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Effect of Using Organic Acids to Substitute Antibiotic Growth Promoters on Performance and Intestinal Microflora of Broilers

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ABSTRACT : A grower broiler experiment (from 14 to 35 days of age) was conducted to study the effect of using two commercial mixtures of organic acids (Galliacid® and Biacid®) to substitute antibiotic growth promoter (Eneramycin®) on performance, carcass characteristics and intestinal microflora. 400 (Ross 308) broiler chicks were used. A basal corn-soybean meal diet were formulated and served as a control treatment. The control diet was supplemented with either 0.06% Galliacid, 0.1% Biacid or 0.02% Eneramycin. Birds fed the Galliacid-supplemented diet had 16% ($p<0.001$) more gain than the control, while those fed the Biacid- or Enramycin-supplemented diets recorded 3 and 5.5% more gain, respectively. Organic acids mixtures and Enramycin supplementation significantly ($p<0.001$) improved feed conversion ratio. These results indicated that birds fed either organic acid mixtures or Enramycin-supplemented diets utilized feed more efficiently than those fed the control diet. Galliacid significantly ($p<0.01$) increased dressing percentage and bursa weight (% body weight). No significant differences were detected on liver, spleen and thymus (% body weight) among treatments. Galliacid or Biacid significantly ($p<0.001$) decreased intestinal *Escherichia coli* and *Salmonella* compared to the control and Enramycin-supplemented diets. Dietary Enramycin significantly ($p<0.001$) decreased *Escherichia coli*, but had no effect on *Salmonella* counts. In conclusion, organic acid mixtures are more efficient than antibiotic growth promoter (Enramycin) in improving broiler performance and decreasing intestinal *Escherichia coli* and *Salmonella* spp., and could be successfully used to substitute antibiotic growth promoters in broiler diets. However, not all of the organic acid mixtures gave the same effect either on performance or intestinal bacterial counts. (**Key Words :** Organic Acids, Antibiotic, Broiler, Performance, Carcass, Intestinal Bacteria)

INTRODUCTION

Poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella* ssp., and *Clostridium*. Pathogenic microflora 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 depresses growth performance and increases incidence of disease.

Antibiotics have been given at sub therapeutic dosage (as feed additive) to stabilize the intestinal microflora and improve the general performances and prevent some specific intestinal pathology promote growth for about 50 years (Dibner and Richards, 2005). The long term and extensive use of antibiotics has resulted in selection of resistant bacterial strains. Resistance among Gram-negative bacteria, like *E. coli* and *Salmonella* spp., has generated the strongest objection to antibiotic use (Gustafson and Bowen, 1997). Nayak and Kenney (2002) showed that 25% of the *Salmonella* isolates from turkey flocks in West Virginia were resistant to one or more antibiotics, including gentamycin, streptomycin, tetracycline, tobramycin, and trimethoprim. Therefore, using antibiotic growth promoters have been under scrutiny for many years (Ratcliff, 2000). Recently, poultry industry has paid more attention towards addressing public concern for environmental and food safety. Thus, the non-prescription use of antibiotics in poultry feeds has been eliminated or severely limited. The European Union banned the use of sub-therapeutic levels of antibiotics to prevent disease or promote growth, starting with a ban on avoparcin in 1997 and a ban on virginiamycin, bacitracin, spiromycin, and tylosin in 1999. Antimicrobials banned by January 2006 included avilamycin, bambermycin, salinomycin and monensin.

So, alternatives to antibiotics are of great interest to the poultry industry (Waldroup et al., 2003). These alternatives

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include acidifiers (organic acids), prebiotics, probiotics, enzymes, herbal products, microflora enhancers, and immuno-modulators.

Most of these alternatives have effects on microflora, directly or indirectly (Richards et al., 2005). Organic acids have strong bacteriostatic effects and have been used as S*almonella*-control agents in feed and water supplies for poultry (Ricke, 2003). Acidification with various weak organic acids to diets such as formic, fumaric, propionic, lactic and sorbic acid 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 supplementation of organic acids to broiler diets increased growth performance, reduced diseases and management problems (Vlademirova and Sourdjiyska, 1996; Runho et al., 1997; Jin et al., 1998; Gunal et al., 2006; Islam et al., 2008; Ao et al., 2009). Most previous studies have used a single organic acid as a dietary supplement. Few studies have been conducted concerning the effect of mixtures of different organic acids and their capability of such mixtures to substitute antibiotic as growth promoters in broiler diets. Therefore, the objective of this study was to determine the effect of using two different commercial mixtures of organic acids to substitute dietary antibiotic growth promoters on performance, carcass characteristics, and intestinal microflora of broiler chickens.

MATERIALS AND METHODS

Birds, diets and housing

A total number of 500 unsexed one day old (Ross 308) broiler chicks were used in this study. Chicks were brooded in a warmed fumigated brooder house and fed on a starter diet to 14 days of age. Birds were then individually weighed and 400 chicks with almost the same body weight were divided into four groups (5 replicates of 20 chicks, each). The average initial live body weight of all replicates was similar. Replicates were randomly allocated in batteries of three-tier system that has 20 compartments (5 replicates×4 dietary treatments).

Two commercial mixtures of organic acids (Galliacid® and Biacid®) as acidifiers and antibiotic (Enramycin®) as growth promoters were used in this study. Galliacid® consisted of a mixture of fumaric acid, calcium format, calcium propionate, potassium sorbate and hydrogenated vegetable oil. These organic acids are coated and protected (microencapsulated) by a matrix of fatty acids. Biacid® consisted of a mixture of citric acid, calcium formate, calcium butyrate, calcium lactate, essential oils and flavoring compounds. Enramycin® is a polypeptide antibiotic produced by *Streptomyces fungicidus.*

A basal grower diet was formulated to cover all the nutrient requirements of (Ross 308) broilers. The basal diet included phytase and xylnase enzyme. The formulation and nutrient composition of the starter and the basal grower diet is shown in Table 1. Phytase addition allows reduction of dietary available phosphorus by 0.1% (Sohail and Roland, 1999) while xylanase allows to reduction of the apparent metabolizble energy by 3 to 4% in feed formulas (Cowan et al., 1996; Zhou et al., 2009).

Four experimental grower diets were used. The basal diet served as a control treatment. The control diet was supplemented with Galliacid® (0.06%), Biacid® (0.10%) or Enramycin® (0.02%). Levels of supplementation were recommended by the producers. Birds were fed the experimental diets for *ad libtum* consumption from 14 to 35 days of age.

Gas heaters were used to keep the required temperature and light was provided 23 h daily during the experiment. Birds were vaccinated against AI, ND, IB and IBD throughout the experimental period. After such medical treatments, a dose of vitamins (AD_3E) was offered in the drinking water for the successive 3 days.

Table 1. Formulation and nutrient composition of starter and grower basal diets

Ingredients %	Starter diet	Grower diet
Yellow corn	54.31	58.50
Soybean meal (48%)	36.50	32.20
Corn gluten meal (60%)	2.00	2.00
Vegetable oil	2.50	3.30
Limestone	1.37	1.40
Dicalcium phosphate	1.80	1.30
Vitamin and mineral mix[1]	0.35	0.35
Salt	0.35	0.35
L-lysine HCl	0.37	0.20
DL-methionine	0.30	0.25
Phytase	0.10	0.10
Xylase	0.05	0.05
Total	100	100
Calculated composition[2] (%)		
Crude protein	24.00	22.00
ME (kcal/kg)	2,950	3,050
Lysine	1.42	1.20
Methionine	0.72	0.64
Methionine+cystine	1.00	0.93
Calcium	1.02	0.90
Nonphytate P	0.45	0.37

[1] Vitamin-mineral mixture supplied per kg of diet: Vit. A, 12,000 IU; Vit. D_3, 2,200 IU; Vit. E, 10 mg; Vit. K_3, 2 mg; Vit. B_1, 1 mg; Vit. B_2, 4 mg; Vit. B_6, 1.5 mg; Vit. B_{12}, 10 μg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 μg; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1 mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg.

[2] Calculated values based on feed composition Tables of NRC (1994).

After fasting overnight, birds were individually weighed and feed consumption was recorded per replicate at 21, 28 and 35 days of age. Body weight gain and feed conversion ratio were calculated weekly and for the entire period (from 14 to 35 days of age).

Carcass measurements

At day 35, ten birds per treatment were randomly taken to study carcass characteristics. Chicks were fasted for approximately 12 h; and then individually weighed, slaughtered, feathered and eviscerated. Weights of carcass, liver, spleen and bursa were recorded. The percentage of carcass and organs (% of live body weight) was calculated.

The preparation of competitive exclusion native gut microflora

Caecal contents were immediately collected from the slaughtered birds and tested for the absence of *Salmonella* ssp. or *E. coli*. Half a gram of such material was inoculated into 10 ml Trypticase Soya broth (Weinack et al., 1979) and incubated at 37°C for 48 h; anaerobically. Using 0.2 ml of the broth culture was then transferred to anther 10 ml tube of Trypticase Soya broth and incubated for 48 h; an aerobically at 37°C. *Salmonella* and *E. coli* colonies counting, (Collins and Lyne, 1984) caecal content specimens were taken a septically one gram of each caecal content was mixed with 9 ml saline for preparation of a ten fold dilution. One ml from each dilution was spread on brilliant green agar plate and incubated at 37°C for 24 h. The total colony count for *salmonella* and *E. coli* was then calculated as the number of colonies by reciprocal of the dilution. The microbial counts were determined as colony forming units (cfu) per gram of sample.

Statistical analysis

Data were statistically analyzed for analysis of variance using the General Liner Model of SAS Institute (1990). Significant differences among treatment means were separated by Duncan's new multiple rang test (Duncan, 1955) with a 5% level of probability.

RESULTS AND DISCUSSION

Table 2 shows the effect of dietary treatments on the productive performance (body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)) recorded weekly and for the entire period (from 14 to 35 days of age). The results of BWG showed that birds fed Galliacid supplemented diet exhibited significant ($p<0.05$ for the first and second periods; and $p<0.001$ for the third and entire periods) more gain than the other groups. At 35 day of age, this group gave 16% more gain compared to the control group. Birds fed the Biacid or Enramycin supplemented diets recorded 3 and 5.5% more gain, respectively, than the control birds.

Feed intake did not differ significantly among treatments during the periods from 14 to 21 and from 22 to 28 days of age. However, during the period from 29 to 35 days of age and the entire period (14-35) birds fed Galliacid supplemented diet consumed significantly ($p<0.05$) more feed than the other groups. No significant differences were detected on either BWG or FI between the control group and those fed Biacid or Enramycin supplemented diets during the different intervals or the entire period. The results of FCR showed that addition of organic acids mixture or Enramycin did significantly ($p<0.01$) improve FCR. Birds fed Biacid or Enramycin supplemented diets gave almost the same values of FCR during the different intervals and the entered period. Birds fed Galliacid supplemented diets showed significant ($p<0.01$) posterior value of FCR compared to the control and the other supplemented diets. Although, birds fed Galliacid supplemented diets consumed significantly ($p<0.05$) more feed than the other group it recorded the best value of FCR. An improvement in value of FCR being 7, 3 or 2% was obtained when the control diet was supplemented with Galliacid, Biacid or Enramycin, respectively. These results indicated the superiority of Galliacid compared to Biacid or Enramycin. Addition of either organic acid mixtures or Enramycin improved the performance of growing broilers expressed as BWG or FCR. Birds fed such supplemented

Table 2. Effect of dietary treatments on growth performance of broiler chicks

Diets	Dietary treatments	14-21 days			22-28 days			29-35 days			14-35 days		
		WG	FI	FCR	WG	FI	FCR	WG	FI	FCR	WG	FI	FCR
Control		347^b	566	1.63^a	407^b	680	1.67^a	458^b	833^b	1.82^a	$1,212^b$	$2,079^b$	1.72^a
Diet 1	0.06% galliacid	374^a	592	1.58^d	457^a	728	1.59^c	574^a	925^a	1.61^c	$1,405^a$	$2,245^a$	1.60^c
Diet 2	0.1% biacid	348^b	559	1.60^c	426^{ab}	702	1.65^b	479^b	836^b	1.75^b	$1,253^b$	$2,097^b$	1.67^b
Diet 3	0.02% enramycin	349^b	562	1.61^b	440^{ab}	727	1.65^b	490^b	856^b	1.75^b	$1,279^b$	$2,145^b$	1.68^b
SE of means		±4.3	±5.7	±0.01	±6.9	±10.6	±0.01	±12.9	±12.8	±0.02	±21	±22.2	±0.02
Significances		*	NS	**	*	NS	**	***	*	***	***	*	***

[a-d] Mean within each column with no common superscript differ significantly ($p<0.05$).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$. NS = Not significant ($p>0.05$).

Table 3. Effect of dietary treatments on dressing percent, and organ weights as percent of live body weight of broiler chicks at 35 days of age

Diets	Dietary treatments	Live body weight	Carcass weight	Dressing %	Liver %	Spleen %	Thymus %	Bursa %
Control		1,564[b]	1,130[b]	72.21[b]	2.27	0.12	0.37	0.105[c]
Diet 1	0.06% galliacid	1,760[a]	1,302[a]	73.98[a]	2.45	0.14	0.39	0.138[a]
Diet 2	0.1% biacid	1,604[b]	1,159[b]	72.26[b]	2.34	0.14	0.38	0.110[b]
Diet 3	0.02% enramycin	1,628[b]	1,179[b]	72.43[b]	2.19	0.12	0.36	0.107[c]
SE of means		±21.24	±19.46	±0.26	±0.065	±0.003	±0.011	±0.004
Significances		***	***	*	NS	NS	NS	**

[a-d] Mean within each column with no common superscript differ significantly (p<0.05).
* p<0.05, ** p<0.01, *** p<0.001. NS = Not significant (p>0.05).

diets utilized feed more efficient than the control diets.

An improvement on values of BWG by 16% and FI by 8% was observed when the control diet was supplemented with Galliacid.

The effects of dietary treatments on carcass characteristics of 35 days old broilers are shown in Table 3. Using Galliacid significantly (p<0.05) increased dressing percentage and bursa weights relative to live body weight. No significant differences were detected on liver, spleen and thymus (% body weight) among treatments. However, there were minor increases in the relative weight of liver, spleen and thymus of birds fed Galliacid or Biacid supplemented diets compared to those fed the control or Enramycin supplemented diets.

The effects of dietary treatments on intestinal microflora (*Escherichia coli* and *Salmonella*) of chicks fed the different dietary treatments are shown in Table 4. The results showed that the addition of Galliacid or Biacid significantly (p<0.001) decreased *Escherichia coli* and *Salmonella* compared to the control and Enramycin supplemented diet. Dietary Enramycin significantly (p<0.001) decreased *Escherichia coli*, but had no effect on *Salmonella*. The pronounced decreased of *Escherichia coli* and *Salmonella* ssp. bacteria counts were observed with galliacid treatment. From the economic point of view, using organic acid mixture costed about 5.25 dollars/ton feed compare to 8.50 dollars for eneramycin antibiotic.

Table 4. Effect of dietary treatments on intestinal bacteria of broiler chicks at 35 days of age

Diets	Dietary treatments	*E. coli* ssp. $\log_{10}$ cfu/g	*Salmonela* ssp. $\log_{10}$ cfu/g
Control		6.392 [a]	5.491 [a]
Diet 1	0.06% galliacid	4.415 [c]	3.690 [c]
Diet 2	0.1% biacid	4.459 [c]	4.194 [b]
Diet 3	0.02% enramycin	5.151 [b]	5.513 [a]
SE of mean		±0.21	±0.21
Significances		***	***

[a-c] Mean within each column with no common superscript differ significantly (p<0.05).
*** p<0.001.

The results of the present study confirmed those obtained by Naidu (2000), Fushimi et al. (2001), Gunes et al. (2001), Gornowicz and Dziadek (2002), Wolfenden et al. (2007) and Abd El-Hakim et al. (2009) who concluded that organic acids could be used in poultry, not only as a growth promoter but also as a meaningful tool of controlling intrinsic pathogenic bacteria (*E. coli* and *Salmonella*). They found that organic acids feeding improved feed conversion ratio, growth performance, enhanced mineral absorption and speeding recovery from fatigue. In addition, Gauthier (2002) reported that, contrary to antibiotics, organic acids have other properties like; lowering of the chyme pH consequently, enhancing of protein digestion. On the other hand, Denli et al. (2003) found that dietary organic acids had no effect on the carcass yield and liver weight of broiler chickens at 42 d old.

Abdel-Fattah et al. (2008) found that broiler chicks fed dietary organic acids had superior improvement in live body weight, body weight gain and feed conversion ratio compared to those of unsupplemented diet. Owens et al. (2008) reported that total live weight gain (12%) and gain: feed (9%) of broiler chicks were significantly improved for diets containing organic acids additives, compared to the control diets. Ao et al. (2009) found that the basal diet supplemented with 2% citric acid of broilers significantly (p<0.05) increased feed intake, weight gain, AME_n of the diets, and retention of CP and neutral detergent fiber (NDF).

Islam et al. (2008**)** concluded that fumaric acid (FA) may promote growth of broilers, 1.25% FA group showed significantly (p<0.05) better weight gain and better feed efficiency than the groups with 5.0 and 7.5% FA. Higher gain was associated with higher feed intake. Similar to the present results, the relative weight of heart, liver and spleen was not affected by the treatment.

The obtained results proved those of Abdel-Fattah et al. (2008) who found that organic acids significantly increased the relative weight of bursa. The resulted improvement in growth performance associated with significant increase in bursa weight proved that addition of Galliacid did positively affect the immune system and resistance of broilers againest desises. In this respect, Katanabdef et al. (1989) reported that the increase in the relative immune organs weight is considered as an indication of the immunological advances.

Other feed additives like B-mannanase (Zou et al., 2006), mashroom extract, probiotics (Willis et al., 2007), ascorbic acid (Amakye-amin et al., 2000) could increase bursa weight and improve growth performance and immunity of broilers. Also, Mos (mannan oligosacchride) exerts a significant growth-promoting effect by enhancing the bird's resistance to enteric pathogens (Ferket, 2004, Mohamed et al., 2008).

Brul and Coote (1999) explained that the key basic principle on the mode of action of organic acids on bacteria is that non-dissociated organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that we call "pH sensitive" meaning that they cannot tolerate a wide internal and external pH gradient. Lee (2005) added that, more likely, the organic acids in poultry might play a direct role on the GIT bacteria population, reducing the level of some pathogenic bacteria and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients.

Dietary acidification inhibits of intestinal bacteria competing with the host for available nutrients, and a reduction of possibly toxic bacterial metabolites, thus improving weight gain of the host animal. Furthermore, the growth inhibition of potential pathogen bacteria and zoonotic bacteria, e.g. *E. coli* and *Salmonella*, in the feed and in the GI- tract are of benefit with respect to animal health. In poultry production organic acids have mainly been used in order to sanities the feed considering problems with *Salmonella* infections (Iba and Berchieri, 1995; Berchieri and Barrow, 1996; Thompson and Hinton, 1997).

The superiority of Galliacid over the Biacid may be because of the microencapsulation the organic acids of Galliacid are coated and protected by a matrix of fatty acids. Thus, the organic acids can reach the intestine without modification, where they are released slowly under the action of lipase secretions. Non dissociated organic acids can be active on bacteria and modulate the intestinal flora.

CONCLUSIONS

It could be concluded that, under the condition of the present study, organic acids mixtures are more efficient than antibiotic growth promoter (Enramycin) on improving broiler performance and decreasing intestinal *Escherichia coli* and *Salmonella ssp*. Not all of the organic acids mixtures gave the same effect either on performance or intestinal bacterial counts. From the obtained results and the forgoing discussion, it could be reported that if organic acids mixtures were used correctly along with nutritional, managerial and biosecurity measures, they can be a powerful tool in maintaining the health of the gastrointestinal tract of poultry, thus improving their performances and successfully used as growth promoters. It is well known that, antibiotics at sub-dosage could prevent necrotic enteritis. Organic acids mixtures proved to have the same effect on stabilizing the intestinal microflora. However, in case of necrotic enteritis challenge organic acids mixtures are not comparable with antibiotics at therapeutic dosage. Further, studies are needed to compare using organic acids mixtures with antibiotics in different experimental conditions.

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