

Effect of Organic Acid Supplementation on the Performance and Ileal Microflora of Broiler During Finishing Period

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Abstract.- This study was conducted to determine the effect of different levels of organic acid on the performance and ileal microflora of broiler during finishing phase. A total of 150 day old broiler chicks were divided into five experimental groups *i.e.*, OA-0, OA-0.5, OA-1, OA-1.5 and OA-2.00 and were supplemented in drinking water at the rate of 0, 0.5, 1, 1.5 and 2% organic acid blend, Aciflex®, respectively. Aciflex® consisted of citric acid 80 g, lactic acid 52 g, CuSO₄ 10 g and phosphoric acid 92 g/l. Each group was further divided into three replicates having 10 chicks per replicate in a completely randomized design. The birds were reared in separate pens for 22 to 42 days in an open sided house. At the end of the experiment, the results revealed that body weight, feed conversion ratio, carcass yield and liver weight increased significantly ($P < 0.05$) in the treated groups showing linear improvement. Similarly, bacterial microflora including *E. coli* and *Salmonella* decreased significantly ($P < 0.05$) in the treated groups. Best antimicrobial results were observed in OA-2 group. We concluded from the results that organic acid improved the production and decreased microbial population in broiler during finishing stage.

Keywords: Broiler, organic acid, performance and microbiota.

INTRODUCTION

Antibiotics use in poultry as a growth promoter is still a common practice in developing countries largely due to an increasing demand of poultry meat and eggs (Aziz-Mousavi *et al.*, 2012; Khan *et al.*, 2012a; Shamlo *et al.*, 2014). In 2006, the European Union (EU) banned antibiotic as growth promoters to be used as feed additives in animal production. The development of antimicrobial resistance of bacteria has become a global health problem. A variety of human food and environmental sources possess bacteria that are resistant to a number of antibiotics which are used in human and veterinary medicine (Khan *et al.*, 2012b; Amaechi and Amaeze, 2012). The nutritionists and researchers are attempting to explore other potential alternatives, claiming to boost the production and growth performance of poultry birds (Chand *et al.*, 2014a&b; Khan *et al.*, 2014).

Organic acids are the substitute as feed additives in animal production (Adil *et al.*, 2011; Khan *et al.*, 2012a). Organic acids maintain cellular integrity of gut lining and improve digestive process

by maintaining normal gut flora and hence can potentially be used as alternative to antibiotic growth promoters. They are generally considered to be safe and play a significant role in increasing the profitability of the poultry production and also by providing healthy and nutritious poultry products to the consumers. Therefore, most of the member states of EU have approved the use of organic acid in the feed for animal production (Patten and Waldroup, 1988).

Organic acids such as citric acid, numeric acid and formic acid enhance the digestibility of protein and amino acid by increasing gastric proteolysis. Organic acid reduces gastric pH which may boost the action of pepsin (Kirchgessner and Roth, 1988) arising proteolysis which activate the release of gastrin and cholecystokinin hormones and regulate the digestion and assimilation of protein (Hayat *et al.*, 2014). They play significant role in better utilization of the available nutrient resulting in improved growth rate and feed conversion efficiency (Denli *et al.*, 2003). Apart from that, they also improve the digestibility of minerals like phosphorus, calcium, zinc and magnesium (Kirchgessner and Roth, 1988).

Organic acid supplementation to broilers has been reported to decrease the colonization of harmful pathogens in the gastrointestinal tract which subsequently decreased the production of toxins

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(Denli *et al.*, 2003). They might distress the veracity of cell macromolecules and microbial cell membrane or impede the transportation of nutrient and energy metabolism which activate bactericidal effect (Ricke, 2003). The non ionized and more lipophilic outward can infiltrate the bacterial cell wall and interrupt the usual physiology of various bacteria (Dhawale, 2005). The antimicrobial activities reduce the pH of digesta, boost up the pancreatic secretion, which distress gastro-intestinal tract (Dibner and Buttin, 2002). The present study was designed to evaluate the effect of different levels of organic acid on the performance and pathogenic microbes in broiler.

MATERIALS AND METHODS

Bird husbandry and experimental protocol

This study was approved by the Use and Care of Animal Committee, University of Agriculture, Peshawar, Pakistan. Before the commencement of experiment, poultry house and floor cages were cleaned and disinfected. Strict biosecurity and hygienic measures were adopted to ensure cleanliness to avoid spread of diseases. Temperature, humidity and optimum ventilation were controlled at standard level of bird needs. One hundred and fifty, 22-days-old broiler birds (Hubbard) were obtained from the birds reared at poultry farm of The University of Agriculture, Peshawar. Broiler birds of similar body weight and physical look were equally and randomly assigned to five replicated. A commercially available organic acid blend (Aciflex®; citric acid 80 g, lactic acid 52 g, CuSO₄ 10 g and phosphoric acid 92 g per Liter) was given in drinking water to broiler birds in group OA-0, OA-0.5, OA-1, OA-1.5 and OA-2 at the rate of 0, 0.5, 1, 1.5 and 2ml/Liter of water, respectively. All birds were given free access to feed and water. Birds were routinely monitored and dead and sick birds, if any, were removed and unusual observations were recorded. The experimental trial was run for 21 days (22-42 days). The feed was provided according to the recommendation of NRC (1994).

Performance traits

Daily and cumulative feed intake was

calculated by providing known amount of feed and measuring feed refusal on daily basis. Birds were initially weighed on day 22nd and then at the end of every week to determine total body weight gain. Feed conversion ratio (FCR) was calculated on weekly basis and adjustment for mortality was made. Two healthy birds were randomly selected from replicates of all groups and were live weighed individually. These birds were humanely slaughtered, skinned off and all internal edible and non-edible organs were removed. Carcass yield was determined and were expressed in term of dressing percentage. Water intake was measured on daily basis by subtracting refused water from the total water offered.

Determination of E. coli and Salmonella load

At the end of the experiment, two birds from each cage were randomly selected for digesta sampling in the lower ileum. Samples were taken in plastic 50-ml Falcon tubes (Corning Incorporated, Corning, N.Y.) and cooled until incubation. The samples were processed quickly after collection. Samples were weighed (1 g) and serially diluted (ten fold) in 0.9% saline, vortexed and 1 ml of each sample was dispensed and spread on selective media in Petri dishes. Brilliant Green Agar media (Oxoid, Basingstoke, UK) was used for *Salmonella* and MacConkey agar plates (Oxoid, Basingstoke, UK) were used for *E. coli*. Microbial suspension from each dilution of a particular sample was transferred through pour plate method (Quinn *et al.*, 1992) and incubated at 37°C for 24 h. The pathogens were identified by growing on specific media and biochemical tests. After then the colonies were counted through colony counter. The total colony count was (expressed as log₁₀ cfu/g of contents) determined by multiplying reciprocal of the dilution factor and average numbers of colonies.

Statistical analysis

Data was subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1981). Duncan's multiple range test was used to find the significance difference (Duncan, 1955). P value less than 0.05 was considered significant.

Table I. Effect of organic acid blend on feed intake (g), body weight (g), water intake (ml) and FCR (g/g) of broiler during finisher phase.

Groups	Feed intake (g)	Body weight (g)	Water intake (ml)	FCR (g/g)
Control	2517.9± 4.44	1210.1 ^b ± 3.56	7180.7± 6.25	2.08 ^a ± 0.02
OA-0.5	2504.0± 4.40	1240.6 ^b ± 4.24	7219.6± 8.34	2.01 ^b ± 0.02
OA-1	2502.4± 4.43	1276.0 ^a ± 3.91	7357.1± 7.49	1.96 ^c ± 0.01
OA-1.5	2496.2± 4.54	1294.3 ^a ± 2.28	7350.1± 6.46	1.92 ^c ± 0.03
OA-2	2492.3± 3.89	1306.9 ^a ± 3.81	7364.8± 6.51	1.91 ^c ± 0.02
P value	0.64	0.004	0.36	0.002

Mean values bearing different superscript differ significantly (P<0.05); OA, Organic acid.

Table II.- Effect of organic acid blend on carcass yield (%), liver weight (g) and heart weight (g) of broiler during finisher phase.

Group	Carcass yield (%)	Liver weight (g)	Heart weight (g)
OA-0	65.32 ^c ±0.55	39.66 ^b ±3.1	10.60±0.25
OA-0.5	66.43 ^{bc} ±0.36	43.00 ^{ab} ±2.1	9.66±0.66
OA-1	67.13 ^b ±1.10	46.33 ^{ab} ±2.7	11.56±0.80
OA-1.5	68.94 ^{ab} ±7.79	50.66 ^a ±1.9	10.76±0.89
OA-2	70.39 ^a ±4.99	51.16 ^a ±2.8	12.36±0.36
P value	0.01	0.02	0.1106

Mean values bearing different superscript differ significantly (P<0.05); OA, Organic acid.

RESULTS

The results of performance traits are given in Table I. The results showed that feed intake did not differ between the groups. Body weight increased significantly in treated groups. A linear increase in body weight was observed with the increasing level of organic acid supplementation. Similarly, FCR was also improved in the treated group in the linearly without effecting the water intake. Carcass yield, liver weight and heart weight are given in Table II. Significantly high carcass yield was observed in OA-2 group. Liver weight was also significantly high in OA 1.5 and OA 2 groups. There was no significant effect of the treatment on the heart weight. The *E. coli* and *Salmonella*

population decreased significantly in the treated groups linearly with increasing the concentration of organic acid, however, there was no significant difference in mortality percentage between the control and treated groups (Table III).

Table III.- Effect of organic acid blend on count of *E. coli*, *Salmonella* and mortality percentage of broiler during finisher phase

Group	<i>E. coli</i> (log ₁₀ cfu/g)	<i>Salmonella</i> count (log ₁₀ cfu/g)	Mortality (%)
OA-0	4.34 ^a ±0.02	3.14 ^a ±0.04	11.11±4.44
OA-0.5	4.01 ^b ±0.04	2.74 ^b ±0.03	8.88±4.44
OA-1	3.91 ^c ±0.02	2.65 ^b ±0.04	8.88±4.44
OA-1.5	3.76 ^d ±0.01	2.54 ^c ±0.02	4.44±2.22
OA-2	3.70 ^c ±0.01	2.52 ^c ±0.02	6.66±3.84
P value	0.0000	0.0000	0.8002

Mean values bearing different superscript differ significantly (P<0.05); OA, Organic acid.

DISCUSSION

Results revealed that the feed intake was not significantly affected by different levels of organic acid during finisher phase in broiler chicks. Our findings regarding feed intake are in agreement with that of Adil *et al.* (2010) who reported non significant effect of organic acids on feed consumption in broiler chicks. Hernandez *et al.* (2006) also reported no effect of organic acids on feed intake in broiler chicks. Feed intake depends on many factors in which palatability is the most important. According to Adil *et al.* (2010) organic acids have strong taste which might have decreased the palatability of feed and resulted in reduced feed intake. Lesson *et al.* (2005) also reported reduction in feed intake due to organic acids in broiler chicks. Organic acids increase the availability of nutrients from the feed which in turn decrease the feed consumption (Pakhira and Samanta, 2006).

According to the results of this study, body weight increased linearly by using different levels of organic acid during finisher phase in broiler chicks which suggest that the organic acid and dose rate in present study have beneficial effect on growth. Adil *et al.* (2011) suggested that the antimicrobial property and low pH of organic acid inhibits the

pathogenic intestinal bacteria and decreases the level of toxic bacterial products. As a result, energy and protein digestibility is improved, thereby increasing the weight gain of broiler chicken. Dibner and Butin (2002) found that organic acids improve the protein digestibility by decreasing endogenous nitrogen losses and production of ammonia as well as other growth depressing metabolites. The organic acid at high dose rate in the present study revealed significantly enhanced weight gain as compared to the other treatments and control group. Similar observations were also recorded by Sultan *et al.* (2014) who found that higher feed conversion with the administration of citric acid in poultry.

Our results are also in agreement with the Denli *et al.* (2003) who reported that different organic acid resulted intestinal cell proliferation and hence the nutrients more available for absorption that is also supported by Samanta *et al.* (2010) who reported that the acidic condition increases the nutrients availability for better performance (Boling *et al.*, 2001).

The organic acid at high dose rate in the present study showed significantly larger liver while heart weight was non significant as compared to the other treatments and control group.

Results of the present study are justified by Islam *et al.* (2008) who reported positive effect of citric acid on liver weight in broilers. Our study is in line with Ali *et al.* (2012) who reported that the organic acid significantly increased the weight of liver and gizzard in Japanese quails.

The organic acid at high dose rate in the present study showed significant reduction in total *Salmonella* and *E. coli* counts as compared to the other treatments and control group. Our results also supported by Chowdhury *et al.* (2009) who reported that the use of citric acid produces acidic environment in the gut thus favors the development of lactobacilli and inhibits the replication of *Salmonella*, *E. coli* and other Gram negative bacteria. The organic acid eliminates the coliforms from the gut by reducing the pH, which is unable for the multiplication of the acid-intolerant species such as *Salmonella* and *E. coli*. Organic acids reduce *E. coli* and other harmful bacteria which may have enhanced poultry growth (Samanta *et al.*, 2010).

The results revealed that dressing percentage numerically higher in group OA-2 followed by OA-1.5, OA-1, OA-0.5 and OA-0 by using different levels of organic acid during finisher phase in broiler chicks. The organic acid and dose rate in present study have beneficial effect on growth. Our findings regarding carcass yield are in well agreement with the previous finding of Kahraman *et al.* (1997) where no significant effect was observed. Furthermore our results also in line with the Islam *et al.* (2008) who reported that the citric acids have positive effect on dressing percentage but statistically non significant in broiler chickens. The results revealed that mortality rate is non significant but decreased numerically. Our result is supported by Simons *et al.* (1990) that citric acid decreases the mortality in broiler chickens.

In conclusion, the present results indicated that the application of organic acid in different concentrations improved the production performance and lowered that pathogenic load in the gut linearly with increasing the levels of organic acids. From the present results, we suggest that 2% organic acid is most useful for optimum results.

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