ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 4 (10): 761-764, 2005
ISSN 1682-8356

Effect of Malic Acid on Visceral Characteristics and Coliform Counts in Small Intestine in the Broiler and Layer Chickens

A. Moharrery[1] and M. Mahzonieh[2]
[1]Animal Science Department, Agricultural College, Shahrekord University, Iran
[2]Pathobilogy Department, Veterinary College, Shahrekord University, Iran

Abstract: Antimicrobial feed additives such as organic acids have made a tremendous contribution to the profitability in the intensive husbandry and providing people with healthy and nutritious poultry products. For examine of this effects of organic acids two experiments were conducted to determine the effects of the malic acid on chicken visceral characteristics and *E. coli* population in the small intestine. First study was performed with male egg type chickens (2 to 21 d of age) which received four levels of malic acid via drinking water. Malic acid was added to the water and offered to chicken freely from first to end of experiment with constant concentration in both experiments. The treatments were zero (as a control), 0.05, 0.10, and 0.15 percent of malic acid which dissolved in water and given to them in waterer pan. The chicks were slaughter on 21 days old and above parameters were measured on visceral organs. In second experiment broiler chicken (male and female from 1 to 56 d of age) was evaluated for same parameters on same treatments as a mentioned for the first experiment. No significant difference ($P > 0.05$) was observed between treatments for weight gain and liver percentage in both experiments. Difference between treatments in relation to gastrointestinal tract (GIT) percentage has been shown in Exp. 1 ($p<0.05$). The results of these trials showed that malic acid have the potential for reduction of *E. coli* population in chicken intestine in both experiments.

Key words: Broiler, malate, visceral, *E. coli*

Introduction

Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the host animal, including the load in the alimentary tract and in the environment (Garrido *et al*., 2004). Poultry possess a limited natural resistance and immunity against colonization or infection by potentially pathogenic microorganisms (Huyghebaert, 2002). In this regard, organic acid feed additives have made a tremendous contribution to the profitability in the intensive husbandry and providing people with healthy and nutritious poultry products (Patten, and Waldroup, 1988).

As a consequence of the increasing concern about the potential for antibiotic resistant strains of bacteria, so many of non-therapeutic alternatives, including enzymes, (in)organic acids, probiotics, prebiotics, herbs and etheric oils and immunostimulants has been used as a feed additives. The impact of acids depends on their chemical characteristics, thereby controlling *in-vitro* and *in-vivo* the microbial flora. The key basic principle on the mode of action of organic acids on bacteria is that nondissociated (non-ionised, more lipophilic) organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (Dhawale, 2005). Malic acid as a one of the organic acids is formed in metabolic cycles in the cells of plants and animals, including chickens. Peripheral malate derives from feed sources and from synthesis in the citric acid cycle (Lehninger, 1978). The evidence by which exogenous malic acid may affect on chick performance is lacking. Therefore, the current study was conducted to determine the effects of malic acid consumption on chicken performance, visceral characteristics and *E. coli* population in chicken intestine.

Materials and Methods

Chickens and treatments: In the first experiment, 1-d-old male egg type chickens were housed in grouped pen and received a corn-based diet. At 2 d of age, twenty chickens were weighed and distributed into four homogenous experimental groups and housed in four pens (five chicks per pen). The pens were 60 × 50 cm. The light was continuous during the experiment. The corn-based diet was formulated according to the nutritional requirements for chickens (NRC, 1994; Table 1). Diet was fed in mesh form and contained no growth factors, coccidiostats, exogenous enzymes, or antibiotics. Malic acid was added to the water and offered to chicken freely from first to end of experiment with constant concentration in entire experiment. The treatments were zero (as a control), 0.05, 0.10, and 0.15 percent of malic acid which dissolved in water and given to them in waterer pan. Feed and water were supplied *ad libitum* throughout the entire experiment.

In the second experiment, one hundred and ninety two broiler chickens (Ross, Iranian agency) sorted and were randomly assigned to 16 pens each consisting of 12

Table 1: Composition of experimental diets

	Exp 1	Exp 2		
	Starter	Starter	Grower	Finisher
Ingredients and analysis	(g/kg)			
Ground yellow corn	597	618	485	615
Soybean meal (44% CP)	308	280	330	190
Fish meal	20	49.5	27	20
Plant oil	20	19	19	25
Wheat bran	14.8	-	84	95
Dicalcium phosphate	18	12	28	28
Oyster shell	12	13	10	10
Sodium chloride	4	1	4.5	4.5
DL- methionine	1	0.5	0.25	0.25
Lysine	0.2	-	-	-
Vitamin/mineral premix[1]	5	7	10	10
Analyses (calculated)[2]				
AME Kcal/kg	2941	3002	2721	2890
Crude protein (%)	20.3	20.8	21.9	16.6
Methionine (%)	0.45	0.44	0.6	0.53
Methionine + Cysteine (%)	0.77	0.75	0.94	0.79
Lysine (%)	1.17	1.21	1.26	0.86

[1]The premix supplied the following (mg/kg diet): retinol 3.6, cholecalciferol .075, biotin 1, dl-α-tocopherylacetate 10, riboflavin 10, pantothenate 20, choline 2000, niacin 100, thiamine 10, pyridoxine 10, menadin sodium bisulphate 1.5, cyanocobalamin .1, folic acid 2, ethoxyquin 150, Mn 100, Fe 100, Cu 10, Co 1, I 1, Zn 100. [2]Estimated from NRC (1994) composition tables.

birds. The room temperature was gradually decreased from 32°C at d1 to 24°C at d 22. The chicks were fed with three type diets consisted starter, grower and finisher (Table 1). The lighting regimen and malic acid treatments were same as experiment 1.

Collection of samples: All birds in Exp. 1 were sacrified at 11.00 hours on d21 and two birds (one male and another female which phenotypically selected) from each pen were killed at 06.00 hours on d56 in Experiment 2, by cutting the carotid artery. Gastrointestinal tracts (GIT) along with livers were excised rapidly, washed in 155 mM NaCl to remove exterior blood and debris. The livers and GIT weighed and GIT used for further study. The samples from GIT were collected from distal parts of small intestine content after isolating their gastrointestinal tracts. Each sample weighted and transferred to sterile tube. Surface plate count method was used for determination of viable numbers. Immediately following sampling, intestinal contents were homogenised in sterile normal saline 10 times (W/V). Then each suspension was serially diluted to prepare tenfold dilutions. A 0.1 ml volume of each dilution streaked on surface of MacConkey agar medium. Plates incubate at 37°C. Bacterial colonies on plates, which showed 30 to 300 colonies on MacConkey agar were counted after 2 days. The number of colony in countable plates multiply by reverse of its' dilution considered as Colony Forming Unit (CFU) of enteric coliform bacteria per gram of intestinal content.

Statistical analysis: The complete randomised model was used to analyse data for weight gain, visceral characteristics and *E. coli* population. Logarithmic transformation used for *E. coli* population. In this regard, four treatments offered to chicken in five (Experiment 1) or four (Experiment 2) replicates individually. The experimental design for visceral characteristics and *E. coli* population (Experiment 2) was a completely randomized one with a 4×2 factorial arrangement of treatments. Each of four treatments was replicated four times per sex (n = 4). The data were analysed using general linear model procedure of SAS (1988). Duncan's multiple range test (SAS, 1988) ($P<0.05$) was used to test the significance of difference between means. Values are given as means, and the homogeneity of variance was checked.

Results and Discussion

Growth performance: Table 2 summarizes the effects of different levels of malic acid on live weight and visceral characteristics in both experiments. These data showed no significant difference ($P>0.05$) between control and malic acid treatment for the live weight or liver percentage. Higher live weight (69 g) in chicken on treatment with 0.05% malic acid concentration (Exp 2) to compare with control group did not showed significant difference ($P = 0.2722$). These results in agreement with Denli *et al.* (2003) who has reported that live weight and liver weight were not affected significantly by organic acid

Table 2: Exp. 1 and 2. Influence of malic acid concentration on the final body weight, and visceral organs

	Malic acid concentration (%)					Sex		
	0	0.05	0.10	0.15	SEM	Male	Female	P*
Exp. 1								
Body weight[1] (g)	129.5	115.9	122.6	118.6	4.56	-	-	-
Liver (%)	3.31	3.30	3.18	3.03	0.114	-	-	-
Gastrointestinal tract (%)	15.8[b]	18.1[ab]	17.7[ab]	18.5[a]	0.82	-	-	-
Exp. 2								
Body weight[2] (g)	2600	2530	2525	2470	54.4	2606	2456	0.0110
Liver (%)	1.89	1.95	1.81	1.89	0.080	1.89	1.88	0.8965
Gastrointestinal tract (%)	4.18	4.21	4.14	4.18	0.053	4.18	4.17	0.8877

[1]Final body weight in 21d old male egg type chicken. [2]Final body weight in 56d old broiler chicken.
[ab]Means in row with no common superscript differ significantly (P<0.05). *Probability.

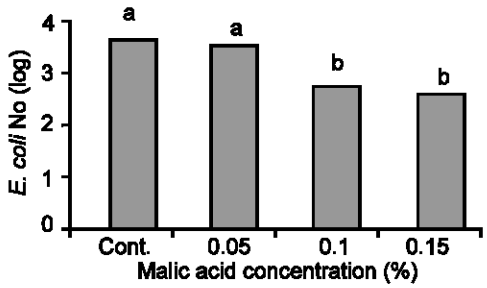

Fig. 1: Effect of malic acid concentration on *E. coli* count in chicken layer intestine

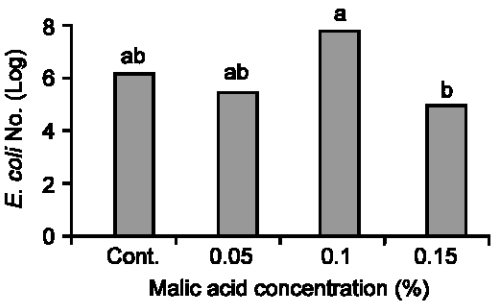

Fig. 2: Effect of malic acid concentration on *E. coli* count in broiler intsetine

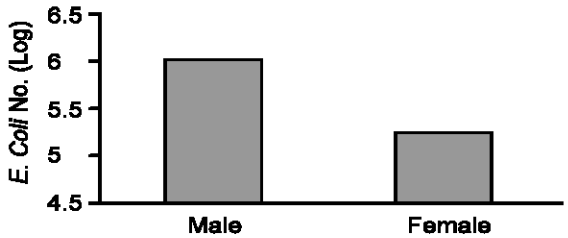

Fig. 3: Difference between male and female broiler chicken for *E. coli* count

treatments in broiler chickens. On the contrary Patten and Waldroup (1988) found that the addition of 1.5% calcium formate in broiler diets reduced weight gain. Besides when given high level of propionic acid in water the acid would strongly decrease palatability and thus intake of water, which reduce feed intake and weight gain (Cave, 1984). In spite of the fact that the domestic fowl does have a sense of taste, but birds have a wide range of tolerance for acidity and alkalinity in their drinking water (Kare and Rogers, 1976). Fuerst and Kare (1962) observed that chicks would accept strong mineral acid solution over extended period of time. On the other hands, Skinner *et al.* (1991) compared the effects of dietary fumaric acid supplementation at 0.125, 0.25 and 0.50% on broiler performance from 0 to 49 d. they found similar results in our present study. However intestinal pH was reduced by level of organic acid mix. As a limit literature for effect of malic acid consumption in poultry, knowledge about the role of dietary malic acid with this type offering is lacking.

The weight of gastrointestinal tract has been significantly (P<0.05) affected by malic acid supplementation in water in Experiment 1 but not in Experiment 2 (Table 2). Denli *et al.* (2003) also reported that the supplementing the diet with the antibiotic, probiotic and organic acid did not only result in the intestinal weight but also in the highest intestinal length at day 42.

Intestinal bacterial counts: The means of the bacterial counts in the distal parts of small intestine of the chickens in Experiment 1 and Experiment 2 has been shown in the Fig. 1 and 2, respectively. The acidified group had much lower counts in higher malic acid concentration than the control group in Experiment 1 (P<0.05; Fig. 1). In Experiment 2 bacterial counts showed increase count in treatment with 0.10% malic acid concentration but the bacteria counts in treatment on 0.15% malic acid concentration was still at lowest rate (P<0.05; Fig. 2). No significant difference between the two sexes was observed (P>0.05; Fig. 3).

The efficacy of organic acids in swine nutrition has been proven time after time (Partanen and Mroz, 1999) but in poultry this innovative approach is in its infancy. The intestinal microenvironment that will influence the

microflora is much dependant on; pH, substrate availability (ex. poorly digested protein, NSPs), redox potential, toxins, antibodies and the presence of other bacteria etc. Malic acid is active against some bacteria and yeasts (Dhawale, 2005). Generally, organic acids with higher pKa values (e.g. Malic acid) are more effective preservatives and their antimicrobial efficacy is generally improved with increasing chain length and degree of unsaturation. The ultimate effect of acids might induce a more balanced intestinal flora by reducing the proliferation of some pathogenic bacteria. Huyghebaert *et al*. (1999) demonstrated that a mixture of organic acids (added to a negative control diet in comparison with a positive control diet on Zn-bacitracin at 50 mg/kg) could only partially compensate for the higher polyserositis (pathogen *E. coli*)-related mortality in broilers.

We can assume that malic acid inhibit pathogen bacteria growth in the chicken intestine, which resulted statistically significant reduction *E. coli* count in chicken on malic acid treatment to compare control group in both experiments.

Conclusion: Malic acid is not antibiotics but, if 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 zootechnical performances. More research is need for better understanding of dietary malic acid role on chicken performance and its interaction with other key substance.

Acknowledgments

A Shahrekord University has funded this study. We are grateful to all the staff from the Section of Research Department in the Shahrekord University. We also thank the staff at the poultry farm in Agricultural College and Microbiology Lab. in Veterinary College in the Shahrekord University.

References

Cave, N.A.G., 1984. Effect of dietary propionic and lactic acid on feed intake by chicks. Poult. Sci., 63: 131-134.

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.

Dhawale, A., 2005. Better eggshell quality with a gut acidifier. Poult. Int., 44: 18-21.

Fuerst, W.F., Jr. and M.R. Kare, 1962. The influence of pH on fluid tolerance and preference. Poult. Sci., 41: 71-77.

Garrido, M.N., M. Skjervheim, H. Oppegaard and H. Sorum, 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. Appl. Environ. Microbiol., 70: 5208-5213.

Huyghebaert, G., 2002. Ovigram as alternative for growth promoting antibiotics in broiler chickens. Proc. 11th Eur. Poult. Conf., (6-10 sept; Bremen, Germany): 108.

Huyghebaert, G., G. De Groote, G. Van De Broek and M. Velzeboer, 1999. Effect on technical results in broilers of several ways of addition organic acids versus addition of an antibiotic growth promoter. Proc. 12th Eur. Symp. Poult. Nutr. (Veldhoven, The Netherlands:15-19 august): 421-423.

Kare, M.R. and J.G. Rogers, Jr., 1976. The special senses. In : P.D. Sturkie, ed., Avian Physiol., Springer, Berlin.

Lehninger, A.L., 1978. Biochemistry. Worth publishing. 509-542.

National Research Council, 1994. Nutrient requirements of poultry. 9th edition. National Academy Press, Washington, DC.

Partanen, K.H. and Z. Mroz, 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev., 12: 117-145.

Patten, J.D. and P.W. Waldroup, 1988. Use of organic acids in broiler diets. Poult. Sci., 67: 178-1182.

SAS, 1988. User's Guide: Statistics, Version 6.03, 4th Ed. SAS Inst., Inc., Cary, NC.

Skinner, J.T., A.L. Izat and P.W. Waldroup, 1991. Fumaric acid enhances performance of broiler chickens. Poult. Sci., 70: 1444-1447.