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Research Article

Lower Illeal Microflora and Growth Performance of Broilers Supplemented with Organic Acid Blend (Aciflex®) During Starter Phase

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ABSTRACT

Organic acids can be replaced as effective feed additives with antibiotics. Organic acids and their salts have the potential to retard the development of pathogens in the diets and thus maintain the microbial equilibrium in the gastrointestinal tract. In this study, 225 day-old broiler chicks were used for a period of 21 days in order to examine the effect of different levels of organic acids (OA) on performance of broiler. The following parameters: feed intake, water consumption, weight gain, feed conversion ratio (FCR), total Salmonella and *E. coli* count, weight of visceral organs and length of intestine were studied during starter phase. The experimental chicks were divided into five groups (n=5, 15 birds/replicate). The chicks in control group OA-0 were offered clean drinking water having no organic acid while those in group OA-1, OA-1.5 and OA-2 were given 1, 1.5 and 2ml of organic acid blend. Each litter of drinking water contained citric acid 80g, lactic acid 52g, phosphorous 92g, copper sulphate 10g, respectively. Organic acid (2ml/L) supplementation significantly ($p<0.05$) increased feed intake (405.37g), weight gain (218g), FCR (1.42), weight of liver (0.02g) and gizzard (0.03g). Similarly in group OA-2 organic acid supplementation significantly decreased ($p<0.05$) the total salmonella ($1.6x10^7$) and *E. coli* ($1.7x10^7$logCFU/g) count in intestinal contents. From present findings it was concluded that organic acid supplementation at level of @ 2ml/L in drinking water had beneficial effect on broiler performance and also decreased the colonization of intestinal bacterial counts.

INTRODUCTION

Poultry production on commercial scale began in Pakistan in 1963. New hybrid varieties were introduced to the poultry. A modern hatchery was established in Karachi by Pakistan International Airlines in shares with the Shaver (Canadian firm) in 1963. A series of layer and broiler farms, feed mills and hatcheries have been established then in the private sector (Mohsin *et al.,* 2008). Poultry, the second biggest industry of Pakistan has developed as an outstanding alternative of beef and mutton. Its value can be recognized from the reality that almost every family in under developed country and every fifth family in developed country is linked directly with poultry sector in one way or the other. Pakistan has produced about 834,000 tons of poultry meat and 13,144 million eggs in 2011-12 (Economic Survey of Pakistan, 2011-12).

Poultry industry is always looking for a new feed supplemented in order to bring improvement in feed effectiveness and chicken health. Organic acids shows potential to be used instead of antibiotics and other feed additives (Hyden, 2000). Organic acids and their salts are able to reduce the growth of microorganism in the feed and thus preserve the microbial balance in the gastrointestinal tract. By modifying intestinal pH, organic acids also enhanced the solubility of the feed ingredient, digestion and absorption of the nutrients (Vogt *et al*., 1981; Patten and Waldroup, 1988; Skinner *et al*., 1991).

Ban on the use of antibiotics as growth promoter in animal nutrition in 2006, the nutritionists and researchers attempted other alternative to improve the performance of broiler chicken. Such alternative was the use of organic acids as feed additive in the animal production.

Organic acids and their salts are generally used in many countries as growth promoter while in many countries the use of antibiotic as a growth promoter is banned in livestock and poultry diets. This initiated the scientists to search for alternatives which were helpful for poultry birds and livestock and having non-significant impact on consumer health. In alimentary canal there are two types of bacterial populations, one is pathogenic and the other is beneficial commensals. The former inhibits growth which is facilitated by the latter one (Samanta *et al.,* 2010).

Organic acid supplementation has been reported to decrease colonies of pathogens and production of toxic metabolites, improve digestibility of protein and minerals serve as substrates in the metabolism (Kirchgessner and Roth, 1998). Dietary supplementation of organic acids increases the feed conversion ratio and body weight in broiler chicken (Gauthier, 2000) and reduces colonization of pathogens on the intestinal wall, thus prevents damage to the epithelial cells (Langhout, 2000). As beside antibiotics, organic acids have properties of lowering pH and consequently enhancing protein digestion (Skinner *et al.,* 1991).

Supplementing poultry diets with organic acids has become an important nutritional strategy aimed to improve performance and health status of poultry fed diets. Organic acids, as feed additives have received increasing attention as alternative. They posses tremendous contribution to the effectiveness in the intensive husbandry and provide people with healthy and nutritious poultry products (Patten and Waldroup, 1988). Organic acids may inspire endogenous enzymes, adjusting gut microbial flora and help in maintaining animal's health. The basic principle on the mode of action of organic acids on bacteria is that no dissociated organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (Dhawale, 2005).

MATERIALS AND METHODS

The research was conducted at the poultry unit of The University of Agriculture, Peshawar. A total of 225- day-old chicks were obtained from commercial market and divided into five groups designated as OA-0, OA-0.5, OA-1, OA-1.5 and OA-2, having 3 replicates of 15 broiler chicks each. Organic acids (i.e. citric acid 80 g, lactic acid 52 g, phosphorus 92 g and $CuSO_4$ 10 g) were offered to the broiler chicks in group OA-0, OA-0.5, OA-1, OA-1.5 and OA-2 at the dose rate of 0, 0.5, 1, 1.5 and 2ml/liter respectively for 21 days.

Isolation and identification of *Salmonella and E. coli*

Samples were collected from lower part of intestine aseptically and transported at 4ºC to the bacteriology laboratory of the Animal Health Department. A loop full of broth was streaked on plates of Brilliant Green Agar (Oxoid, Basingstoke, UK) for S*almonella* and MacConkey agar (Oxoid, Basingstoke, UK) for *E. coli*. The plates were incubated at 37ºC for 24 hrs and suspected colonies of *Salmonella* from each plate was collected for presumptive identification based on their morphological characteristics and various biochemical tests and the bacteria were counted on bacterial counting machine.

Preparation of culture media

Commercially available media ready to use were rehydrated in distilled water. The following media were prepared and used during this study.

MacConkey's agar

MacConkey's agar was used as a selective medium for the isolation and purification of Coliform organism to distinguish lactose fermenting characteristics of bacteria from non- lactose fermenting bacteria.

Preparation

Commercially prepared dehydrated medium was used at the concentration of 52g in every 1000ml of distilled water. The prepared agar was sterilized in the autoclave for 15 min under 15 lb pressure at 121ºC. The media was allowed to cool at 50ºC and then 15ml each were distributed into sterilized petri dishes.

Identification of *E. coli* and *Salmonella*

A smear of the culture was prepared on glass slide and fixed for 2 to 3 times over flame. Crystal violet dye (basic stain) was poured on smear for 1 min and gently washed with water. Then Gram's iodine solution (mordant) was applied on the smear for 1 min and gently washed with water. For decolorization with ethyle alcohol, it was poured on smear for 15 sec and gently washed with water. Counter stain was done by taking few drops of Safronine for 0.5-1 min and gently washed with water. Blot was air dried and then examined under microscope using 100x oil immersion lens. Pink color and rod shaped organisms were indication of *E. coli* and *Salmonella.*

Feed Intake and water consumption

Daily feed intake was used to work out weekly feed intake. Feed intake was calculated by feed offered – feed refused and water consumption was calculated by subtracting daily water refused from water offered.

Weight gain and feed conversion ratio (FCR)

Weekly weight gain was calculated by subtracting initial weight from final weight of each week and weekly FCR was calculated by dividing weekly feed consumed by weekly weight gain.

Weight of carcass and visceral organs and length of intestine

Two birds were randomly selected from each replicate at the end of experimental period and were slaughtered. The head, legs, and visceral organs were removed to work out carcass weight**.** Visceral organs i.e. liver and heart were weighted separately by using an electric digital balance. Intestinal content was removed and length of intestine from duodenum to caecum was measured.

Statistical analysis

The relative data was analyzed through standard procedure of analysis of variance (ANOVA) using complete randomized design (CRD) as suggested by Steel and Torie (1981). The statistical package SAS (1997) was applied to complete the data analysis.

RESULTS AND DISCUSSION

This research was performed to evaluate the effect of organic acids on the performance of broiler chicks. The following results were obtained.

Water Intake and feed consumption

Mean data showed no (P>0.05) effect on water intake from the first day of the experiment till end (Table 1). However, numerical variation was observed among the groups treated with organic acid to that of control. Higher water intake was observed in group that was fed with organic acid at rate of 2ml/lit of drinking water and followed by the group at the rate of 1.5ml/lit of drinking water. It seems that organic acid has improved water quality by minimizing microbial load that normally disturbs drinking quality and smell. Due to better water taste the birds may have consumed more water than non- treated water.

Table 1: Mean water intake (ml) in broiler chicks for the three weeks treated with organic acid

Groups	Mean ± SE			
	Week 1	Week 2	Week 3	Over all
Control	399.99±8.81	766.66±43.33	1269.11±61.69	811.62±37.94
OA-0.5	406.00±19.25	869.33±4.44	1381.55±41.48	885.62±21.72
OA-1	373.33±21.16	813.33±33.38	1384.45±41.82	857.03±32.12
OA-1. 5	418.08±11.22	878.89±9.49	1435.56±65.47	910.84±28.72
OA-2	411.11±8.01	876.00±28.00	1471.11±53.51	919.40±29.94
P-Value	0.3145	0.0649	0.1669	

Means with different superscript in column are significantly different at α=0.05

Data showed that feed consumed by the birds throughout the experimental period was significantly different (P<0.05) in all treatments (Table 2). The organic acid at high dose rate in the present study showed significantly (P<0.05) higher feed consumption. Highest feed consumption (405.37 g) was recorded for treatment OA-2, while lowest feed consumption (265.29 g) was noted in control group. Similarly, highest weekly feed consumption (585.08 g) was achieved on 3rd week, while lowest feed consumption (139.94 g) was observed on 1st week. Results of this study are justified with Mohamed and Bahnas (2009), who reported significant effect (P<0.05) on feed intake by using malic acid supplementation as a growth promoter. Organic acids increase gastric proteolysis and enhance digestibility of protein and amino acids, decrease the pH of the gut which improves the bird performance (Samanta *et al.,* 2010). However, present results are in contrast to the findings of Sacakli *et al.* (2006) who reported no-significant (P<0.05) effect on feed intake while using organic acid which might be due to different acids used in the studies.

Table 2: Mean feed consumed (gm) in broiler chicks treated with organic acid.

Group	Mean±SE			
	Week 1	Week 2	Week 3	Mean
Control	123.771.89	1 72.20^{c}±10.29	499.91^{cd}±8.44	265.29^{e} ±6.87
OA-0.5	123.37^{c}±4.71	267.89^{b}±18.40	486.42^{d}±34.44	292.59^{d} ±19.183
OA-1	141.31^{b}±2.63	285.93^{ba}±11.58	554.71^{c}±18.68	327.31^{c} ±10.96
OA-1.5	150.17^{b}±2.56	273.71^{ba}±10.44	638.53^{b}±38.49	354.13^{b} ± 17.16
OA-2	161.08^{a}±3.41	309.17^{a}±8.79	745.86^{a}±11.89	405.37^{a} ± 8.03
P-Value	0.0001	0.0001	0.0001	0.001

Means with different superscript in column are significantly different at α=0.05

Weight gain

The organic acid at high dose rate showed significantly (P<0.05) higher weight gain as compared to the other treatments and control group (Table 3). Pooled data showed that highest weight gain (218.03 g) was recorded for treatment OA-2, while lowest weight gain (182.67 g) by the chicks was noted in control group. Similarly, in the case of week highest weight gain (357.28 g) by the chicks was achieved on 3rd week, while lowest weight gain (98.08 g) was noted on 1st week.

Table 3: Mean weight gain (g) of broiler chicks treated with organic acid.

Group	Mean±SE			
	Week 1	Week 2	Week 3	Overall
Control	89.13^{c}±2.07	130.13±4.69	328.77^{d}±7.20	182.67^{e}±4.65
OA-0.5	87.11^{bc}±4.41	159.44±5.02	350.55^{c}±2.89	199.03^{d}±4.10
OA-1	99.86^{ba}±8.98	157.13±13.05	359.77^{cb}±0.77	205.58^{c}±7.6
OA-1.5	104.28^{ba}±3.24	146.31±18.16	367.66^{a}±3.02	206.08^{b}±8.14
OA-2	110.06^{a} ±4.11	164.38±9.56	379.66^{a}±1.67	218.03^{a}±5.11
P-Value	0.0440	0.2865	0.0001	0.001

Means with different superscript in column are significantly different at α=0.05

The present results are in line with the findings of Dibner and Buttin (2002), who showed that organic acids improve protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of subclinical infections and secretion of immune mediators, and by reducing production of ammonia and other growth-depressing microbial metabolites. Adil *et al*. (2011) also found the same results and mentioned that the antimicrobial and pH reducing properties of organic acids have resulted in inhibition of intestinal bacteria leading to the reduced bacterial competition with the host for available nutrients and decreasing the level of toxic bacterial products resulting in improvement of protein and energy digestibility, thereby increasing the weight gain and performance of broiler chicken. Our results match with the following authors (Patten and Waldroup, 1988; Skinner et al., 1991; Mujdat *et al.,* 1999; Adil et al., 2010) who stated that weight gain was significantly affected on week 3 by organic acid.

Feed conversion ratio (FCR)

The feed conversion ratio by the birds in week 2 and week 3 are significantly different (P<0.05) while non-significant in week 1 (p>0.05) (Table 4). The organic acid at high dose rate in the present study showed significantly (P<0.05) highest feed conversion ratio (1.38) on week 1st for treatment O A - 2 , while lowest feed conversion ratio (1.46) for control. Similarly, on 2nd week highest feed conversion ratio (1.32) was achieved for treatment OA-2, while lowest feed conversion ratio (1.88) was noted for control. On week 3rd highest feed conversion ratio (1.38) was noted for treatment OA-1.5, while lowest feed conversion ratio (1.96) was noted for control. The feed conversion ratio results in present study are supported by Samanta *et al.* (2010) who stated that organic acids reduce *E. coli* and other harmful bacteria which increase poultry growth. It also produces acidic condition that makes the nutrients more available for better performance (Boling *et al.,* 2001). Similarly, our result are also in line with Hassan *et al.* (2010) who stated that organic acids significantly (p<0.001) improved feed conversion ratio. These results also agreed with the findings of Isabel and Santos (2009); Parker (2011), who investigated that organic acid has significantly affected the FCR. However, present results are contrasted by Sacakli *et al.* (2006) who reported no significant effect (P>0.05) on FCR by phytase and organic acid.

Table 4: Mean feed conversion ratio (FCR) of broiler chicks fed with organic acid.

Group	Mean ± SE			
	Week 1	Week 2	Week 3	Over all
Control	1.46±0.02	1.88^{a}±0.09	1.96^{a}±0.02	1.76^{a}±0.04
OA-0.5	1.44±0.01	1.87b^{a}±0.06	1.73^{b}±0.08	1.68^{b}±0.05
OA-1	1.41±0.13	1.81^{b}±0.04	1.54^{a}±0.09	1.58^{b}±0.14
OA-1. 5	1.41±0.04	1.68^{b}±0.04	1.38^{d}±0.03	1.49^{d}±0.03
OA-2	1.38±0.02	1.32^{c}±0.08	1.52^{a}±0.15	1.40^{c}±0.08
P-Value	0.9309	0.0302	0.0006	0.001

Means with different superscript in column are significantly different at α=0.05

Total *salmonella* and *E. coli counts*

Total salmonella count (TSC) was significantly decreased on day 21 (P<0.05) but no effect on day 0 (P>0.05 (Table 5). The organic acid at high dose rate showed significantly (P<0.05) lower TSC as compared to the other treatments including control groups. Highest TSC (2.9) was recorded in control treatment, while lowest TSC (2.2) were noted for OA-1.5 and OA-2 respectively. Similarly, TSC was higher (2.8) at beginning of experiment while lower (1.6) at the end. The organic acids eliminate the coliforms from the gastrointestinal tract by reducing the pH, which is unsuitable for the multiplication of the acid-intolerant species such as *E. coli* and *Salmonella* (Panda *et al.,* 2009). Our results are in line with Hassen (2011); Park (2011), who stated that total s*almonella* and *E. coli* counts were significantly affected by organic acid. Organic acids reduce *E. coli* and other harmful bacteria which may have enhanced poultry growth (Samanta *et al.,* 2010).

Table 5: Mean of Total Salmonella count (TSC) in broiler chicks treated with organic acid (Log CFU/g)

Group	Mean ± SE		Over all
	TSC 0	TSC 21	
Control	2.9±0.33	2.4^{a}±0.33	2.6^{a}±0.33
OA-0.5	2.9±0.57	1.9^{a}±0.88	2.4^{b}±0.72
OA-1	2.8±1.15	1.9^{b}±0.57	2.3^{c}±0.86
OA-1.5	2.7±1.20	1.7^{c}±0.57	2.2^{cd}±0.88
OA-2	2.8±0.33	1.6^{c}±0.57	2.2^{cd}±0.45
P-Value	0.5995	0.0001	0.001

Means with different superscript in column are significantly different at α=0.05

Total *E. coli* count (TEC) was significantly decreased on day 21 (P<0.05) (Table 6). The organic acid at high dose rate showed significantly (P<0.05) lower TEC as compared to the other treatments and control groups. While highest TEC (2.6) was recorded in control treatment.

Table 6. Mean of Total E. coli Count (TEC) in broiler chicks treated with organic acid (Log CFU/g)

Group	Mean ± SE		Over all
	TEC 0	TEC 21	
Control	2.9±0.57	2.4^{a}±0.88	2.6^{a}±0.72
OA-0.5	2.7±0.88	2.3^{a}±0.66	2.5^{ab}±0.77
OA-1	2.9±0.66	2.1^{b}±0.57	2.5^{ab}±0.61
OA-1.5	2.8±0.66	1.8^{c}±0.88	2.3^{c}±0.77
OA-2	2.9±0.66	1.7^{d}±0.57	2.3^{c}±0.16
P-Value	0.2804	0.0001	0.001

Means with different superscript in column are significantly different at α=0.05

Gizzard, Liver and carcass weights and Intestine length

The gizzard and liver weights of the birds were significantly different (P<0.05) while intestine length and carcass weight were non-significant on day 21 (p>0.05) (Table 7). The organic acid at high dose rate in the present study showed significantly (P<0.05) larger gizzard (0.03g) and liver weight (0.021 g) as compared to the other treatment and control group, while lowest gizzard weight (0.024g) and lowest liver weight (0.01g) for control was achieved for control. Highest intestine length (4.86 Inc) was recorded in OA-0.5 treatment, while lowest intestine length (4.50 Inc) was noted in control. Highest carcass weight (458.66 g) was found in OA-2, while lowest carcass weight (368.66 g) was noted in control treatment.

Table 7: Mean of gizzard (g), liver (g) and carcass wt (g) and intestine length (inch) broiler chicks treated with organic acid.

Group	Mean ± SE			
	Gizzrad wt (g)	Liver wt(g)	Intestine length (inch)	Carcass wt (g)
Control	0.024^{a}±0.001	0.01^{b}±0.00	4.50±0.2886751	368.66±59.62
OA-0.5	0.03^{b}±0.002	0.01^{a} ±0.001	4.86±0.18	404.00±17.15
OA-1	0.03^{a}±0.002	0.021^{a}±0.001	4.40±0.23	420.00±2.51
OA-1.5	0.02^{ab}±0.001	0.02^{a}±0.00	4.70±0.35	417.00±10.21
OA-2	0.03^{ab}±0.001	0.02^{a}±0.00	4.83±0.24	458.66±19.36
P-Value	0.0031	0.0499	0.6746	0.3657

Means with different superscript in column are significantly different at α=0.05

Results of the present study are justified by Islam *et al.* (2008) who reported positive effect (P<0.05) on liver weight by citric acid and acetic acid in broilers. Our results do not match with Adil *et al.* (2011) who stated that intestine length was significantly affected by organic acid. Similarly, the findings of Mujadat *et al.* (1999) match with our results, who stated that carcass was non-significantly affected by organic acid. Our results are in contrast with the observations recorded by Denli *et al.* (2003) who reported significant increase in intestinal length by supplementation of different organic acids in broiler chicken. This could be increase in the goblet cell size and villus height that was improved due to organic acid supplementation (Çelik and Mutluay, 2007). This indicates improved intestinal length. That led to enhanced growth performance of broiler birds. The difference of the result may be due to the difference of acid used by the previous authors.

CONCLUSIONS

It was concluded from present results that organic acid has significantly increased feed intake, body weight gain, FCR, gizzard and liver weight in broiler chicks. Organic acid at the level of OA-2 (2ml/1L drinking water) improved the gut efficacy and significantly decreased the total *Salmonella* and *E .coli* Counts as compared to control group.

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