain of broiler chicks and concluded that this increase was remarkable when prebiotic was used. They also showed that addition of the growth promoters significantly decreased feed conversion ratio.[10]

Overall, results of the present study in broilers are compatible with those of studies that showed addition of prebiotic to diet improves growth performance. The lack of compatibility in results of the present study with those of others could be due to difference in type and concentration of growth promoters, type of intestinal microflora, flock health and management.

The morphological changes in the intestine could be as a result of effects of growth promoters on absorption area and changes in performance of the broilers. Length and width of intestinal villi are of histomorphometrical indices and any increase in the values end up increase in absorptive surface of intestine. Several studies have shown that addition of prebiotic to the diet of broiler leads to improved performance through improving gut microflora and gut histological parameters.[10,14,18,19]

Extensive proliferation of intestinal bacteria in the chicks fed with diets lacking effective growth promoters on microbial population could lead to destruction of intestinal mucosa and explain reduction in dimensions of villi. On the other hand growth promoters like prebiotics reduce pathogenic bacterial population through rising useful intestinal microflora (Lactobacilli and Bifidobacteria), increasing production of fatty acids and reduction in intestinal pH. Therefore, intestinal tissue health and growth are achieved.[11,16]

Regarding maintenance of intestinal tissue health, antibiotics may be less effective than prebiotics because the antibiotics decrease population of both useful and pathogenic bacteria.[13] In the present study the prebiotic increased villi height in duodenum and villi width in both duodenum and ileum. However, the antibiotic increased villi height only in duodenum.

Intestinal epithelial cells are changed constantly and compensate villi cell loss through proliferation and maturation inside crypts and upward migration. Crypts depth is correlated with the intestinal cells turnover rate and increase in crypts depth indicates the need for enterocyte replacement and higher tissue turnover.[10,16] Such a need could be because of increase in dimensions of villi or maintenance of the dimension as a result of increased destruction. In the present study increased depth of the duodenal crypts in the antibiotic group could be explained by increased height of intestinal villi and also effects of the antibiotic on reduction of useful intestinal microflora and subsequent need for intestinal cells turnover. On the other hand increase in the population of useful intestinal microflora provides better conditions for longer enterocyte life and reduces the intestinal mucous cells turnover rate.[10] Hence, depth of the crypts remains unchanged or decreases. Increased replacement of enterocytes requires more energy and protein that limits growth and the development of other tissues. Thus, decrease in depth of crypts leads to reduction in the need for replacement of enterocytes and subsequently increases growth rate of the chick.[10] In the present study decreased depth of crypts in the prebiotic group was more considerable than that of antibiotic group.

Studies have shown different responses for intestinal histomorphometry by dietary additives. Marković *et al.*, showed that using antibiotic growth promoter, probiotic and prebiotic in the diet of broilers accompanied improved growth performance, increased height and width of intestinal villi, and decreased depth of crypts at the end of the study and were similar to those of results of the prebiotic used in our study.[10] Solis *et al.*, have reported an increase in the height of duodenal villi in broilers fed with diet contained prebiotic that matched with our result.[14] Baurhoo *et al.*, showed that height of jejunal crypts decreased using antibiotic growth promoter compared to control and prebiotic groups. In the antibiotic growth promoter and prebiotic groups population of useful bacteria was lower and more than other groups, respectively.[13] In our study, there was no significant effect on jejunum, however, in the antibiotic growth promoter group height of jejunal crypts were yltnacifingisni lower than those of the prebiotic and control groups ($P > 0.05$). Gunal *et al.*, showed that addition of probiotic to diet of broilers increased height of villi in jejunum and ileum compared to the control group, however, using antibiotic and organic acid did not end up significant difference. The width and depth of crypts also were not affected in any groups.[20] In their study no significant effects of antibiotic in jejunum and ileum on height and width of the villi and depth of crypts are in agreement with our results.

Although beneficial effects of the most growth promoters are revealed particularly when flocks encounter stress conditions, [12] in the present study regarding effects of the growth promoters on growth performance and intestinal histomorphometry, it might be concluded that prebiotic could be considered as a suitable alternative for antibiotic as a growth promoter.

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References

1. Dibner JJ, Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 2005; 84:634-643.
2. Gustafson RH, Bowen RE. Antibiotic use in animal agriculture. J Appl Microbiol 1997; 83:531-541.
3. Caprioli A, Busani L, Martel JL, et al. Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int J Antimicrob Agents 2000; 14:295-304.
4. Pelicano ERL, Souza PA, Souza HBA, et al. Productive traits of broiler chickens fed diets containing different growth promoters. Braz J Poult Sci 2004; 6:177-182.
5. Van den Bogaard AE. Antimicrobial resistance-Relation to human and animal exposure to antibiotics. J Antimicrob Chemother 1997; 40:453-454.
6. Van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents 2000; 14:327-335.
7. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Clin Nutr 1995; 125:1401-1412.
8. Fuller R. probiotics in man and animals: A review. J Appl Bacteriol 1989; 66:365-387.
9. Iji PA, Saki A, Trivey DR. Intestinal structure and function of broiler chickens on diets supplemented with a mannanoligosaccharide. J Sci Food Agric 2001; 81:1186-1192.
10. Marković R, Šefer D, Krstić M, et al. Effect of different growth promoters on broiler performance and gut morphology. Arch Med Vet 2009; 41:163-169.
11. Sandikci M, Eren U, Onol AG, et al. The effect of heat stress and the use of *Saccharomyces cerevisiae* or (and) bacitracun zinc against heat stress on the intestinal mucosa in quails. Revue Med Vet 2004; 155:552-556.
12. Hooge DM. Meta-analysis of broiler chicken pen trials evaluating dietary mannanoligosaccharides, 1993-2003. Int J Poult Sci 2004; 3:163-174.
13. Baurhoo B, Phillip L, Ruiz-Feria CA. Effect of purified lignin and mannanoligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci 2007; 86:1070-1078.
14. Solis de los Santos F, Farnell MB, Tellez G, et al. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. Poult Sci 2005; 84:1092-1100.
15. Santin E, Maiorka A, Macari M, et al. Performance and intestinal mucosa

development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. J Appl Poult Res 2001; 10:236-244.
16. Oliveira MC, Rodrigues EA, Marques RH, et al. Performance and morphology of intestinal mucosa of broilers fed mannan-oligosaccharides and enzymes. Arq Bras Med Vet Zootec 2008; 60:442-448.
17. Mohamed MA, Hassan HMA, El-Barkouky EMA. Effect of mannanoligosaccharide on performance and carcass characteristics of broiler chicks. J Agri Soc Sci 2008; 4:13-17.
18. Xu ZR, Hu CH, Xia MS, et al. Effects of dietary fructooligosaccharide on digestive enzyme activities. Intestinal microflora and morphology of male broilers. J Anim Sci 2003; 82:1030-1036.
19. Spring P, Wenk C, Dawson KA, et al. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the caeca of *Salmonella*-challenged broiler chicks. Poult Sci 2000; 79:205-211.
20. Gunal M, Yayli G, Kaya O, et al. The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int J Poult Sci 2006; 5:149-155.