African Journal of Agricultural Research Vol. 6(12), pp. 2794-2799, 18 June, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.1031
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Comparative study of the effect of alternative and antibiotic feed additives on the performance and intestinal histomorphometrical parameters of broiler chickens

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Accepted 29 April, 2011

A four replicate study was conducted to compare the effects of alternative feed additives (prebiotic, ECOCELL® and butyric acid, Baby c4®) to an antibiotic growth promoter (bacitracin) on the performance and histomorphometrical parameters of the small intestine of 192, 1-day-old Ross broiler chicks. The used chicks were randomly assigned to one out of four dietary treatments for 6 weeks (each treatment contained four replicates, 12 birds each). Replicate dietary treatments were as follow: 1- control (basal diet), 2-basal diet + prebiotic, 3-basal diet + butyric acid and 4-basal diet + antibiotic growth promoter. The results showed that body weight, weight gain and feed intake were not affected by dietary treatments. Prebiotic had significantly improved ($p<0.05$) feed conversion ratio (FCR) in 22-42 days and total period compared with the control nontreated and butyric acid fed broilers. The addition of either prebiotic or antibiotic significantly increased the villus height in duodenum ($P<0.001$), while prebiotic increased duodenum and ileum villus width compared with other treatments. The duodenal crypt depth was significantly increased by antibiotic or organic acid compared with prebiotic and control ($P<0.01$). In conclusion, prebiotic displayed a greater efficacy as growth promoter for broilers, but butyric acid glycerides do not promote broiler's growth.

Key words: Prebiotic, organic acid, antibiotic, performance, histomorphometry, small Intestine, broiler chicken.

INTRODUCTION

The health of alimentary tract is an important factor in performance of poultry production due to its crucial role in absorption of nutrients and a barrier against everlasting attack of pathogenic microbes. Intensive and industrial farming has provided a good condition for residing of harmful microflora in alimentary tract because of gradual colonization of natural microflora in intestine of newly hatched chicks (Fuller, 1989). Therefore, antibiotics as growth stimulants in poultry production have been commonly used. However, application of antibiotics has been declining because of their harmful effects, residues in carcass, rising of resistance to microorganisms and causing hypersensitivity (Smith et al., 2003). On the other hand, due to breakthrough in genetics and nutritional sciences, improvement in vaccines, bio-security and vaccination programs application of antibiotics as prophylactic and growth promoters have been decreased (Baurhoo et al., 2007). Moreover, in some countries antibiotic application is prohibited (Cervantes, 2006; Michard, 2008). Nowadays, efforts for finding proper alternatives, such as probiotic, prebiotic and organic acid to replace antibiotics have been extensively increased (Hertrampf, 2001; Langhout, 2000).

Prebiotic is a suitable alternative because it is an indigestible foodstuff that induces growth and activity of one or more types of bacterial flora of alimentary tract (Baurhoo et al., 2007; Gibson and Roberfroid, 1995). Therefore, it can improve health of the host (Gibson and Roberfroid, 1995) by the following: increasing of population of useful bacteria such as *Lactobacilli* and *Bifidobacteria*, competing pathogens for linking to intestinal

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cells, increasing production of volatile fatty acids, decreasing intestinal pH, producing antimicrobial compounds, improving immune system, providing digestive enzymes and improving morphologic indices (Fuller, 1989; Marković et al., 2009; Oliveira et al., 2008;Sandikci et al., 2004).

On the other hand, organic acids exert their stimulating influences on host growth through decreasing of pH in feedstuff and alimentary tract, decreasing production of bacterial poisonous compounds, decreasing aggregation of intestinal pathogens and stimulating growth of useful intestinal flora (Antongiovanni et al., 2007; Hooge, 2004). Butyric acid is a short-chain fatty acid that is proposed to be a main source of energy for intestinal cells and is reported to be essential for the correct development of the gut associated lymphoid tissue (GALT) (Friedman and Bar-Shira, 2005). Since free butyric acid is characterized by a strong unpleasant, penetrating smell, it is almost impossible to cope with it in the feed manufactory and results in poor intakes of the treated feed. In recent years, using glycerides of butyric acid has solved the problem (Antongiovanni et al., 2007).

Others reported that addition of prebiotic to the ration has improved broiler's performance (Hooge, 2004; Marković et al., 2009). On the contrary, there are some reports stated that prebiotics did not influence poultry performance (Baurhoo et al., 2007; Oliveira et al., 2008). Also, organic acids can be used to control intestinal microbial growth and increase the performance (Antongiovanni et al., 2007; Jin et al., 1998). Application of organic acids did not only influence the performance of broilers but in some cases had harmful effects (Cave, 1984; Gunal et al., 2006; Patten and Waldroup, 1988).

In the present study effects of antibiotic growth promoter, prebiotic and organic acid (butyric acid glycerides) on growth performance and histomorphometery of small intestine in broilers were compared.

MATERIALS AND METHODS

Experimental chicks

One hundred and ninety two (192) day-old Ross 308 chicks were randomly divided into four groups (48 chicks each). Each group was further divided into 4 replicates (12 chicks each) for 42 days.

Feeding

All chick groups were fed on the same basal starter, grower and finisher rations based on corn and soybean according to NRC (1994).

Feed additives

The prebiotic (ECOCELL®, Impextraco) used in this study was derived from cell wall of *Saccharomyces cerevisiae* and composed of mannanoligosaccharide and β-glucan (contains > 40% of mannose and > 50% of glucose). It was mixed with basal ration in the rate of 0.1% according to producers' instructions. The used organic acid was butyric acid (Baby c4®). According to the producer (SILO), it was added to ration in rat of 0.3% in the first week and 0.2% up to 25 days. Bacitracin was used as an antibiotic to promote the growth. For starter, 50 g/ton; and for grower and finisher diets, 25 g/ton was added to the ration.

Performance parameters

Broiler performance including average body weight, body weight gain, feed intake and feed conversion ratio (FCR) were evaluated (Oliveira et al., 2008).

Intestinal histomorphometric parameters and morphometric analyses

Histomorphometeic parameters of collected chicken intestines were determined (Marković et al., 2009). 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 were made at 6-7 µm and they were stained with hematoxylin-eosin (Luna, 1968). Using digital photography and light microscope the photos were taken and morphometric analyses were performed by means of an image analysis program (Image J software). In each of the five sections taken from the tissues, the villus height and width and crypt depth were determined by examining randomly 6 villi and 6 crypts. Later, the average of 30 values obtained for each chick was taken (Rezaian et al., 2007).

Experimental design

Experimental chick groups were given the growth promoter as follows: Gr.1- was fed on basal diet as control non-treated, Gr. 2- basal diet + prebiotic, Gr. 3- basal diet + organic acid and Gr. 4- basal diet + antibiotic.

All chicks were weighed upon arrival and afterward were weighed weekly. Feed intake and feed conversion ratio were determined for each pen. At the end of the experiment chicks were randomly taken to determine histomorphometric parameters and morphometric analyses. The obtained results are shown in Tables 1 to 3.

Statistical analysis

The calculated data were analyzed using the statistical software package SAS (1996). The results were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. Statistical significance was established at $P<0.05$ where the non significant data were further estimated as $P<0.01$ and $P<0.001$.

RESULTS

Performance

The effects of dietary prebiotic, organic acid and antibiotic as growth promoter on broiler performance (Tables 1 and

Table 1. Average live body weights and body weight gains of treated as compared with control group.

Group number	Treatment	Live body weight (g) at day of age			Body weight gain (g) at day of age		
		1	21	42	0- 21	22- 42	0- 42
1	Control	48.12	590.31	2267.1	542.19	1676.8	2219.0
2	Antibiotic	47.39	568.83	2274.3	521.44	1705.5	2226.9
3	Prebiotic	47.91	581.35	2352.1	533.44	1770.7	2304.2
4	Organic acid	48.43	564.88	2091.8	516.44	1526.9	2043.3
SEM		0.373	18.234	93.227	18.130	86.833	93.091
P-value		NS	NS	NS	NS	NS	NS

SEM: Pooled standard error; means within a column lacking a common superscript differ (P<0.05). NS: Not significant (P> 0.05).

Table 2. Average feed intakes and feed conversion ratios (FCR) of treated and control group.

Group number	Treatment	Feed intake (g) at day of age			FCR (g:g) at day of age		
		0-21	22-42	0-42	0-21	22-42	0-42
1	Control	837.15	3359.80	4196.90	1.55	2.02 ab	1.90 ab
2	Antibiotic	815.71	3062.70	3878.40	1.57	1.80 bc	1.75 bc
3	Prebiotic	846.90	3106.90	3953.80	1.59	1.76 c	1.72 c
4	Organicacid	850.10	3171.40	4021.50	1.65	2.08 a	1.97 a
SEM		16.218	102.000	104.995	0.046	0.074	0.056
P-value		NS	NS	NS	NS	*	*

SEM: Pooled standard error; means within a column lacking a common superscript differ (P<0.05). NS: Not significant (P> 0.05).

2) proved that there were no significant differences (P>0.05) among experimental groups in average weight and weight gain at 21 and 42 days of age. However, at the end of the study (42 days) prebiotic and antibiotic fed groups both average weight and weight gain were insignificantly higher than those of control group. On the other hand, group fed with organic acid indexes were lower than the control group. The feed intake did not show any significant difference among experimental groups. FCR from 1 to 21 days was not affected in all groups, while in 22-42 days and total period FCR showed a significant improvement at P<0.05 in prebiotic group compared to control and organic acid group, and in antibiotic group compared to organic acid group. Also, in these periods feed conversion ratio in organic acid group was insignificantly higher than the control group.

Histomorphometry

Villi height was only significant (P<0.001) in duodenum where usage of antibiotic or prebiotic resulted in the most increase in villi height compared to control and organic acid groups (Table 3). While, there was no significant difference between the other groups. Prebiotic increased (P<0.01) villi width of duodenum and ileum compared with other treatments and there was no significant difference among other groups. Duodenal Crypts depth was the only affected segment in experimental groups. Organic acid and antibiotic increased (P<0.01) crypts depth of duodenum compared to prebiotic and control groups. While, no significant difference could be seen between the results of other groups.

DISCUSSION

Nowadays, broiler poultry industry tends to use the alternative growth promoter to replace antibiotics to avoid its adverse effect on public health (Hertrampf 2001; Langhout, 2000). Although the available literature does not provide us with clear cut result, the previous results of growth promoter in diets of broiler were different. Therefore our study aims to explore this area, that is, demonstration of the effects of dietary addition of prebiotic, organic acid and antibiotic as growth promoter on broiler's performance.

In the present study, there were no significant differences (P>0.05) among experimental groups in average weight, weight gain and feed intake at 21 and 42 days of age (Tables 1 and 2). This result agree with those of Oliveira et al. (2008) who showed that addition of

Table 3. Major morphometrical and histomorphometrical traits of small intestine of treated and control examined birds at 42 days of age (µm).

Group number	Treatment	Villus						Crypt depth		
		Height			Width					
		D	J	I	D	J	I	D	J	I
1	Control	1557 [b]	1260	928	174 [b]	111	117 [b]	167 [b]	165	149
2	Antibiotic	1903 [a]	1295	863	173 [b]	110	128 [b]	202 [a]	152	146
3	Prebiotic	1800 [a]	1338	988	204 [a]	117	146 [a]	179 [b]	160	155
4	Organicacid	1545 [b]	1220	1004	165 [b]	117	127 [b]	209 [a]	164	150
SEM		60.628	51.014	39.689	7.257	4.293	4.813	8.066	6.418	6.843
P-valu		***	NS	NS	**	NS	**	**	NS	NS

D: Duodenum; j,jejunum, I, ileum. SEM: Pooled standard error; means within a column lacking a common superscript differ (P<0.05). NS: Not significant (P> 0.05) *: P<0.05; **: P<0.01; ***: P<0.001.

prebiotic to the broilers diet was ineffective. Gunal et al. (2006) also showed that addition of antibiotic growth promoter and organic acid to the diet of broilers did not have significant (P>0.05) effect on body weight and feed intake.

Both average weight and weight gain of prebiotic and antibiotic fed groups at the end of the study were insignificantly higher than those of control nontreated group. These findings can be supported by those of Markovic et al. (2009) and Mohamed et al. (2008), who demonstrated that prebiotic or antibiotic increased weight gain. Baurhoo et al. (2007) reported that while prebiotic or antibiotic was added to broiler's diet, the weight was significantly decreased compared with that of control group.

Based on our results, prebiotic had significant (p<0.05) effect on improvement of feed conversion ratio in 22-42 days and total period compared with the control group. While, antibiotic or butyric acid had no effect on the feed conversion ratio compared with the control group. These findings are in agreement with those of Markovic et al. (2009) and Taherpour et al. (2009), who reported that feed conversion ratio was improved when prebiotic was used. While, Baurhoo et al. (2007) and Mohamed et al. (2008), showed that prebiotic had no influence on the feed conversion ratio of broilers. Our results are also in agreement with those of other researchers such as Antongiovanni et al. (2007), Baurhoo et al. (2007), Gunal et al. (2006), Leeson et al. (2005) and Mohamed et al. (2008), who reported that feed conversion ratio remained unchanged when antibiotic or organic acid was used. However, contrary to our results, Markovic et al. (2009) showed that addition of antibiotic to broiler diet decreased feed conversion ratio. Taherpour et al. (2009) also reported that feed conversion ratio was improved when butyric acid was used.

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.

Studies have also shown different responses for intestinal histomorphometry by dietary additives.

In the present study, villi height increased when prebiotic or antibiotic was used. This result can be supported by those of Markovic et al. (2009), Oliveira et al. (2008) and Solis de los Santos et al. (2005), who demonstrated that addition of prebiotic or antibiotic to the broilers diet increased villi height. But contrary to our result, Gunal et al. (2005) and Pelicano et al. (2007) showed that addition of antibiotic growth promoter or prebiotic to the diet of broilers did not have significant effect on villi height. Baurhoo et al. (2007) also reported that the villus height in the jejunum was shorter in birds given antibiotic growth promoter.

No difference in villi height was observed between the control and organic acid group. This result agrees with those of Gunal et al. (2005) and Leeson et al. (2005), who demonstrated that addition of organic acid to the broilers diet was ineffective on villi height. Antongiovanni et al. (2007) reported that when organic acid was added to broiler's diet, the villi height was decreased compared with that of control group.

In the present study, prebiotic increased villi width of duodenum and ileum compared with other treatments and there was no significant difference (P>0.05) among other groups. These findings are in agreement with those of Gunal et al. (2005), who did not find any differences in villi width between the control group and groups fed antibiotic and organic acid. Marković et al. (2009) also showed that using prebiotic in the diet of broilers increased width of intestinal villi, but contrary to our results, antibiotic growth promoter increased villi width. In the current study, the duodenal crypt depth was increased by antibiotic or organic acid compared with prebiotic and control groups and there was no significant difference (P>0.05) between the other groups. In agreement with our results, Baurhoo et al. (2007) showed that prebiotic had no influence on the crypt depth of broilers' intestine. However, these results disagree with those of Leeson et al. (2005), who reported that addition

of antibiotic growth promoter to the broilers diet decreased depth of crypts and organic acid was ineffective. Marković et al. (2009) also showed that using prebiotic in the diet of broilers decreased depth of crypts.

Length and width of intestinal villi are of histomorphometrical indices and any increase in the values ends up increasing the absorptive surface of intestine (Marković et al., 2009; Pelicano et al., 2007). 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 (Marković et al., 2009; Oliveira et al., 2008). On the other hand, growth promoters such as prebiotics reduce pathogenic bacterial population through rise in useful intestinal microflora (*Lactobacilli* and *Bifidobacteria*), increasing production of fatty acids and reduction in intestinal pH. Therefore, healthy intestinal tissue and growth are achieved (Oliveira et al., 2008; Sandikci et al., 2004).

Regarding maintenance of healthy intestinal tissue, antibiotics may be less effective than prebiotics because antibiotics decrease population of both useful and pathogenic bacteria (Baurhoo et al., 2007). In the present study the prebiotic increased villi height in duodenum and villi width in both duodenum and ileum. However, 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 to cell replacement rate and increase in crypts depth indicates the need for enterocyte replacement and higher tissue turnover (Marković et al., 2009; Oliveira et al., 2008). 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 duodenal 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 tissue turnover (Marković et al., 2009). 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 (Marković et al., 2009). In the present study decreased depth of crypts in the prebiotic group is more considerable than that of antibiotic and organic acid groups. The effect of butyric acid to increase depth of crypts along with no effect on the dimensions of villi may be due to the fact that butyric acid could not selectively increase useful intestinal microflora compared to the prebiotic. The results of other works have demonstrated that organic acid did not have positive effect on performance of broilers and in some cases the effects were negative (Cave, 1984; Gunal et al., 2006; Patten and Waldroup, 1988). The results of growth performance in our study also showed that improved feed conversion ratio was observed only in prebiotic group and butyric acid did not affect performance.

Conclusion

Regarding our results, it can be concluded that prebiotic could be considered as a suitable alternative for antibiotic as a growth promoter through increasing absorption area and improving growth performance. However, butyric acid could not perform as growth promoter.

ACKNOWLEDGMENTS

This manuscript is obtained from DVSc thesis of Reza Sayrafi at Urmia University, Urmia, Iran. We would like to express thanks for support provided by the Urmia University, Urmia, Iran.

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