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Influence of Feed Sanitation on Zootechnical Performance, Prevalence, Immune Status and Carcass Trait of *Salmonella typhimurium* Infected Broiler Chickens

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ABSTRACT

An experiment was conducted to determine the effects of a commercial acidifier feed additive product containing formaldehyde on performance, *Salmonella typhimurium* prevalence and immune status of broiler chickens. Three hundreds, day-old chicks (Hubbard breed) were randomly distributed into three treatment groups (4 replicates each) using 25 chicks per replicate on floor pens. Control (C) birds were offered non-supplemented basal diets. Treatment groups 1 and 2 (T1 and T2) were fed diets containing product at 250 and 500 g t^{-1} feed, respectively. Feed and water were offered *ad-libitum* for 35 days experimental period. Feed consumption and body weight were recorded weekly to calculate body weight gain and feed conversion. Blood samples were collected by time intervals to evaluate the immune status of the birds against some vaccines. At day 21 of age,20 birds were chosen randomly from each group (5 from each replicate) and were challenged orally with 1 mL containing 10^6 colony forming unit (CFU mL^{-1}) *Salmonella typhimurium* and were kept under observation for two weeks. At the end of the experimental period, 20 birds were chosen randomly from each group (5 from each replicate) to compare carcass yield. The results revealed that body weight gain was significantly ($p<0.05$) improved in chicks fed on diets containing product compared with the control one. The best feed conversions were recorded in T2. The results of the *Salmonella typhimurium* challenge experiment showed that both doses of the products significantly ($p\leq0.05$) reduced the signs, mortalities, gross lesions, shedding rate and re-isolation of *Salmonella typhimurium*. Dressing percentage and liver weight were non-significantly different between groups. Supplementation of the broiler diets significantly enhanced the immune responses measured against the vaccines used. It can be concluded that, using acidifiers and formaldehyde as feed additives for feed sanitation reflected positively on the zootechnical performance of broiler chickens, reduced the incidence of salmonellosis and enhanced the immune status of broiler chickens.

Key words: Formaldehyde, broilers, acidifiers, salmonellosis, performance, immunity

INTRODUCTION

Salmonella persist for long periods in a wide range of feed materials and considered as major microbial hazard. Feed pelleting reduces but may not completely eliminate, *Salmonella* contamination because of limitations of the process or recontamination after thermal processing

(Jones, 2011). When *Salmonella* are consumed by animals or birds, the adapted strains tend to multiply within the host, causing a diseased or carrier state, whereas less adapted strains either are maintained and shedding to affect environment and/or human health. Consequently, it is important to endeavour to control *Salmonella* contamination wherever it is found. Chemicals used to control *Salmonella* in feeds have primarily consisted of blends of organic acids (mainly formic and propionic acids) and formaldehyde (Ricke, 2005). Superior decontamination of feed by formaldehyde, compared with acid products, has been demonstrated (Smyser and Snoeyenbos, 1979). Some commercial formaldehyde based products may also contain organic acids or other antimicrobial compounds to minimize the effects of evaporation on antimicrobial activity (Carrique-Mas *et al*., 2007). In addition, formaldehyde products have been used at higher levels on a carrier (i.e., grain, soybean meal or wood chips) to decontaminate the interior surfaces of inaccessible equipment (Torroella *et al*., 1987). However, Food and Drug Administration allowed the use of formaldehyde as an antimicrobial feed additive for maintaining *Salmonella* free poultry feeds for up to 14 days. The allowed limit of its use is 2.5 kg t^{-1} (37% aqueous solution) of feed. Up to now the impact of using acidifier feed additive products containing formaldehyde on productive performance and *Salmonella* prevalence in poultry is still unclear.

Thus, the main objective of this experiment was to study and compare the efficacy of a commercial acidifier feed additive product containing formaldehyde on zootechnical performance, *Salmonella typhimurium* prevalence, carcass trait and immune status of broiler chickens.

MATERIALS AND METHODS

Tested product: A commercial product containing a mixture of short chain organic acids and formaldehyde was used. Each kilogram contains 100 g propionic acid (99%), 100 g ammonium propionate, 100 g formic acid (85%) and 200 g formaldehyde (40%). The product is manufactured by Dex Iberica, S.A. Animal nutrition experts, Spain.

Experimental birds: Three hundred days old chicks (Hubbard breed) of both sexes were obtained from a commercial local hatchery. Chicks were weighed and randomly distributed into three dietary treatment groups (4 replicates each) using 25 chicks per replicate on floor pen at the Poultry Experimental station, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt. Birds in all experimental groups were vaccinated against Newcastle Disease (ND), Infectious Bronchitis (IB) and Gumboro (IBDV) diseases "Intervet, Inc., Egypt" as recommended.

Diets and feeding program: Corn-soybean meal basal diets were formulated to cover the nutrient requirements for Hubbard broilers. Three stages diets (starter, grower and finisher) in the form of mash and water were provided *ad-libitum* during the 35 days experimental period (Table 1). Control (C) birds were offered non-supplemented basal diets. Treatment groups 1 and 2 (T1 and T2) were fed diets containing the product at 250 and 500 g t^{-1} feed, respectively. Feed and water were offered *ad-libitum* for 35 days experimental period. Feed consumption and body weight were recorded weekly to calculate body weight gain and feed conversion. The individual body weights in all replicates as well as the rest of feed were recorded weekly. The consumed feed for each replicate was divided by the number of birds per replicates to calculate consumption/bird/week. Body weight gain and feed conversion were calculated.

Blood samples: Blood samples were collected from all groups (5 samples/replicate) weekly. Antibody titres against Newcastle (NDV) using Haemagglutination Inhibition (HI) test were

Table 1: Composition percentage and calculated nutrients profile of the basal diets

Ingredients (%)	Starter (1-20 days)	Grower (21-30 days)	Finisher (31-35 days)
Yellow corn	51.70	56.15	61.15
Corn gluten meal	5.00	5.00	5.00
Soybean meal (44% CP)	37.30	31.50	25.90
Soy oil	2.20	3.50	4.00
Dicalcium phosphate	1.60	1.60	1.70
Limestone	1.40	1.45	1.44
Common salt	0.40	0.40	0.40
DL-Methionine	0.05	0.05	0.06
L-Lysin	0.05	0.05	0.05
Vitamin and mineral premix*	0.30	0.30	0.30
Calculated analysis			
ME (kcal kg^{-1})	2951.80	3049.55	3124.07
Crude protein (%)	23.20	21.29	19.00
Crude fat (%)	6.00	6.92	8.00
Crude fibre (%)	4.50	4.80	5.20
Calcium (%)	1.00	1.00	1.00
Non-phytate phosphorus (%)	0.45	0.45	0.45

*Per kg premix vit A: 1 200 000 IU, vit D_3: 350 000 IU, vit E: 4 000 mg, vit B_1: 250 mg, vit B_2: 800 mg, vit. B_6: 600 mg, vit B_{12}: 3.2 mg, vit K_3: 450 mg, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, 40 mg Co

measured. Antibody titres against Gumboro disease (IBDV) using ELISA kit according to the manufacturer's instructions (QIAGEN Leipzig GmbH, Germany) were detected.

Challenge inoculum of *Salmonella typhimurium*: Chicken's field strain of *Salmonella typhimurium* containing 10^6 colony forming unit CFU mL^{-1} was prepared according to the method mentioned by Bjerrum *et al*. (2003). At day 21 of age, 20 birds from each group (5 birds/replicate) were isolated and bacteriological swabs were taken from cloaca and were tested for any inherent *Salmonella typhimurium* before challenge. The isolated birds were challenged orally with 1 mL broth culture containing 10^6 CFU mL^{-1} of the local isolate of *Salmonella typhimurium*. Infected birds were kept under complete daily observation for 15 days post experimental infection for recording clinical signs or mortalities. Dead birds were macroscopically examined for *Salmonella typhimurium* lesions (O'Brien, 1988). At 35 day of age the challenged birds were euthanized, bacteriological swabs were taken from the liver and from the intestine to examine the prevalence of *Salmonella typhimurium* (Gama *et al*., 2003).

Evaluation of humeral immune response: Detection of antibodies titer to NDV vaccine using Haemagglutination Inhibition (HI) test according to Beard (1989) with chicken RBCs and 4 units of NDV antigen, then geometric mean titers were calculated. Detection of Antibodies titer to IBDV Vaccine using ELISA kits (BioChek) according to the manufacture's instruction.

Evaluation of innate immunity: Assay of phagocytosis was performed according to Bos and de Souza (2000) with some modification where phagocytic percent (number of phagocytic macrophages/total number of macrophages) and phagocytic index (number of macrophages engulf ≥3 *Candida* spores/total no of phagocytic macrophages). Lysozyme activity was measured by agarose gel plate lyses assay according to Peeters and Vantrappen (1977). Nitric Oxide (NO) assay was carried out according to Yang *et al*. (2010).

Carcass yield: At 35 days of age 20 birds from each group (5 birds/replicate) were chosen randomly. The chosen birds were weighed, slaughtered and dissected to compare live body weights, dressing percentage, liver weights and abdominal fat.

Statistical analysis: All data was statistically analyzed using IBM SPSS® version 19 software. Means were compared by one way ANOVA ($p<0.05$) using Post Hoc test according to Snedecor and Cochran (1980).

RESULTS

Zootechnical performance: The effects of different levels of feed additive on the productive performance of broiler chickens are shown in Table 2. Broiler chickens fed the diets supplemented with low and high doses of feed additive attained higher ($p<0.05$) body weights and body weight gains compared to non supplemented group. Highest body weight gains were achieved in the birds fed on diets containing 500 g t^{-1} of the product. Also, the effect of dietary organic acids and formaldehyde mixture feed additive on body weight gain was significantly different ($p<0.05$) than that of the control group. The feed consumption was found statistically non-significant ($p>0.05$) among all the treatment groups (Table 2). Chicks fed the diets supplemented with organic acids showed improvement in the FCR compared with the chicks fed control diet (Table 2). The best feed conversions were recorded in T2.

Challenge of *Salmonella typhimurium*: The results of challenges of broiler chickens with *Salmonella typhimurium* at 3 weeks of age are tabulated in Table 3 and 4. All the challenged birds

Table 2: Performance parameters measured at day 35 of age (Mean±SD)

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Body weight (g $bird^{-1}$)	1985.00±65.5[a]	2000.50±25.3[b]	2110.30±18.5[c]
Body weight gain (g $bird^{-1}$)	1945.00±41.5[a]	1960.50±20.5[b]	2070.30±22.5[c]
Feed Intake (g $bird^{-1}$)	3425.50±25.5[a]	3435.50±20.9[a]	3478.50±15.9[a]
FCR	1.76	1.75	1.68

Figures in the same row with different letters are statistically significantly different ($p<0.05$), FCR: Feed conversion ratio

Table 3: Number of broiler chickens and relative (%) frequencies of *Salmonella typhimurium* positive pre and post-challenge (positive bird/total number per group)

			Treated groups			
	Control group (C)		T1 (250 g t^{-1} feed)		T2 (500 g t^{-1} feed)	
Parameters	No.	%	No.	%	No.	%
Pre-challenge	0/20	0	0/20	0	0/20	0
Post-challenge	20/20	100	13/20	65	10/20	50
Shedding rate	17/20	85	14/20	70	10/20	50
Mortality	11/20	55	4/20	20	2/20	10

Table 4: Re-isolation rate and relative (%) frequencies of *Salmonella typhimurium* in broiler chickens (positive sample/total number of samples)

			Treated groups			
	Control group (C)		T1 (250 g t^{-1} feed)		T2 (500 g t^{-1} feed)	
Parameters	No.	%	No.	%	No.	%
1st week Post-challenge						
Intestine	10/10	100	7/10	70	7/10	70
Liver	8/10	80	3/10	30	1/10	10
2nd week Post-challenge						
Intestine	7/10	70	5/10	50	5/10	50
Liver	5/10	50	2/10	20	0/10	0

showed signs of anorexia and watery diarrhea 4 days post infection. The severity of signs was recorded in the control group (C) than those consumed feed containing product by low or high doses. The mortality rate was 55% in the control group reduced to 20 and 10% in the T1 and T2 groups respectively. Post-mortem examination of dead birds revealed severe enteritis with mucoid intestinal contents and congestion of the intestinal mucosa.

The lowest shedding rate was observed in T2 group (50%), while it is recorded 85% in the control group. The results of *Salmonella typhimurium* re-isolation from intestine and liver of sacrificed birds are shown Table 4. Higher *Salmonella typhimurium* re-isolation rate was in the control group than treated ones. The lowest re-isolation rate was present in birds treated with high dose of feed additive.

Humeral immune response: The results of serum antibody titers against NDV and IBDV are shown in Fig. 1 and 2, respectively. Higher antibody titers were significantly noticed in treated groups (T1 and T2) on days 28 and 35 of age (7 and 14 days after 2nd vaccination with NDV) compared with the control groups (Fig. 1). The ELISA (OD) results showed a significant increase in the treated groups on day 35 of age (7 days after 2nd vaccination with IBDV) in comparison with the control (Fig. 2).

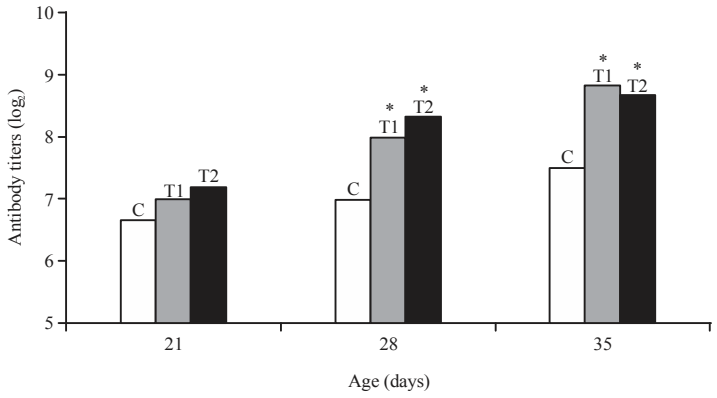

Fig. 1: HI titers of NDV, *Significant difference ($p \leq 0.05$)

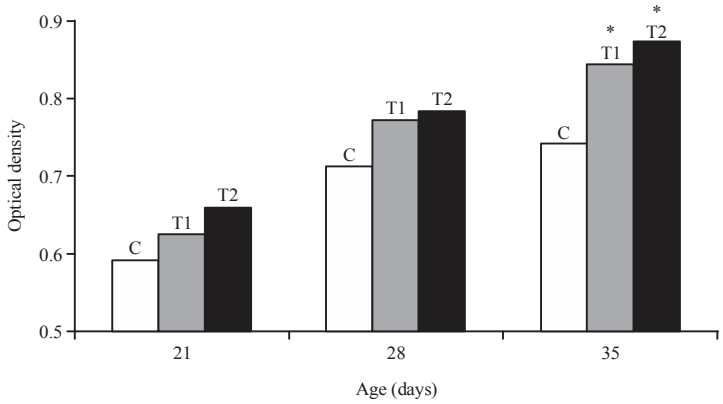

Fig. 2: ELISA Optical Densities (OD) of IBDV, *Significant difference ($p \leq 0.05$)

Innate immunity: The results of innate immunity investigated are illustrated in Table 5 and 6. Samples investigated before *Salmonella typhimurium* challenge showed significant increase in the phagocytic percent (T2) and in phagocytic index (T1 and T2) compared with the control group. The obtained results revealed that in T1 and T2 groups the phagocytic percent was high at 7 and 14 days post infection. Phagocytic index was high at 3 days post infection comparing with the control group.

The results of lysozymes activity are shown in Table 7. The results showed that before infection with *Salmonella*, birds in T1 and T2 groups showed increase in lysozyme activity compared with control group.

Table 8 shows a significant increase in NO production at 3 and 7 days post infection with *Salmonella typhimurium* in all groups. By the end of the experiment there was a significant decrease in NO in control group this may be due to apoptosis of macrophages induced by *Salmonella* infection and this confirmed by increase in bacterial cell count in this group comparing with T1 and T2 groups.

Carcass yield: Table 9 shows the results of carcass yield. Carcass yield was not influenced by the additive used. Dressing weight showed the same results as live body weight results, dressing weight of broilers in the treated groups are significantly increased ($p<0.05$) compared to the control group. On the other hand, dressing percentage, liver weight and abdominal fat are not affected by the dietary treatments.

Table 5: Phagocytic percent of peripheral blood mononuclear cells before and after *Salmonella typhimurium* challenge

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Pre-challenge	50.00±0.58^{a}	56.00±1.91^{b}	61.00±1.62^{c}
Post-challenge (days)			
3	70.33±0.83^{a}	75.67±1.53^{b}	78.67±0.88^{c}
7	63.00±0.58^{a}	73.00±0.58^{b}	75.33±1.34^{b}
14	41.00±0.55^{a}	62.00±0.69^{b}	65.00±1.67^{b}

Figures in the same row with different letters are statistically significantly different ($p<0.05$)

Table 6: Phagocytic index of peripheral blood mononuclear cells before and after *Salmonella typhimurium* challenge

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Pre-challenge	0.33±0.014^{a}	0.50±0.06^{b}	0.50±0.06^{b}
Post-challenge (days)			
3	0.47±0.09^{a}	0.63±0.07^{b}	0.67±0.03^{c}
7	0.63±0.03^{a}	0.67±0.07^{b}	0.70±0.03^{b}
14	0.40±0.06^{a}	0.43±0.07^{a}	0.50±0.09^{b}

Figures in the same row with different letters are statistically significantly different ($p<0.05$)

Table 7: Effect of feed additive on serum lysozyme (μg mL^{-1})

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Pre-challenge	26.00±0.63^{a}	37.33±0.84^{b}	38.33±1.70^{c}
Post-challenge (days)			
3	18.00±0.58^{a}	32.33±6.33^{b}	34.67±5.55^{c}
7	20.67±1.33^{a}	33.67±5.55^{b}	34.33±0.67^{b}
14	22.67±2.33^{a}	35.33±4.84^{b}	37.33±4.33^{c}

Figures in the same row with different letters are statistically significantly different ($p<0.05$)

Table 8: Effect of feed additive on serum nitric oxide (μmol mL^{-1})

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Pre-challenge	18.20±0.39[a]	21.33±0.42[b]	22.17±1.48[c]
Post-challenge (days)			
3	25.00±0.88[a]	27.00±1.15[b]	29.00±2.08[c]
7	23.67±0.57[a]	25.33±0.96[b]	26.67±0.58[c]
14	15.00±1.15[a]	22.00±0.58[b]	23.00±0.24[c]

Figures in the same row with different letters are statistically significantly different ($p<0.05$)

Table 9: Carcass yield measured at day 35 of age (Mean±SD)

		Treated groups	
Parameters	Control group (C)	T1 (250 g t^{-1} feed)	T2 (500 g t^{-1} feed)
Live body weight (g)	1990.00±15.3[a]	2010.50±33.5[b]	2130.50±26.2[c]
Dressing weight (g)	1462.60±22.5[a]	1471.70±18.9[b]	1559.52±21.3[c]
Dressing weight (%)	73.50	73.20	73.20
Liver weight (g)	48.60±2.5[a]	48.70±3.2[a]	48.60±3.1[a]
Abdominal fat (g)	10.80±3.8[a]	10.52±2.8[a]	10.65±2.9[a]

Figures in the same row with different letters are statistically significantly different ($p<0.05$)

DISCUSSION

The effects of feed additive on the productive performance of broiler chickens revealed that supplemented birds with low and high doses showed higher ($p<0.05$) body weights and body weight gains than non supplemented ones. Birds fed on supplement at 500 g t^{-1} attained the highest body weight gains. These results are in harmony with the previous investigations of Owens *et al*. (2008), who reported that supplementation of organic acids in broiler chicken improved the live body weight and body weight gain of broiler chickens. In the present study, birds fed on a mixture of organic acids and formaldehyde revealed significantly different ($p<0.05$) body weight gain compared with non treated control group which confirming earlier results of Senkoylu *et al*. (2007). The improvement in body weight gain might be due to direct antimicrobial effect of organic acids and formaldehyde as reported by Ricke (2003). Feed sanitation using formaldehyde decreased the microbial contamination of feeds. Moreover, organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism causing bactericidal effect. Gunal *et al*. (2006) also reported that the use of organic acid mixture significantly decreased the total bacterial and gram negative bacterial counts in broiler chicken. Furthermore, organic acids supplementation has pH reducing properties in various gastro-intestinal segments of broiler chicken as was observed by Abdel-Fattah *et al*. (2008). The lowered pH is conducive for the growth of favourable bacteria simultaneously hampering the growth of pathogenic bacteria which grow at relatively higher pH. Together the direct antimicrobial and pH reducing properties of organic acids might have resulted in inhibition of intestinal bacteria leading to the reduced bacterial competition with the host for available nutrients and diminution in the level of toxic bacterial metabolites as a result of lessened bacterial fermentation resulting in the improvement of protein, energy and other nutrients digestibility, thereby ameliorating the weight gain and performance of broiler chicken.

The results of feed consumption are in agreement with Hernandez *et al*. (2006), who found no difference in the cumulative feed consumption between the groups fed organic acids and the control group. The improvement in FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain in the birds fed organic acids supplemented diets. These results are in concordance with the reports of earlier researchers (Runho *et al*., 1997), who reported that dietary supplementation of organic acids improved the feed conversion ratio in broiler chicken.

Considering signs and lesions after *Salmonella typhimurium* challenge, similar results were reported by Lee *et al*. (2003), who found that affected broilers with *Salmonella typhimurium* exhibited signs of depression and diarrhea. The effect of organic acids and formaldehyde on reducing the mortality rate in chickens was reported by Berchieri and Barrow (1996), who found that mixture of organic acids treatment at 0.68% reduced mortality rate from 77-33% in chicks given treated feed. Al-Tarazi and Alshawabkeh (2003) reported that the addition of formic and propionic acids mixture in a total concentration of 2.0% or more to the diet of newly hatched infected layer chicks with *S. pullorum* reduced the chick mortality rate. Moreover, Al-Natour and Alshawabkeh (2005) found that supplementation various levels of formic acid decreased mortality rate in all treatments of experimentally infected chicks.

The shedding rate was 50% in T2 group (50%) but 85% in control one. Tony *et al*. (2014) and Fernandez-Rubio *et al*. (2009) confirmed that a butyric acid based feed additive was effective in decreasing the faecal shedding of *Salmonella enteriditis* in infected broilers and also had a positive effect on bird health by preventing *Salmonella* colonization at the intestine.

Regarding the re-isolation rate of *Salmonella typhimurium*, it was higher in control chickens than in treated ones. The lowest re-isolation rate was present in birds treated with high dose of feed additive. Previous study by Sterzo *et al*. (2007) demonstrated that chickens received ration containing different levels of acidifiers as formic and propionic acids had a lower incidence of *Salmonella* intestinal colonization than those received the untreated control diets. Al-Natour and Alshawabkeh (2005) reported that supplementing the feed with formic acid reduced *S. gallinarum* colonization and lowered the pH in the contents of the crop, small intestine, large intestine and caecum. Similarly, Van Immerseel *et al*. (2006) mentioned that feeding of chickens with diets containing 0.3% caproic acid resulted in significant decrease in caecal and internal organs colonization by *Salmonella enteritidis*. The bacteriostatic activity of organic acids is thought to derive from the penetration into the bacterial cell and their dissociation into anion and protons. The protons are responsible for the acidification of the cytoplasm, decreasing intestinal pH (Barcellos *et al*., 2004), interfere with bacterial metabolism by decreasing the cytoplasmatic pH, interfering thus with most cellular functions, whereas the anions may inhibit DNA synthesis to varying degrees depending on the specific compound used (Jordan *et al*., 2009). It has been proposed that a possible explanation of the effect of the organic acids on *Salmonella* may derive from creation of an acidic environment in the crop of the chickens or reducing the uptake of *Salmonella* (Ricke, 2003). The formaldehyde-containing treatment was the most effective in most conditions. In commercial poultry, formaldehyde is widely applied in higher concentrations in feed without showing any adverse effects under experimental conditions (Khan *et al*., 2006). Formaldehyde treatment contains terpenes, which have recently been shown to have antimicrobial activities (Trombetta *et al*., 2005) and may partly explain the greater efficacy of this product.

Changes of serum antibody titers can accurately and directly reflect the state of humoral immunity which can neutralize and eliminate extracellular microbes and microbial toxins (Abbas and Lichtman, 2004). Kazempour and Jahanian (2011) found that antibody titer against Newcastle disease virus was markedly increased by dietary organic acid supplementation in laying hens. Ozek *et al*. (2011) found that dietary formic and propionic acids supplementation had a positive effect on antibody titers against NDV and IBDV in laying hens. In broiler chickens, Dehghani and Jahanian (2012) showed a significant improvement in antibody titers against Newcastle disease, infectious bronchitis and Gumboro virus vaccines by dietary organic acids supplementation. Abdel-Fattah *et al*. (2008) concluded that the dietary addition of organic acids

resulted in a higher immunity response due to higher level of globulin in their serum. They suggested that improvement in bird immunity could be related to the inhibitory effects of organic acids on gut system pathogens.

The results of innate immunity demonstrated significant increase in the phagocytic percent (T2) and in phagocytic index (T1 and T2) in comparison with control group before *Salmonella typhimurium* challenge. These results are in agreement with Lee *et al*. (2007), who found that organic acids intake for 28 and 46 days enhanced peripheral blood mononuclear cells proliferation. These findings may be due to reduction in gut pH, caused by organic acids supplementation. Organic acids could inhibit the growth of pathogenic microorganisms and increase the growth of lactic acid bacteria which are able to grow at relatively low pH. Diaz-Rosales *et al*. (2006) reported that lactic acid bacteria increase the activities of phagocytes. Alun *et al*. (2002) found that neutrophils and macrophages increased 10 and 3 fold, respectively after oral *Salmonella* infection in mice. The obtained results revealed that in T1 and T2 groups the phagocytic percent was high at 7 and 14 days post infection. Phagocytic index was high at 3 days post infection comparing with the control group. These recorded results are in agreement with Higgins *et al*. (2007), who noticed a significant increase in phagocytosis of Abdominal Exudate Cells (AEC) obtained from chicks treated with a lactobacillus based probiotic and infected by *Salmonella enteritidis*.

Lysozymes are an important component of the innate immune system of birds that hydrolyze peptidoglycan, the major bacterial cell wall constituent (Yum *et al*., 2009). Obtained results revealed that before infection with *Salmonella*, birds in T1 and T2 groups showed increase in lysozyme activity compared with control group. This finding may be due to increasing in intestinal lactic acid bacteria as mentioned by Carroll and Martinez (1979).

Nitric oxide (NO) is an important mediator produced from immunologically activated macrophages which have a highly potent microbicidal activity and play a major role in the intracellular microbial killing as mentioned by Withanage *et al*. (2005). These results are in accordance with He *et al*. (2006), who found that exposure of primary peripheral blood mononuclear cells (PBMC) to *Salmonella* (*Salmonella typhimurium* or *S. enteritidis*) increased NO production. Nearly the same dynamic response has been reported in chicken macrophages cell line which produces strong oxidative burst and NO in response to *Salmonella typhimurium*, *S. enteritidis* and *S. gallinarum* infection (Ibuki *et al*., 2011). By the end of the experiment there was a significant decrease in NO in control group this may be due to apoptosis of macrophages induced by *Salmonella* infection and this confirmed by increase in bacterial cell count in this group comparing with T1 and T2 groups.

Carcass yield was not influenced by the additive used. Dressing and live body weight were the same, while dressing weight of the treated broilers was significantly increased ($p<0.05$) than control ones. However, dressing percentage, liver weight and abdominal fat were not affected. These results are in agreement with Thirumeigmanam *et al*. (2006), who found no effect on the carcass characteristics of broiler chicken fed organic acid based diets.

CONCLUSION

Using organic acids with their salts and formaldehyde mixture as feed additives for feed sanitation reflected positively on the productive performance, reduced the incidence of salmonellosis, enhanced the immune status and improved carcass trait of broiler chickens.

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