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Influences of different growth promoters on intestinal morphology of broiler chickens

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ABSTRACT

The aim of this study was to determine the influences of different growth promoters (prebiotic, probiotic, synbiotic and acidifier) for a period of 42 days on the growth and intestinal morphology of broiler chickens. A total of one hundred and sixty 1-d-old Ross 308 broiler chickens were randomly allocated into five dietary groups with four replicates. A corn-soybean-based diet was used as a basal diet. The dietary groups were 1- Control (basal diet), 2- basal diet plus prebiotic (1kg of ActiveMOS/ton) 3- basal diet plus probiotic (150,100,50gr of Protexin/ton of the starter, grower and final diets respectively) 4- basal diet plus synbiotic (1kg of Amax4x/ton) 5- basal diet plus acidifier (2 liter of Globacid/ton). Performance of broilers improved in experimental groups compared with control group at the end of the experiment. So that groups supplemented with prebiotic, synbiotic and acidifier had a significant (P<0.05) effect on broiler performance. whereas, probiotic group had not significant (P>0.05) effect on broiler performance. There was no difference (P>0.05) in the weight of internal organs between groups except spleen that increased significantly (P<0.05) in probiotic group compared with control group. Also, dietary groups influenced the histomorphological measurements of small intestine. Villus height, Crypt depth and Villus height to Crypt depth ratio were differed significantly (P<0.05) between experimental groups. So that synbiotic and acidifier groups had more effects on histomorphological parameters than other groups.

Keywords: growth promoters, small intestine, Broiler chicken

INTRODUCTION

Over the last decade, the importance of gastrointestinal tract health in broiler chicken has been increasingly recognized due to its contribution to their overall health and performance [1, 2]. Antibiotics as growth promoters in food animal production have been used since 1946 throughout the world [3]. They are used in poultry production to improve performance, for therapeutic and prophylactic to stabilize [4], and are thought to stabilize the intestinal microbial flora and to prevent some specific intestinal pathogens [5]. In recent years, concerns about antimicrobial resistance have grown, but the main concerns have been focused specifically on resistance within the food supply [6, 7]. Based on these statements, using alternative growth promoters such as prebiotic, probiotic, synbiotic and acidifier become important every day.

Probiotics are "live microorganisms which, when administered in adequate amount, confer a health benefit on the host" [8]. The use of living microorganisms as probiotics is recommended as an alternative to antibiotics as prophylactic, therapeutic and growth-promoting agents in livestock production [9, 10, 11, 12]. A prebiotic was defined as nondigestible food ingredients that beneficially affect the host, selectively stimulating the growth or activity, or both, of one or a limited number of bacteria in the colon [13]. Several studies reported that prebiotics have beneficial effects on growth performance [14, 15, 16, 17]. Synbiotics are combination of prebiotics and probiotics which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract [18]. The acidifiers can modify the PH of both the feed and the animal`s digestive tract and can disrupt the normal cell function and protein synthesis of various gut microorganisms [19]. Reduction in gastric pH that occurs following organic acid feeding may increase pepsin activity [20]. Moreover, peptides arising from pepsin proteolysis and triggers the release of hormones, including gastrin and cholecystokinin that regulate the digestion and absorption of protein [21]. Additionally, it has been suggested that lowering the pH by organic acids improves nutrient absorption [22]. Some studies demonstrated the beneficial effects of acidifiers on improvement of growth performance [23, 24, 25, 26, 27]. The objectives of the present study were to compare the influences of prebiotics, probiotics, synbiotics and acidifiers as growth promoters on the growth and small intestinal morphology in the broiler chickens.

MATERIALS AND METHODS

Birds, experimental design and husbandry

We used one hundred and sixty one-day-old broiler chickens (Ross 308) for a period of 42 days. The chickens were randomly allocated into one of five groups. Each group consisted of four replicates with 8 chickens in each. The birds were housed in separate floor pens (1.2 × 0.9) with a wood shaving floor and had free access to feed and water. During the 42 days of experimental period, environmental factors such as lightning, temperature, humidity, ventilation and etc maintain on optimal levels recommended for Ross 308 broiler chickens. A corn-soybean-based diet was formulated for chickens. Experimental groups were: 1- Control group: basal diet 2- Prebiotic group: basal diet plus prebiotic (Active MOS, Brazil) 1 kg/ton 3- probiotic group: basal diet plus probiotic (Protexin, England) 150gr/ton of the starter diets, 100gr/ton of the grower diets and 50gr/ton of the final diets 4- Synbiotic group: basal diet plus synbiotic (Amax4x, USA) 1 kg/ton 5- Acidifier group: basal diet plus acidifier (Globacid DW, France) 2 liter/ton.

Histomorphological and Performance measurements

All birds were weighed individually at 1, 14, 28 and 42 days of age. The weight of chickens in the first day (after arriving to the experimental farm) was known as initial weight and also the weight of chickens in 42 days of age (the end of experimental period) was known as final weight. Feed intake, feed conservation ratio and daily weight gain was calculated for the starter, grower and finisher phase of the experiment. At the end of experiment, 8 birds per group were randomly selected and euthanized by cervical dislocation. Then, gastrointestinal tract was removed and internal organs including proventriculus, gizzard, liver, spleen and bursa of fabricius were separated and weighed individually. Also, duodenum part of small intestine, from the gizzard outlet to the end of pancreatic loop separated. After that, two centimeter long segments were taken from the central part of the duodenum and fixed in 10% buffered formalin. These samples were sent to "Danesh Pathobiology Center 2" laboratory for preparing histological slides and staining by haematoxylin and eosin method. Then slides were examined under light microscope (Nikon, Japan) for measuring villus height, crypt depth and villus height to crypt depth ratio.

Statistics

All data were subjected to one-way ANOVA using the Statistical Package for Social Science (SPSS for Windows Version 15) to determine if variables differed between groups. Mean values of dietary groups were compared by Duncan's multiple range test. Probability values of less than 0.05 ($P<0.05$) were considered significant.

RESULTS

The influences of dietary inclusion of prebiotic, probiotic, synbiotic and acidifier on body weight, daily weight gain, feed intake and feed conversion ratio are presented in Table 1. There was no significant ($P>0.05$) difference in body weight of broilers between experimental groups on day 14. However, the body weight of broilers supplemented with synbiotic was significantly ($P<0.05$) higher than broilers in control group on day 28. At the end of the experiment (day 42), broilers supplemented with prebiotic, synbiotic and acidifier had higher body weight in compare of control group (*P<0.05*). However, the difference in body weight of broilers between probiotic and control groups was not significant (*P>0.05*). Between days 1-14, there was no significant (*P>0.05*) difference in daily weight gain of broilers between experimental groups. But, daily weight gain of broilers on days 15-28, increased significantly (*P<0.05*) in experimental groups compare to the control group. Also, between days 29-42, daily weight gain of broilers in experimental groups was significantly (*P<0.05*) higher than control group. During the whole period of experiment (1-42), daily weight gain of broilers in prebiotic, synbiotic and acidifier groups were significantly (*P<0.05*) higher than control group. However, there was no significant difference (*P>0.05*) between probiotic and control groups. Feed intake of broilers did not differ significantly (*P>0.05*) between experimental groups on days 1-14. Whereas, between days 15-28, feed intake of broilers in prebiotic, probiotic and synbiotic groups was significantly (*P<0.05*) higher than control group. Also, on days 29-42, feed intake increased significantly (*P<0.05*) in prebiotic group compare to the probiotic and synbiotic groups. However, at the end of experimental period, there was not any significant (*P>0.05*) difference in feed intake of broilers between groups. Feed conversion ratio (FCR) did not show any significant (*P>0.05*) difference between groups on days 1-14. However, between days 15-28, there was a significant (*P<0.05*) decrease in feed conversion ratio of broiler chickens in synbiotic and acidifier groups compared with the control group. Between days 29-42, feed conversion ratio in Synbiotic and Acidifier groups were significantly (*P<0.05*) lower than control group. At the whole experimental period (1-42), feed conversion ratio in Synbiotic and Acidifier groups were significantly (*P<0.05*) lower than control group However, there was no significant differences in prebiotic and probiotic groups compared with each other, comparing the control group and also compared to synbiotic and acidifier groups (*P>0.05*).

Table 1: Influences of different growth promoters on body weight, daily weight gain, feed intake and feed conversion ratio

	Experimental groups					Statistics	
	Control	Prebiotic	Probiotic	Synbiotic	Acidifier	SEM	P Value
Body weight							
d 1 (g)	42.70	43.10	42.45	41.95	42.90	0.56	0.981
d 14 (g)	324.07	324.56	323.3	328.77	330.51	3.12	0.949
d 28 (g)	1004.92^{a}	1041.91^{ab}	1029.73^{ab}	1059.04^{b}	1051.35^{ab}	7.50	0.163
d 42 (g)	2011.26^{a}	2097.91^{bc}	2075.57^{ab}	2153.78^{c}	2128.35^{bc}	14.25	0.004
Daily Weight Gain							
d 1-14 (g)	20.09	20.10	20.06	20.48	20.54	0.12	0.660
d 15-28 (g)	48.63^{a}	51.02^{b}	50.45^{b}	52.16^{b}	51.48^{b}	0.35	0.007
d 29-42 (g)	71.88^{a}	75.42^{bc}	74.70^{b}	78.19^{c}	76.92^{bc}	0.61	0.002
d 1-42 (g)	46.87^{a}	48.92^{b}	48.40^{ab}	50.28^{b}	49.65^{b}	0.36	0.016
Feed Intake							
d 1-14 (g/bird)	410.02	409.22	408.80	413.01	412.53	1.41	0.862
d 15-28 (g/bird)	1182.29^{a}	1224.51^{c}	1206.78^{bc}	1210.72^{bc}	1194.95^{ab}	4.20	0.005
d 29-42 (g/bird)	2406.15^{ab}	2441.20^{b}	2381.45^{a}	2369.03^{a}	2401.44^{ab}	8.16	0.034
d 1-42 (g/bird)	3998.46	4074.98	3997.08	3992.80	4008.96	14.28	0.349
Feed Conversion Ratio							
d 1-14	1.265	1.261	1.264	1.256	1.248	0.01	0.988
d 15-28	1.736^{b}	1.707^{ab}	1.708^{ab}	1.658^{a}	1.657^{a}	0.01	0.025
d 29-42	2.391^{c}	2.311^{bc}	2.289^{abc}	2.164^{a}	2.229^{ab}	0.02	0.014
d 1-42	1.988^{b}	1.942^{ab}	1.925^{ab}	1.853^{a}	1.883^{a}	0.01	0.041

$^{a-c}$*Means in the same row with different superscripts differ significantly (P < 0.05)*

The influences of dietary inclusion of prebiotic, probiotic, synbiotic and acidifier on weight of internal organs are presented in Table 2. At the end of experiments, the weight of Proventriculus, Gizzard, Liver and Bursa did not show any significant (*P>0.05*) difference between experimental groups. However, the weight of Spleen increased significantly (*P<0.05*) in probiotic group compared with control group, although there was no significant (*P>0.05*) difference in weight of spleen in other groups.

Table 2: Influences of different growth promoters on weight of internal organs at the end of experimental period

	Experimental groups					Statistics	
Organ	Control	Prebiotic	Probiotic	Synbiotic	Acidifier	SEM	P Value
Proventriculus (g)	8.42	8.51	9.05	8.40	8.22	0.16	0.640
Gizzard (g)	43.12	46.15	43.07	44.02	42.57	1.19	0.913
Liver (g)	64.77	66.65	61.72	62.15	63.92	1.36	0.823
Spleen (g)	1.87^{a}	1.98^{ab}	2.11^{b}	2.06^{ab}	1.96^{ab}	0.03	0.104
Bursa (g)	2.28	2.14	2.36	2.25	2.09	0.04	0.222

$^{a-b}$*Means in the same row with different superscripts differ significantly (P < 0.05)*

The influences of dietary inclusion of prebiotic, probiotic, synbiotic and acidifier on duodenum morphology are presented in Table 3. There was a significant (*P<0.05*) difference in Villus height between groups. So that broilers supplemented with acidifier had the highest villus and also villus height in synbiotic and acidifier groups had a significant (*P<0.05*) increase compared with control and probiotic groups. Also, the difference of crypt depth between experimental groups were significant (*P<0.05*). Synbiotic and acidifier groups had a significant decrease in crypt depth compare to the control and probiotic groups. Furthermore, Villus height to Crypt depth ratio showed a significant (*P<0.05*) difference between experimental groups. So that, Villus height to Crypt depth ratio in acidifier and synbiotic groups was more than control and probiotic groups (*P<0.05*). And also the difference between prebiotic and probiotic groups was significant (*P<0.05*).

Table 3: Influences of different growth promoters on duodenum histomorphometry

	Experimental groups					**Statistics**	
	Control	Prebiotic	Probiotic	Synbiotic	Acidifier	SEM	P Value
Villus height (µm)	1636.52^{a}	1693.62^{ab}	1610.90^{a}	1732.60^{b}	1760.22^{b}	16.97	0.008
Crypt depth (µm)	186.3^{b}	182.6^{ab}	185.04^{b}	177.5^{a}	179.0^{a}	1.12	0.028
Villus height:Crypt depth	8.78^{ab}	9.27^{bc}	8.68^{a}	9.76^{c}	9.83^{c}	0.13	0.001

$^{a-c}$*Means in the same row with different superscripts differ significantly (P < 0.05)*

DISCUSSION

Following the supplementation of prebiotic, synbiotic and acidifier to basal diets, body weight increased significantly compared with control group. However broilers supplemented with probiotic did not show any significant difference in compare of control group. These results are in agreement with earlier studies [3, 18, 25, 26, 27]. Zakeri and Kashefi (2011) found that dietary supplementation of mannanoligosaccharide increased body weight of broilers in compare of control group. Ortiz et al. (2009) did not observe any effect by dietary inclusion of inulin as a prebiotic on body weight in broiler chickens, whereas EL-Banna et al. (2010) found that dietary inclusion of two different prebiotics increased body weight significantly at the end of the experiment. It has been reported that dietary inclusion of synbiotic had a beneficial effect on body weight of broilers [18, 29, 30]. Chowdhury et al. (2009) found that citric acid supplementation as an acidifier caused a significant increase on body weight in broiler chickens, whereas Bonos et al. (2012) observed no effect on body weight of Japanese quail by addition of acidifiers to diets. In agreement of our findings, Abdel-Fattah et al. (2008) found that the addition of dietary citric acid, acetic acid, or lactic acid improved body weight of broiler chickens compared with control group. Similar results were found by other researchers [25, 26]. Awad et al. (2009) reported that addition of probiotic to broilers diet did not show any significant effect on body weight compared with control group. In contrast, Mountzouris et al (2010) observed that diets containing 10^{8} cfu probiotic/kg increased body weight of broilers significantly in compare of control group. In agreement with our findings, it's reported that dietary supplementation of probiotic did not affect body weight of broilers [29, 32, 33].

Also, daily weight gain of broilers in prebiotic, synbiotic and acidifier groups was significantly more than control group, whereas probiotic group did not differ significantly compare to the control group. In agreement with our findings, Awad et al. (2009) found that dietary inclusion of synbiotic increased daily weight gain of broilers significantly, whereas; addition of probiotic had no significant effect. In contrast, Jung et al (2008) found that addition of galacto-oligosaccharides and Bifidobacterium lactis had no significant effect on daily weight gain of broiler chickens. Similar findings were reported by other researchers [29, 30]. Chowdhury et al. (2009) reported that addition of citric acid to broilers diet increased daily weight gain significantly compare to the control group.

Feed intake of broilers did not show any significant difference between experimental groups at the experimental period. Samli et al (2007) found that feed intake of broilers did not differ significantly by dietary inclusion of probiotics. Similar results were found by Jung et al. (2008) who found that addition of prebiotic and probiotic did not have any significant effect on feed intake of broiler chickens. Nezhad et al. (2007) found that the addition of citric acid did not affect feed intake in broilers supplemented with citric acid and similar results were found by Chowdhury et al. (2009). However, this observation was not found by the findings of Moghadam et al. (2006), who reported that the effects of citric acid on feed intake of broilers were significant. Also, Salianeh et al. (2011) reported that dietary inclusion of prebiotic significantly decreased feed intake in broiler chickens compared with control group, whereas, addition of probiotic did not have the same effect as prebiotic

In the present study, dietary inclusion of synbiotic and acidifier significantly decreased FCR in compare of control group during the whole experimental period, whereas addition of prebiotic and probiotic had not a significant effect on FCR. In agreement with our findings, Chowdhury et al. (2009) found that dietary inclusion of citric acid significantly decreased feed conversion ratio in broiler chickens compared with control group. Similar results were found by other researchers [20, 23, 27]. It has been reported that addition of synbiotic to broilers diet significantly decrease feed conversion ratio in broiler chickens [30]. Also, Awad et al. (2009) reported that dietary supplementation of synbiotic significantly decreased feed conversion ratio, while addition of probiotic had no significant effect. In another study, Jung et al. (2008) reported that dietary inclusion of prebiotic and probiotic had no significant effect on feed conversion ratio in broiler chickens and similar results were found by Ortiz et al. (2009). In contrast, Talebi et al. (2008) reported that addition of probiotic to broiler chicken diets decreased feed conversion ratio significantly. Salianeh et al. (2011) observed that addition of prebiotic decreased feed conversion ration significantly, however, probiotic supplementation did not affect feed conversion ratio in broiler chickens.

The present study did not show any significant effect by addition of prebiotic, probiotic, synbiotic and acidifier on the weight of Proventriculus, Gizzard, Liver and Bursa between groups. In agreement with our findings, it's reported that weight of Gizzard did not affect significantly by addition of prebiotic [36], probiotic [18, 29, 36, 37] and synbiotic [18]. Also, it has been reported that dietary inclusion of prebiotic, probiotic and synbiotic had no significant effect on Liver weight [36, 38, 39]. In agreement with our findings, it's reported that weight of Bursa did not show any significant difference by dietary supplementation of prebiotic [36], probiotic [18,] and synbiotic [18]. In our study, the weight of Spleen increased significantly ($P<0.05$) in probiotic group compare to the control group. However, Awad et al. (2009) reported that addition of probiotic and synbiotic to broilers diet did not show any significant effect on spleen weight compared with control group, whereas the weight of spleen was significantly different between probiotic and synbiotic group. It has been reported that addition of probiotics to broilers diet did not have any significant difference on spleen weight [36, 38, 39, 40].

Small intestinal histomorphology including villus height, crypt depth and their ratio are one of important indications of gut health in broiler chickens. Increased villus height and villus height to crypt ratio are directly correlated with an increased epithelial turnover [41], and longer villi are associated with activated cell mitosis [42]. Whereas, shortening of villus and deeper crypts lead to poor nutrient absorbtion, increased secretion in gastrointestinal tract and reduced performance [43]. The present study showed a significant effect on the intestinal morphology concerning the villus height, crypt depth and villus height to crypt depth ratio, as well as positive effects on growth performance. In agreement with our findings, Sen et al. (2012) reported that supplementations of Bacillus subtilis LS 1-2 did not affect villus height, crypt depth and villus height to crypt ratio in duodenum significantly compared with control group. However, Awad et al. (2010) fount that addition of Lactobacillus to broiler diet, increased villus height and villus height and crypt depth ratio in duodenum. Awad et al. (2008) found that dietary inclusion of synbiotic did not have any significant effect on villus height, crypt depth and villus height to crypt ratio in duodenum.

CONCLUSION

In Conclusion, addition of prebiotics, probiotics, synbiotics and acidifiers to basal diets improved performance and intestinal morphology of broiler chickens. Among these Synbiotic had the greatest effect on growth performance as well as intestinal morphology in comparison with other experimental groups. Furthermore, experimental groups had not any significant effect on the weight of internal organs weights, except spleen that was greater for the probiotic group compared with control group.

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