ISSN 1682-8356
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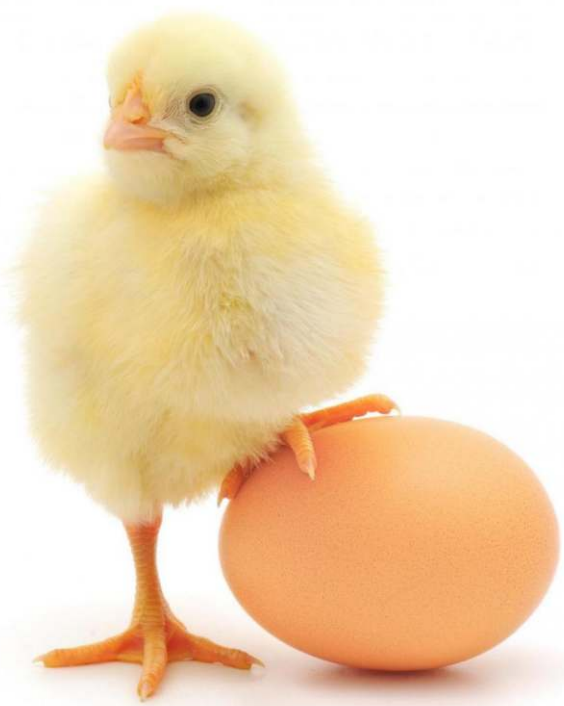

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 5 (2): 149-155, 2006
ISSN 1682-8356

The Effects of Antibiotic Growth Promoter, Probiotic or Organic Acid Supplementation on Performance, Intestinal Microflora and Tissue of Broilers

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Abstract: Effects of an antibiotic growth promoter (flavomycin), a probiotic mixture (protexin) or a mixture of organic acids including plant extract and mineral salts (genex) on performance, intestinal microbial flora and tissue morphology have been examined in 160 day-old Ross 308 broiler chicks. Commercial corn-soybean-based broiler starter and grower diets were formulated as basal diets for control treatment. Basal diets were supplemented with a probiotic (0.1% protexin), an antibiotic growth promoter (0.1% flavomycin), an organic acids mixture (0.2% genex) or a combination of a probiotic with an organic acids mixture (0.1% protexin+0.2% genex). In total, five dietary treatments were employed in the trial. Live weight gain, feed intake, feed conversion ratio and mortality were not affected by dietary treatments throughout the experiment. However, relative weight of the small intestine of antibiotic treatment had significantly less than that of the basal diet. Intestinal microbial flora and tissue were determined at 21th and 42th days. In both periods, antibiotic or organic acids mixture treatments significantly decreased total bacteria counts. In addition to that all treatments significantly decreased gram negative bacteria counts compared to the basal diet. Probiotic treatment significantly increased ileum and jejunum villus height, whereas antibiotic treatment significantly decreased muscularis thickness compared to the basal diet.

Key words: Antibiotic, probiotic, organic acid, broiler, performance, intestine

Introduction

The poultry industry has developed in several areas such as nutrition, genetics, management to maximizing the efficiency of growth performance and meat yield. However, nowadays, the poultry industry has focus more attention towards addressing public concern for environmental and food safety. Animals including poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli, Salmonella ssp., Clostridium perfringens* and *Campylobacter sputorum*. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al*., 2000). This leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies (Truscott and Al-Sheikhly, 1977; Miles *et al*., 1984; Waldroup *et al*., 1985). However, the antibiotic growth promoters have been under scrutiny for many years and have been removed from the market in many countries (Ratcliff, 2000). Their usefulness has seldom been contested, it is their relatedness with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that causes concerns (Philips, 1999). In light of that situation, the feed manufacturers and the animal growers have been actively looking to an efficacious alternative to antibiotic growth promoters. Probiotics and organic acids are the most promising alternative to antibiotics. Probiotics are viable microbial additives which assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes (Green and Sainsbury, 2001). It was reported that probiotics benefit the host animal by stimulating synthesis vitamins of B-groups, improving immunity stimulation, preventing harmful microorganisms, providing digestive enzymes and increasing of production of volatile fatty acids (Fuller, 1989; Rolfe, 2000; Coates and Fuller, 1977). However, acidification with various weak organic acids to diets such as formic, fumaric, propionic, lactic and sorbic have been reported to decrease colonization of pathogen and production of toxic metabolites, improve digestibility of protein and of Ca, P, Mg and Zn and serve as substrates in the intermediary metabolism (Kirchgessner and Roth, 1988). Several studies demonstrated that the supplementation of organic acid or probiotic to broiler diets increased the growth performance, reduced diseases and management problems (Vlademirova and Sourdjiyska, 1996; Jin *et al*., 1998; Vogt *et al*., 1981; Runho *et al*., 1997).

The intestinal epithelial layer constitutes a barrier that

Table 1: Ingredients and nutrient contents of the basal diets

Ingredients	Starter diet, 0-21 days	Grower diet, 22-42 days
Maize	460	530.40
Soyabean meal	354	304
Wheat	80	70
Fish meal	40	20
Vegetable oil	37.7	46.6
Limestone	11.5	12.5
Dicalciumphosphate	8.8	9
Vitamin premix*	2.5	2.5
Trace mineral premix**	1	1
Salt	3.5	3.5
DL-methionine	1	0.5
Total	1000	1000
Calculated values		
Crude protein, g/kg	230	200
Metabolizable energy, MJ/kg	12.97	13.39
Ca, g/kg	10	9
P (Total) , g/kg	6.9	6
Methionine, g/kg	5.1	4
Lisin, g/kg	12.3	10.3

* Vitamin premix provided the following per 2.5 kg of diet: vitamin A 15.000 IU, vitamin D_3 1.500 IU, vitamin E 20 mg, vitamin K_3 5 mg, vitamin B_1 3 mg, vitamin B_2 6 mg, niacin 25 mg, Ca-D- pantothenate 12 mg, vitamin B_6 5 mg, vitamin B_{12} 0.03 mg, folic acid 1 mg, D-biotin 0.05 mg, choline cloride 400 mg and carophyll-yellow 25 mg. **Trace mineral premix provided the following per kg of diet : Mn 80 mg, Fe 60 mg, Zn 60 mg, Cu 5 mg, Co 0.2 mg, I 1 mg and Se 0.15 mg.

protects the host against luminal pathogens (Deitch *et al.*, 1995). Reduced epithelial cell proliferation and mucosal atrophy of the intestine allow various pathogens in the intestinal lumen to invade. Feed additives such as antibiotic, probiotic or organic acids can help intestinal tissue, since supplementation of their to diets decrease pathogens.

The main objective of the study was to determine the performance, small intestinal microbial flora and tissue morphology of broiler chickens fed an antibiotic growth promoter, a probiotic or a mixture of organic acids.

Materials and Methods

A total of 160 day-old mixed sex broiler chicks (Ross 308) were used in the study conducted at the Suleyman Demirel University, Atabey-Isparta in Turkey. Chicks were individually weighed and randomly assigned into wire floor battery type experimental cages. Feed and water were provided *ad-libitum* and illumination was 24-hour florescent lighting. The trial was planned as randomized block design during a period of 42 days. Thus, each of dietary treatment had 8 replications in which 4 birds were assigned. Experimental cages were kept in environmentally controlled room. The basal diet was formulated to meet the nutrient needs suggested by the NRC (1994). The composition of the basal diet is shown in Table 1. For each period 1 to 21, 22 to 42 days, a large batch of the basal diet was mixed and aliqouts were used to mix test diets. Protexin, flavomycin, genex were used as a probiotic, an antibiotic growth promoter and an organic acids mixture, respectively. Thus, feed treatments were: 1) basal diet-no additives 2) basal diet + 0.1 % protexin 3) basal diet + 0.1 flavomycin 4) basal diet + 0.2 % genex 5) basal diet + 0.1 protexin + 0.2 genex. Protexin included *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Streptococcus thermophilus, Aspergillus orizea, Bifidobacterium bifidum, Enterococcus faecium, Candida pintolepesii* with a minimum of 6 x 10^7 cfu / g of the product. Genex consisted of a mixture of propionic and formic acid salts, plant extracts, vegetable essential oil and mineral salts. Body weight and food consumption were monitored weekly and feed conversion ratio was calculated as feed intake consumed per unit of gain. Mortality was recorded daily. Randomly chosen 3 males and 3 females from each treatment chicks were killed by cervical dislocation on days 21 and 42. Small intestine was opened immediately after killing and was weighed. The weight of small intestine was expressed as a percentage of live body weight. Ileum was defined as extending from Meckel's diverticulum to a point 4 cm to distal. Jejunum was defined as midway between the end of duodenum and Meckel's diverticulum. Digesta were obtained from ileum and caecum. Digesta samples were homogenized with 1 ml serum physiologic. Five µL aliqout was mixed with blood agar and eosin methylene blue (EMB) and incubated at 37°C for 24 h. After incubation total bacteria colonies were counted in blood agar whereas gram (-) bacteria colonies were counted in EMB agar. The microbial counts were determined as colony forming units (cfu) per gram of samples. For histopathologic and morphometric analysis, 2-cm tissue samples from the jejunum and ileum were obtained and fixed in 10 % buffered formalin (100 mL of 40 % formaldehyde, 4 g phosphate, 6.5 g dibasic sodium phosphate and 900 mL of distilled water) for 24-48 h. Tissues were dehydrated by transferring through a series of alcohols with increasing concentrations, placed into xylol and embedded in paraffin. A microtome was used to make 5 cuts that were 5 µm. The cuts were stained with hematoxylin-eosin. The values were measured using a light microscope. Measurements of villus height, width, crypt depth and muscularis thickness were determined at a magnification of 10X. A minimum 3 measurements per slide were made for each parameter and averaged into one value. The results were evaluated using SPSS® (1999) program. Statistical differences among treatments means were separated using the Duncan's Multiple Range Test with a % 5 probability (Duncan, 1955).

Results and Discussion

Some performance parameters of experimental diets are given in Table 2. Live weight gain, feed intake, feed

Table 2: The effects of a probiotic (protexin), an antibiotic growth promoter (flavomycin), an organic acids mixture (genex) or a probiotic + an organic acids mixture (protexin+genex) treatments to broiler diets on some performance parameters, (n=32)

Criteria	Basal diet	Probiotic	Antibiotic	Organic Acids mix	Organic acids mix + probiotic	SEM
Initial body weight,g	41.93	41.9	41.87	41.78	41.87	0.08
Body weight gain, g						
1 to 21 d	762.82	751.17	779.49	767.79	755.6	12.14
22 to 42 d	1570.43	1577.68	1613.38	1591.68	1600.51	14.03
1 to 42 d	2333.25	2328.84	2392.86	2359.47	2356.11	19.38
Feed intake, g						
1 to 21 d	1057.25	1030.13	1045.27	1036.29	1025.21	14.45
22 to 42 d	3149.55	3234.94	3254.32	3222.93	3287.28	43.17
1 to 42 d	4206.8	4265.07	4299.59	4259.22	4312.5	42.2
Feed conversion ratio, g:g						
1 to 21 d	1.39	1.38	1.33	1.36	1.34	0.02
22 to 42 d	2.01	2.05	2.01	2.03	2.05	0.02
1 to 42 d	1.8	1.83	1.8	1.81	1.83	0.01
Mortality, %	6.25	6.25	-	3.12	3.12	

conversion ratio and mortality were not affected (P>0.05) by dietary treatments throughout the experiment. These results are in an agreement with those of Izat *et al.* (1990); Cave (1984); Vale *et al.* (2004); Watkins and Kratzer (1984); Lee *et al.* (1993); Panda *et al.* (2000); Ozturk and Yildirim (2004); Engberg *et al.* (2000) and Ceylan *et al.* (2003) who reported that the supplementation of an organic acid, a probiotic or an antibiotic growth promoter did not have any effect on weight gain and feed conversion ratio. However, the results on the beneficial effects of these additives on weight gain and feed conversion ratio were reported by several researchers (Manickam *et al.*, 1994; Yeo and Kim 1997; Gunes *et al.*, 2001; Vogt *et al.*, 1981; Runho *et al.*, 1997; Henry *et al.*, 1987). There are some reports that high levels of organic acids have detrimental effects on performance of broilers. Cave (1984) reported that increasing level of propionic acid depressed feed intake and weight gain, increased mortality. Patten and Waldroup (1988) also found that the addition of 1.5% calcium formate in broiler diets reduced weight gain. In the present study, the observed lack of effects of a growth promoter effect may be associated with environmental conditions. Well-nourished healthy chicks do not positively respond to growth promoters when they are housed under clean conditions and at a moderate stoking density. Several researchers reported that when chicks were housed in a clean environment, growth promoters such as probiotic, organic acid or antibiotic were unaffective on performance (Miller, 1987; Lyons, 1987; Anderson *et al.*, 1999).

Ileal and caecal muscularis thickness were affected (P<0.05) by dietary treatments (Table 3). Antibiotic treatment decreased (P<0.05) muscularis thickness in jejunum and ileum at 21th or 42th days compared to the non-supplemental basal diet. These results are probably due to gram negative bacteria counts. During a pathogenic bacteria infection, lymphocytes accumulate to kill the pathogens and cause inflammation which in turn increases muscularis thickness. Antibiotics reduce microbial population numbers and their production of toxin and by products in the lumen. Likewise, a positive relationship between pathogen infection and caecal muscularis thickness was determined by Tellez *et al.* (1994). Wostmann *et al.* (1960) compared penicillin-fed birds to germ-free birds and found that conventional birds consuming the antibiotic treatment had reduced amounts of ileal lamina propia and reticulo-endothelial components almost similar to level seen in the germ-free birds. Visek (1978) suggested that intestinal enteric microflora may increase tissue turnover rate up to 40 %. In the present study, antibiotic treatment decreased (P<0.05) relative intestinal weight compared to the basal diet. The reduction of intestine weight observed by the antibiotic treatments may be associated with the reduction of muscularis thickness in jejunum and ileum at 21th or 42th days. The results on the intestine weight by antibiotic treatments are similar to that observed by Dafwang *et al.* (1985) and Stutz *et al.* (1983). Henry *et al.* (1987) also obtained that a 19 % decrease in intestinal weight in broiler chicks from dietary inclusion of virginiamycin.

In the present study, probiotic or organic acids mixture alone or in combination reduced numerically muscularis thickness in jejunum and ileum at 21th or 42th days and relative intestine weight compared to the basal diet. However, these reductions were not statically significant (P>0.05), which is parallel to the findings of Jin *et al.* (1998) having found that supplementing *Lactobacillus* to diets did not affect the weights of intestine. A similar observation was obtained by Fethiere and Miles (1987). They found that a mixture of *Lactobacillus* added to the diet did not change the weight of small intestine of broilers. On the other hand, Tortuero (1973) reported that a decrease in the weight of the caecum in chicks after the intake of a probiotic based *Lactobacillus acidophilus*.

Table 3: The effects of a probiotic mixture (protexin), an antibiotic growth promoter (flavomycin), an organic acids mixture (genex) or a probiotic + an organic acids mixture (protexin+genex) treatments to broiler diets on some intestinal parameters, (n=6)

Criteria	Basal diet	Probiotic	Antibiotic	Organic acids mix	Organic acids mix + probiotic	SEM
42 d						
Small intestine, g /kg body weight	3.24^{a}	3.14^{ab}	2.58^{b}	2.85^{ab}	2.97^{ab}	0.14
Jejunum, 21 d						
Villus height, µm	680.00^{a}	811.67^{b}	671.67^{a}	692.22^{a}	722.01^{ab}	23.15
Villus width, µm	95.56	108.89	04.44	100.42	102.78	4.82
Crypt depth, µm	114.44	125	106.11	111.67	112.01	5.57
Villus height/cypt depth, µm/µm	5.96	6.55	6.43	6.22	6.47	0.29
Muscularis thickness, µm	137.78^{a}	124.44^{ab}	110.56^{a}	121.11^{ab}	120.83^{ab}	5.74
Ileum, 21 d						
Villus height, µm	528.89^{a}	665.00^{b}	523.33^{a}	552.78^{a}	563.33^{ab}	26.18
Villus width, µm	90	98.83	87.22	95	96.11	4.98
Crypt depth, µm	103.33	110.56	101.67	100.56	109.44	5.15
Villus height/cypt depth, µm/µm	6.16	6.08	5.17	5.57	5.22	0.36
Muscularis thickness, µm	140.56^{a}	125.33^{ab}	115.56^{b}	120.561^{ab}	122.78^{ab}	5.33
Total bacteria, log_{10} cfu/g	8.03^{ab}	7.93^{a}	6.61^{c}	7.05^{cd}	7.33^{abd}	0.16
Gram negative bacteria,log_{10} cfu/g	7.28^{a}	6.71^{b}	6.11^{c}	6.33^{bc}	6.55^{bc}	0.12
Caecum, 21 d						
Total bacteria, log_{10} cfu/g	8.68^{a}	8.53^{a}	7.10^{b}	7.63^{bc}	8.05^{ac}	0.15
Gram negative bacteria,log_{10} cfu/g	8.10^{a}	7.45^{b}	6.81^{c}	6.98^{bc}	7.15^{bc}	0.13
Jejunum, 42 d						
Villus height, µm	435.56^{a}	546.11^{b}	431.67^{a}	452.78^{a}	492.78^{ab}	18.52
Villus width, µm	66.67	71.11	66.67	67.78	67.22	4.8
Crypt depth, µm	69.44	75.76	68.89	73.89	73.33	5.3
Villus height/crypt depth, µm/µm	6.38	7.34	6.35	6.49	6.95	0.6
Muscularis thickness, µm	91.11^{a}	82.11^{ab}	68.89^{b}	75.01^{ab}	81.15^{ab}	4.57
Ileum, 42 d						
Villus height, µm	309.32^{a}	456.24^{b}	293.89^{a}	329.44^{a}	375.89^{ab}	25.2
Villus width, µm	59.49	61.94	53.23	52.22	61.67	4.57
Crypt depth, µm	61.46	69.14	54.44	57.78	63.59	4.37
Villus height/crypt depth, µm/µm	5.4	6.62	5.47	5.79	6.07	0.48
Muscularis thickness, µm	100.56^{a}	89.88^{ab}	73.89^{b}	84.52^{ab}	88.66^{ab}	5.3
Total bacteria, log_{10} cfu/g	8.20^{a}	7.95^{a}	6.16^{b}	7.03^{c}	7.31^{ac}	0.18
Gram negative bacteria,log_{10} cfu/g	7.58^{a}	6.93^{b}	5.61^{c}	6.63^{b}	6.83^{b}	0.14
Caecum, 42 d						
Total bacteria, log_{10} cfu/g	8.46^{a}	8.13^{ac}	6.53^{b}	7.65^{c}	7.83^{ac}	0.16
Gram negative bacteria,log_{10} cfu/g	7.95^{a}	7.33^{b}	6.06^{c}	7.06^{b}	7.28^{b}	0.14

^{a,bcd}Row means with common superscripts do not differ (P<0.05) SEM: standard error of mean

Henrique *et al*. (1998) obtained that antibiotic, probiotic or organic acid includes fumaric acid supplementation to diets had no effect on ileum or jejunum weights of broilers.

The counts of total bacteria and gram negative bacteria were affected (P<0.05) by dietary treatments (Table 3). Antibiotic or organic acids mixture treatments decreased (P<0.05) ileal and caecal total and negative bacteria counts at 21th or 42nd days compared to the basal diet. These results show that antibiotics or organic acids reduce total and gram negative bacteria counts. The inhibitory effects of antibiotic or organic acid on microbial flora colonizations reported in previous researches. Smulikowska *et al*. (2005) obtained decreasing of *Clostridium* count in faeces with the antibiotic treatments. Engberg *et al*. (2000) found that supplementation with salinomycin and zinc bacitracin alone or in combination to diets resulted in significantly lower counts *C. perfringens* as well as *Lactobacillus salivarius*. Alp *et al*. (1999) recorded that the inclusion of an antibiotic and an organic acids mixture which contains lactic, fumaric, propionic, citric and formic acid separately or combined reduced *Enterobacteriaceae* count in the ileum of broilers. Thompson and Hinton (1997) reported that an organic acids mixture includes formic and propionic acid treatment decreased *Salmonella* and lactic acid-producing bacteria counts in hens's crops. In the present study, probiotic alone or a combination of probiotic with organic acid mixture treatments to diets decreased (P<0.05) on ileal and caecal negative bacteria counts at 21nd or 42nd days. These results concur with the results of Ceylan *et al*. (2003) who reported that a probiotic based *Enterococcus*, Cylactin, treatments to diets reduced aerobic and coliform bacteria counts. A similar observation was reported by Ghadban *et al*. (1998). They reported that spray application of probiotic by water reduced *Salmonella* and *E. coli* colonizations in caecum from 38.8% to 9.72%, from 51.4% to 22.2% respectively. However, Ozturk and Yildirim (2004) found that a probiotic based *Lactobacillus* treatments had no effect on ileal and caecal gram negative bacteria counts.

Jejunum and ileum crypt depth, the ratio of villus height to crypt depth and villus width at 21^{th} or 42^{th} days of age were not affected by treatments (P>0.05). However, probiotic treatment increased (P<0.05) jejunum and ileum villus height at 21^{th} or 42^{th} days of age compared to non-supplemented basal diet. These results confirm the fact that probiotic treatments to diets increase villus height. A similar result was reported by Samanya and Yamauchi (2002). They reported that villus height in duodenum and ileum significantly increased in 28-day old chicks fed *Bacillus subtilis*. Santin *et al.* (2001) recorded that fed *Saccharomyces cerevisiae* broilers were higher villus height than that of control group during the first 7^{th} day. These results are most probably due to enhanced short chain fatty acids formation induced by probiotics. It has been reported that under *in vitro* probiotics increase the levels of the short chain fatty acids whilst decreasing the production of ammonium (Sakata *et al.*, 1999). The short chain fatty acids which are by products of bacterial fermentation stimulate the proliferation of epithelial cells of the bowel (Ichikawa *et al.*, 1999). Sakata *et al.* (1999) obtained that *Lactobacillus casei* increased the crypt cell production rate of the ileum by 40% in rats. In the present study, antibiotic or organic acids mixture treatments had no effect (P>0.05) villus height at 21^{th} or 42^{th} days of age compared to the basal diet. These results concur with the results of Maiorka *et al.* (2004) who found that a mixed organic acid includes fumaric, lactic, citric and ascorbic acids or a growth promoter antibiotic had no effects in villus height and crypt depth 21-day broiler chickens. Similarly, Sun (2004) also recorded that antibiotic or organic acid supplementation to broiler diets did not affect villus height.

The results of the present study showed that an antibiotic growth promoter (flavomycin), a probiotic (protexin), an organic acids mixture (genex) or in combination of probiotic and organic acids mixture (protexin+genex) treatments to broiler diets have no beneficial effects on the growth performance of broilers. However, all treatments to diets helped to decrease gram negative bacteria counts in intestine. Probiotic treatment to diets helped to improve villus height.

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