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 spp., 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
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 Salmonella-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 Soudjyska, 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 formate, 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 xylanase 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 metabolizable 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 libitum 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 (AD₃E) 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 | 36.50 | 32.20 |
| Corn gluten meal | 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 | 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 (%), 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. D₃, 2,200 IU; Vit. E, 10 mg; Vit. K₂, 2 mg; Vit. B₁, 1 mg; Vit. B₂, 4 mg; Vit. B₆, 1.5 mg; Vit. B₁₂, 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
Cecal 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 another 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

| Diets          | Dietary treatments | 14-21 days |    |    |    | 22-28 days |    |    |    | 29-35 days |    |    |    | 14-35 days |    |    |    |    |    |    |    |    |
|----------------|--------------------|------------|----|----|----|------------|----|----|----|------------|----|----|----|------------|----|----|----|----|----|----|----|----|
| Control        |                    | 347±       | 566| 1.63| 407±       | 680| 1.67| 458±       | 833| 1.82| 1,212±      | 2,079| 1.72| 1,054      | 3,753| 1.72| 2,849| 6,275| 1.72| 1,054| 3,753| 1.72|
| Diet 1         | 0.06% galliacid    | 374±       | 592| 1.58| 457±       | 728| 1.59| 574±       | 925| 1.61| 1,405±      | 2,245| 1.60| 1,102      | 3,802| 1.60| 2,603| 6,275| 1.60| 1,102| 3,802| 1.60|
| Diet 2         | 0.1% biacid        | 348±       | 559| 1.60| 426±       | 702| 1.65| 479±       | 836| 1.75| 1,253±      | 2,097| 1.67| 1,135      | 3,675| 1.67| 2,603| 6,275| 1.67| 1,135| 3,675| 1.67|
| Diet 3         | 0.02% enramycin    | 349±       | 562| 1.61| 440±       | 727| 1.65| 490±       | 856| 1.75| 1,279±      | 2,145| 1.68| 1,141      | 3,825| 1.68| 2,603| 6,275| 1.68| 1,141| 3,825| 1.68|

In 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).
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 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, AMEn 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 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    |                   | 1,564b          | 1,130b        | 72.21b     | 2.27    | 0.12     | 0.37     | 0.105b  |
| Diet 1     | 0.06% galliacid   | 1,760a          | 1,302a        | 73.98b     | 2.45    | 0.14     | 0.39     | 0.138c  |
| Diet 2     | 0.1% biacid       | 1,604b          | 1,159b        | 72.26b     | 2.34    | 0.14     | 0.38     | 0.110b  |
| Diet 3     | 0.02% enramycin   | 1,628b          | 1,179b        | 72.43b     | 2.19    | 0.12     | 0.36     | 0.107c  |

SE of means ±21.24 ±19.46 ±0.26 ±0.065 ±0.003 ±0.011 ±0.004

Significances *** *** * NS NS NS **

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.

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 | Salmonella ssp. log 10 cfu/g |
|------------|-------------------|--------------------------|-----------------------------|
| Control    |                   | 6.392a                   | 5.491a                      |
| Diet 1     | 0.06% galliacid   | 4.415c                   | 3.690c                      |
| Diet 2     | 0.1% biacid       | 4.459c                   | 4.194b                      |
| Diet 3     | 0.02% enramycin   | 5.151b                   | 5.513a                      |

SE of mean ±0.21 ±0.21

Significances *** ***

Mean within each column with no common superscript differ significantly (p<0.05).

*** p<0.001.
growth performance associated with significant increase in bursa weight proved that addition of Galliacid did positively affect the immune system and resistance of broilers against 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), mushroom 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 oligosaccharide) exerted 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 sanitise 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 foregoing 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.

REFERENCES

Abd El-Hakim, A. S., G. Cherian and M. N. Ali. 2009. Use of organic acids, herbs and their combination to improve the utilization of commercial low protein broiler diets. Int. J. Poult. Sci. 8(1):14-20.

Abdel-Fattah, S. A., M. H. El-Sanhoury, N. M. El-Mednay and F. Abdel-Azeem. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7(3):215-222.

Amakye-amin, J., T. L. Lin, P. Y. Hester, D. Thiagarajan, P. A. Watkins and C. C. Wu. 2000. Ascorbic acid supplementation improved antibody response to infection bursal disease vaccination in chicks. Poult. Sci. 79:680-688.

Ao, T., A. H. Cantor, A. J. Pescatore, M. J. Ford, J. L. Pierce and K. A. Dawson. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult. Sci. 88:111-117.

Berchieri, A., Jr. and P. A. Barrow. 1996. Reduction in incidence of experimenal fowl typhoid by incorporation of a commercial formic acid preparation (Bio-Ad[g] into poultry feed. Poult. Sci. 75:339-341.

Brul, S. and P. Coote. 1999. Preservative agents in foods, mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50:1-17.

Collins, C. H. and P. M. Lyne. 1984. Microbiological methods. Fifth Edition, Butterworth’s, London, 331-345.

Cowen, W. D., A. Korsbak, T. Hastrup and P. B. Rasmussen. 1996. Influence of added microbial enzymes on energy and protein availability of selected feed ingrediants. Anim. Feed Sci. Technol. 60:311-319.

Denli, M., F. Okan and K. Celik. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pak. J. Nutr. 2:89-91.

Dibner, J. J. and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84:634-643.

Duncan, D. B. 1955. Multiple range test and multiple F tests. Biometrics 11:1-42.

Engberg, R. M., M. S. Hedemann, T. D. Leser and B. B. Jensen.
2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79:1311-1319.

Ferket, P. R. 2004. Alternatives to antibiotics in poultry production: response, practical experience and recommendations. In: Biotechnology in the Feed and Food Industry (Ed. T. P. Lyons and K. A. Jacques), Proceedings of Alltech’s 20th Annual Symposium, pp: 57-66. Nottingham University Press, UK.

Fushimi, T., K. Tayama, M. Fukaya, K. Kitakoshi, N. Nakai, Y. Tsukamoto and Y. Sato. 2001. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. J. Nutr. 131:1973-1977.

Gauthier, R. 2002. Intestinal health, the key to productivity (The case of organic acids) XXVII Convention ANECA-WPDSA Puerto Vallarta, Jal. Mexico. 30 April 2002.

Gornowicz, E. and K. Dziadek. 2002. The effects of acidifying preparations added to compound feeds on management conditions of broiler chickens. Ann. Anim. Sci. (Suppl. 1):93-96.

Gunal, M., G. Yayli, O. Kaya, N. Karahan and O. Sulak. 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci. 5(2):149-155.

Gunes, H., H. Cerit and A. Altinel. 2001. Etilik pilicilerin verim ozellikleri üzerine pre-probiotigin (Fermacto-500) etkisi. Ist. Univ. Vet. Fak. Derg. 27:217-229.

Gustafson, R. H. and R. E. Bowen. 1997. Antibiotic use in animal agriculture. J. Appl. Micro. 83:531-541.

Iba, A. M. and A. JR. Berchieri. 1995. Studies on the use of a formic acid propionic acid mixture (Bio-add™) to control experimental Salmonella infection in broiler chickens. Avian Pathol. 24:303-311.

Islam, K. M. S., A. Schuhmacher, H. Aupperle and J. M. Gropp. 2008. Fumaric acid in broiler nutrition: a dose titration study and safety aspects. Int. J. Poult. Sci. 7(9):903-907.

Jin, L. Z., Y. W. Ho, N. Abdullah, M. A. Ali and S. Jalaluddin. 1998. Effects of adherent Lactobacillus cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. Anim. Feed Sci. Technol. 70:197-209.

Katanbaf, M. N., E. A Proning and P. B. Siegel. 1989. Restricted feeding in early and late feathering chickens, growth and physiological responses. Poult. Sci. 68:344-351.

Kirchhoff, V. M. and F. X. Roth. 1988. Ergotrope effekte durch Katanbaf, M. N., E. A Dunington and P. B. Siegel. 1989. Effects of adherent 

Mohamed, M. A., H. M. A. Hassan and E. M. A. El-Barkouky. 2008. Effect of mannan oligosaccharide on performance and carcass characteristics of broiler chicks. J. Agric. Soc. Sci. 4:13-17.

Naidu, A. S. 2000. Natural food antimicrobial systems. CRC Press USA, pp. 431-462.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy press, Washington, DC.

Nayak, R. and P. B. Kenney. 2002. Screening of Salmonella isolates from a turkey production facility for antibiotic resistance. Poult. Sci. 81:1496-1500.

Owens, B., L. Tucker, M. A. Collins and K. J. McCracken. 2008. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. Br. Poult. Sci. 49(2):202-212.

Ratcliff, J. 2000. Antibiotic bans-a European perspective. Pages: 135-152 in Proceeding of the 47 Maryland Nutrition Conferences for Food the Manufacturers, March 22-24.

Richards, J. D., J. Gong and C. F. M. de Lange. 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: Current understanding, possible modulations and new technologies for ecological studies. Can. J. Anim. Sci. 85:421-435.

Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632-639.

Runho, R. C., N. K. Sakomura, S. Kuana, D. Banzatto, O. M. Juenquera and J. H. Stringhini. 1997. Uso do acido organico (acido fumarico) nas races de frangos de corte. Revista Brasileira de Zootecnia, 26:1183-1191.

SAS Institute. 1990. SAS®/STAT User’s Guide: Statistics, Version 6, 4th edition. SAS Institute Inc, Cary, NC.