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Effects of Dietary Zinc Oxide and a Blend of Organic Acids on Broiler Live Performance, Carcass Traits, and Serum Parameters

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ABSTRACT

This experiment was carried out to evaluate the effect of different dietary supplementation levels of zinc oxide and of an organic acid blend on broiler performance, carcass traits, and serum parameters. A total of 2400 one-day-old male Ross 308 broiler chicks, with average initial body weight 44.21±0.19g, was distributed according to a completely randomized design in a 2 x 3 factorial arrangement. Six treatments, consisting of diets containing two zinc oxide levels (0 and 0.01% of the diet) and three organic acid blend levels (0, 0.15, and 0.30%) were applied, with eight replicates of 50 birds each. The experimental diets were supplied *ad libitum* for 42 days. There were significant performance differences among birds fed the different zinc oxide and organic acid blend levels until 42 d of age ($p<0.01$). The result of this experiment showed that the organic acid blend did not affect feed intake, but zinc oxide increased feed intake. Carcass traits were not influenced by the experimental supplements. Zinc oxide supplementation increased serum alkaline phosphatase level ($p<0.01$). The organic acid blend reduced serum cholesterol and triglyceride levels ($p<0.05$). No interactions were found between zinc oxide and the organic acid blend for none of the evaluated parameters. We concluded that zinc oxide and the evaluated organic acid blend improve broiler performance.

INTRODUCTION

It is well-documented that zinc (Zn) is an essential nutritional trace mineral and the first unequivocal evidences that zinc is required for growth and health were obtained in laboratory animals (Todd *et al*., 1934). According to previous literature studies, the primary role of Zn in the body appears to be related to its association with enzymes and proteins, both as part of their molecule and as an activator. Numerous studies have provided a detailed description of one thousand known proteins associated with Zn (Balas *et al*., 1994). Under severe Zn deficiency, the activities of the enzymes plasma alkaline phosphatase; liver, retina and testicular alcohol dehydrogenase; connective tissue and fetal thymidine kinase; pancreatic carboxypeptidase A; and liver nuclear DNA dependent RNA-polymerase may be depressed. Many authors have suggested that Zn deficiency greatly reduces synthesis of DNA, RNA, and proteins and, hence, impairs cellular division, growth, and repair (Prask & Plocke, 1971; Balas *et al*., 1994). Fetal abnormalities occur and hatchability of eggs is reduced (Bao *et al*., 2007; Golden, 1988). Zinc supplementation of poultry, swine and dairy cattle diets improves the performance as measured by final body weight or feed efficiency ratio (Feng *et al*., 2009). The inclusions of zinc in broiler diets are commonly based on the NRC (1994) recommendations (40 mg/kg), and this is often criticized for not representing the needs of modern strains of commercial broilers (Leeson, 2003). It is a common practice in the broiler industry to formulate diets to contain 100–120 mg supplemental Zn/kg (Feng *et al*., 2009), and therefore, commercial feed manufacturers use much higher levels than those recommended by the NRC to achieve maximum performance. The common forms of zinc used to supplement poultry fed are zincoxide (ZnO) and feed grade zinc sulfate ($ZnSO_4.7H_2O$). Comparisons of the relative bioavailability in broilers have shown that feed-grade sources of ZnO are 44 or 61% of the value of analytical-grade $ZnSO_4$, depending on the index (tibia zinc or broiler growth) (Wedekind and Baker, 1990; Wedekind *et al*., 1992). Sandoval *et al*. (1998) reported that ZnO was about 60% available compared with reagent-grade $ZnSO_4$ and that highly available Zn sources are more toxic when consumed at high levels.

Due to the ban of antibiotic growth promoters in poultry diets in different countries, it is of interest to investigate potential alternatives to maintain good growth performance. Several organic acids have been reported to improve growth performance, feed efficiency, and mineral absorption when supplemented in non-ruminant diets (Langhout, 2000). Organic acids, such as acetic, butyric, lactic, and propionic acids, can modify intestinal microbial growth and this prebiotic effect results in the production of short-chain fatty acids by bacteria. Short-chain fatty acids are thought to have an antibacterial effect by decreasing luminal pH (Corrier *et al*., 1990). Other organic acids, such as citric, fumaric, and malic acids, have been used as growth promoters in chickens. Their mode of action is believed to rely on their ability to acidify the diet and ultimately the contents of the digestive tract. This is primarily important in young poultry, which endogenous acid production is low (Golden, 1988; Biggs & Parsons, 2008). Afshrmanesh & Porreza (2005) suggested that the reduction of gastric pH after organic acid consumption may increase pepsin activity. Several studies demonstrated that the supplementation of organic acids to broiler diets increased growth performance and reduced diseases and management problems (Ao *et al*., 2009; Ricke, 2003). However, detailed information is still lacking.

The general purpose of the current study was to examine the effects of different levels of zinc oxide and of an organic acid blendes possible alternatives to antibiotics in order to improve broiler chicken growth performance, FCR, and meat and serum parameters.

MATERIAL AND METHODS

Test design, Test period and Feeding method

A trial was conducted to examine the effect of zinc oxide and organic acid blend on broiler performance, carcass characteristics, and serum parameters. The protocol for this experiment was reviewed and approved by the University of Tabriz Animal Care Committee, and birds were cared for according to the 2006 Guidelines for Animal Care of the Agriculture Organization of East Azerbaijan, Tabriz, Iran. The2400 one-day-old Ross 308male broiler chickens used in this experiment were obtained from a commercial producer and sexed at a local commercial hatchery (Behparvar, Tabriz, Iran). Broiler chicks were randomly distributed into six treatments with eight replicates each, with50 chicks per replicate (initial weight 44.21±0.19 g). The diets were provided as mash and fed during the experimental period, from 0 to 42 d of age. Feed and water were available *ad libitum*. In this experiment, initial room temperature was 32°C and this was reduced by 1°C at 2-d intervals to 24°C. Room humidity was set at 70% for the duration of the experiment and lighting cycle was fixed at 23L: 1D during the whole experimental period.

Experimental diets

The basal diets formulated for the starter and the grower stages contained 20.7% CP, 2890 kcalME/kg, and 18.4% CP and 2960 kcal ME/kg, respectively (Table 1). The following six dietary treatments were applied: 1) a control diet with no additive supplementation and the dietary addition of2) 0% ZnO + 0.15% OA, 3) 0% ZnO + 0.30% OA, 4) 0.01% ZnO + 0% OA, 5) 0.01% ZnO + 0.15% OA, and 6) 0.01% ZnO + 0.30% OA. The commercial organic acid blendcontained15%formicacid, 15%malicacid,15% tartaric acid,20%citric acid,30% lactic acid, and 5% orthophosphoric acid. The feeds were mixed at 2-day intervals to maintain their stability. The test feeds were mixed for 3.5 min in a V-type mixer with a 500-kg capacity.

MEASUREMENTS

Broiler performance, carcass traits and serum parameters.

At the end of the experimental period (day 42), feed was removed from the feeders and weighed. After a 12-h fasting period, Broilers were weighed per pen. Out these measurements, broiler live weight gain (g),

Table 1 – Composition of experimental diets

	Starter (0-21 d)						Grower (22-42 d)					
Ingredient (%)	1	2	3	4	5	6	1	2	3	4	5	6
Ground yellow corn	63.14	63.05	62.94	63.12	63.05	62.94	69.32	69.22	69.12	69.32	69.22	69.12
Soybean meal (48%)	30.48	30.42	30.37	30.49	30.41	30.36	24.63	24.58	24.52	24.62	24.57	24.51
Fish meal, menhaden (60% CP)	3.00	3.00	3.00	3.00	3.00	3.00	2.50	2.50	2.50	2.50	2.50	2.50
Di-calcium phosphate	1.01	1.01	1.02	1.01	1.01	1.02	1.14	1.14	1.15	1.14	1.14	1.15
Oyster shell	1.32	1.32	1.32	1.32	1.32	1.32	1.36	1.36	1.36	1.36	1.36	1.36
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Mineral premix [1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.05	0.05	0.05	0.05	0.05	0.05
L-lysineHCL	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20	0.20	0.20
Coccidiostats[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Zinc oxide [3](mg/kg)	-	-	-	100	100	100	-	-	-	100	100	100
Organic acid blend[4]	-	0.15	0.30	-	0.15	0.30	-	0.15	0.30	-	0.15	0.30
Calculated analysis												
ME, kcal/kg	2890	2890	2890	2890	2890	2890	2960	2960	2960	2960	2960	2960
CP %	20.7	20.7	20.7	20.7	20.7	20.7	18.4	18.4	18.4	18.4	18.4	18.4
Calcium%	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Available phosphorus%	0.41	0.41	0.41	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.40	0.40
Methionine%	0.5	0.5	0.5	0.5	0.5	0.5	0.36	0.36	0.36	0.36	0.36	0.36
Lysine%	1.11	1.11	1.11	1.11	1.11	1.11	0.95	0.95	0.95	0.95	0.95	0.95

[1]Vitamin Mineral premix provided per kg of diet: vitamin A, 8,250 IU; vitamin D_3, 1,000 IU; vitamin E, 11 IU; vitamin B_{12}, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin,5.5 mg; Mn. 55mg; Zn, 60mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; Na, 1.6 g.[2] salinomycin.[3] Zinc oxide is a product of Merck Co. (Germany) with 99% purity,[4] Organic acids is a commercial product of Sunzen Co. (Malaysia) that is containing formic acid, malic acid, tartaric acid, citric acid, lactic acid, and orthophosphoric acid.

feed intake (g), and feed conversion ratio (FCR) were calculated. After final body weight was recorded, eight birds per pen were randomly selected and tagged, feed fasted (water was still available) for 12 h on day 42. Birds were weighed and sacrificed by severing both the right and left carotid artery and the jugular vein with a single cut and bled for 180s. After slaughter, carcass weight was determined as chilled carcass weight after removal of feathers, head, lungs, gastrointestinal tract, liver, kidney, abdominal fat. Carcass, breast meat, thighs, heart, liver, and abdominal fat yields were calculated as a percentage of fasted live body weight. On day 42, eight birds were randomly selected and 2-mL blood samples were collected from the right jugular vein. Blood samples were allowed to clot and then centrifuged at 3000×g (Beckman Avanti J.) for 10 min. Serum cholesterol (mg/dL), triglyceride (mg/dL), total protein (g dL^{-1}), albumin (g dL^{-1}), alkaline phosphatase (u/L), lactate dehydrogenase(u/L), calcium (mg/dL), and phosphorous (mg/dL) levels were determined in an auto-analyzer (ALCYON 300-Abbott, USA) using commercially available kits.

Statistical analysis

A completely randomized design in a2 x 3factorial arrangement with two levels (0 and 100 mg/kg of diet) of zinc oxide (ZnO) and three levels (0, 0.15, and 0.30%) of an organic acid blend (OA) was applied.

The obtained data were submitted to analysis of variance and treatment means, if significant, were compared by Duncan Multiple range test using SAS, version 9.1, statistical software (2000).

RESULTS

Our findings showed that zinc and organic acid supplementation had a significant effect on broiler weight gain (Table 2). From 0 to 42 d, both ZnO and OA a tall levels improved broiler growth rate when compared with the control diet ($p<0.01$). During the experimental period, birds fed diet6 (0.01% ZnO + 0.30% OA) presented the highest ($p<0.01$) weight gain. Birds fed the diets containing 0.01% ZnO presented higher weight gain than those fed the diets with no ZnO ($p<0.01$). On the other hand, all diets supplemented with OA significantly increased the weight gain of broilers when compared with those fed the control diets ($p<0.01$). Our findings show that, although weight gain was affected by the diets, significant feed intake differences ($p<0.05$) were only observed in ZnO supplemented diets. All organic acid blend levels had no effect on feed intake of broiler chicks. The study results provided some interesting findings relative to FCR. The diets supplemented with ZnO and OA significantly influenced FCR during the experimental period (ZnO; 0.05 and OA; $p<0.01$). The

Table 2 – Effects of dietary ZnO and OA levels on cumulative growth performance (1-42 days of age).

Treatments	1	2	3	4	5	6	SEM
Feed intake (g)	3870	4038	4015	4043	4180	4335	47.57
Weight gain (g)	1973	2136	2159	2120	2235	2339	31.16
FCR	1.96	1.88	1.86	1.9	1.87	1.85	0.01

Source of variation	ZnO	OA	ZnO*OA
Feed intake	*	Ns	Ns
Weight gain	**	**	Ns
FCR	*	**	Ns

Ns: no significant effect, *: Significant effect at p<0.05 and **: Significant effect at p<0.01.

Dietary treatments: 1: a control diet without additive supplementation, 2: 0% ZnO + 0.15% OA, 3: 0% ZnO + 0.30% OA, 4: 0.01% ZnO + 0% OA, 5: 0.01% ZnO + 0.15% OA and 6: 0.01% ZnO + 0.30% OA.

birds fed diet 3 (0% ZnO + 0.30% OA) and 6 (0.01% ZnO + 0.30% OA) presented lower FCR than those fed the other diets. The best FCR was observed when diets containing 0.3% of the organic acid blend was fed. No significant interaction ZnO × OA interaction was detected for the evaluated performance parameters.

The carcass trait results are shown in Table 3. None of the evaluated by the dietary treatments; however, the birds fed diet6 (0.01% ZnO + 0.30% OA) presented numerically higher empty carcass weight and breast yield than the other diets. The effects of dietary zinc oxide and organic acids levels on serum parameters are summarized in Table 4. The dietary addition of ZnO significantly influenced alkaline phosphatase (p<0.01) levels, but not the other evaluated serum parameters. On the other hand, the addition of the organic acid blend to the diets significantly reduced serum cholesterol (p<0.01) and triglyceride (p<0.05) levels, and increased phosphorus (p<0.01), total protein (p<0.05), and albumin (p<0.01) concentrations. The ZnO × OA interaction was not significant for serum parameters.

DISCUSSION

In this study we investigated the effects of the supplementation of zinc oxide and of an organic acid blend to a standard diet on the live performance, carcass traits, and serum parameters of broilers between one and 42 days of age. The Ross 308 (2007) management guide establishes broiler zinc requirement during the grower period at a level of 120 mg Zn/kg diet, whereas the NRC (1994) reports that the zinc requirement of broilers during the starter period is 40 mg Zn/kg. In the present experiment, average zinc supplementation in the control diet from the trace mineral premix was 60 mg Zn/kg. We hypothesized that when conventional corn-soybean meal diets are fed, the Zn content in the mineral premix often meets the requirement for broiler growth. It was shown that Zn supplementation did not significantly change broiler weight gain (Wedekind *et al.*, 1992). Some previous studies have documented that dietary zinc concentrations of 45 to 48 mg/kg were adequate for broilers (Mohanna & Nys, 1999; Huang *et al.*, 2009). However, Burrell *et al*. (2004) reported

Table 3 – Effects of dietary zinc oxide and organic acids levels on carcass traits

Treatments	1	2	3	4	5	6	SEM
Empty carcass %	67.33	69.20	69.62	69.65	69.99	71.06	0.39
Breast %	25.17	27.68	27.13	27.73	27.31	28.37	0.35
Thighs %	30.85	30.60	31.13	30.86	31.62	31.50	0.18
Abdominal fat%	2.11	1.88	2.00	1.68	1.80	1.78	0.07
Liver%	2.43	2.07	2.28	2.08	2.14	2.31	0.06
Heart%	0.57	0.6	0.57	0.58	0.54	0.51	0.1

Source of variation	ZnO	OA	ZnO*OA
Empty carcass %	Ns	Ns	Ns
Breast %	Ns	Ns	Ns
Thighs %	Ns	Ns	Ns
Abdominal fat%	Ns	Ns	Ns
Liver%	Ns	Ns	Ns
Heart%	Ns	Ns	Ns

Ns: no significant effect, *: significant effect at p<0.05 and **:significant effect at p<0.01.

Chilled Carcass traits are as a percentage of fasted body live weight before slaughter.

Dietary treatments: 1: a control diet without additive supplementation, 2: 0% ZnO + 0.15% OA, 3: 0% ZnO + 0.30% OA, 4: 0.01% ZnO + 0% OA, 5: 0.01% ZnO + 0.15% OA and 6: 0.01% ZnO + 0.30% OA.

Table 4 – Effects of dietary zinc oxide and organic acids levels on serum parameters

Treatments	1	2	3	4	5	6	SEM
Cholesterol(mg/dL)	157.17	140.83	136.50	151.00	138.17	126.50	2.47
Triglyceride(mg/dL)	84.67	60.67	68.00	70.33	66.00	58.33	2.53
Calcium(mg/dL)	9.77	9.93	9.95	9.87	10.20	10.30	0.08
Phosphorus(mg/dL)	6.12	6.42	6.45	6.25	6.45	6.57	0.04
Total protein(g/dL^{-1})	3.83	3.97	3.95	3.90	3.98	4.08	0.02
Albumin(g/dL^{-1})	1.78	1.90	1.92	1.82	1.92	1.93	0.02
Alkalinephosphatase(u/L)	296.47	301.52	329.02	334.02	339.30	383.57	8.77
Lactatedehydrogenase(u/L)	318.77	336.98	346.43	351.67	345.77	363.32	6.02

Source of variation	ZnO	OA	ZnO*OA
Cholesterol	Ns	**	Ns
Triglyceride	Ns	*	Ns
Calcium	Ns	Ns	Ns
Phosphorus	Ns	**	Ns
Total protein	Ns	*	Ns
Albumin	Ns	**	Ns
Alkaline phosphatase	**	Ns	Ns
Lactatedehydrogenase	Ns	Ns	Ns

Ns: no significant effect, *: significant effect in p<0.05 and **: significant effect at p<0.01.

Dietary treatments: 1: a control diet without additive supplementation, 2: 0% ZnO + 0.15% OA, 3: 0% ZnO + 0.30% OA, 4: 0.01% ZnO + 0% OA, 5: 0.01% ZnO + 0.15% OA and 6: 0.01% ZnO + 0.30% OA.

that optimum weight gain was achieved with110 mg supplemental Zn/kg in conventional corn-soybean meal diets. In the present experiment, there were significant differences in weight gain between 100 mg ZnO diets and the control diet. This result is consistent with the findings of Golden (1988) and Alcicek *et al*. (2004), who reported that broiler live performance improved when a zinc complex ($ZnSO_4$) was added above 100 ppm to the diet.

On the other hand, Rossi *et al*. (2007) reported that diets with low zinc levels lead to depressed appetite, resulting in reduced feed intake and weight gain. In contrast with our results, Zn bioavailability was ~40-80% in feed grade ZnO relative to feed-grade $ZnSO_4$, using weight gain as response variable (Sandoval *et al*., 1997; Edwards & Baker, 1999). The result of Collins & Moran (1999) differ from that obtained in the present experiment: those authors reported that body weight and feed efficiency were generally not influenced by feeding excessive levels of supplemented Zn. Zinc is involved in several biochemical reactions, and the live performance improvements detected in the present study may be explained by the fact that the supplemented Zn levels met the requirements of enzymes that have a main role in the synthesis of DNA, RNA, and body protein (Balas *et al*., 1994; Karamouz *et al*., 2010).

Many additives, including organic acids, have been researched to determine their efficacy in improving the performance of broilers in order to reduce or replace dietary antibiotic growth promoters (Waldroup, 1995). The organic acid blend used in this study effectively improved weight gain and FCR, and did not present any adverse effects on the other evaluated parameters. However, its effect on feed intake was not significant. Hassan *et al*. (2010) suggested that the addition of organic acid blend in broilers diets may improve live performance (weight gain or FCR), but also found that it significantly increased the feed intake of broilers. Also in contrast with our results, Rafacz *et al*. (2005) reported that organic acids significantly increased feed intake in most experiments (Snow *et al*., 2004). The beneficial effect of organic acids on performance is related to a more efficient utilization of nutrients, which in turn results in improved FCR (Cave, 1982). However, Hernández *et al*. (2006) showed that, when formic and propionic acids (5,000 or 10,000 ppm) were added to broiler diets, performance was not affected.

Interestingly, we found that, although broiler performance improved with the dietary supplementation of ZnO, none of the evaluated carcass traits were influenced by the treatments. The results of the present study are in agreement with Denli *et al*. (2003), who reported that organic acid supplemented diets had no effects on carcass traits. On the other hand, Hassan*et al*. (2010) showed an increased carcass dressing percentage in 35-d-old broilers with the dietary addition of organic acids.

In the present experiment, serum parameters were not affected by the dietary treatments, except for alkaline phosphatase level, which increased with the dietary addition of 100 mg ZnO/kg. This finding contrasts with the result of Watkins and Southern (1993), who reported no differences in alkaline phosphatase activity when dietary Zn level increased. Uyanik *et al*. (2001) and Karamouz *et al*. (2010) showed that zinc supplementation had no significant effect on serum alkaline phosphatase activity, but with increasing Zn levels, serum alkaline phosphatase levels were reduced earlier than normal Zn level feeding. Feng *et al*. (2009) showed that a zinc-glycine chelate increased serum total protein and calcium concentrations, but had no effect on albumin or phosphorus levels, whereas Barman *et al*. (2009) did not report any significant effect of different dietary zinc supplementation levels on serum protein concentration.

The dietary addition of an organic acid blend significantly reduced serum cholesterol and triglyceride levels in the study of Abdel-Fattah *et al*. (2008) and Abdo (2004). On the other hand, Abdel-Azeem *et al*. (2000) did not find any significant blood lipid profile changes in broilers fed citric acid. Powell (2000) reported that the reduction obtained in cholesterol levels may be the result of the increasing breakdown of cholesterol by bile acids and of the inhibitory effect of organic acids on micelle formation in the low pH of digesta content. In present experiment, the addition of organic acids statistically increased serum phosphorous levels of broilers. A possible explanation for this effect may be attributed to the reduction of the intestinal pH, increasing the absorption of such mineral from the gut into the blood stream (Abdel-Fattah *et al*., 2008). Abdel-Fattah *et al*. (2008) reported that the supplementation of organic acids in broiler diets increased serum calcium and phosphorous concentrations, and Abdo (2004) observed an increase of blood calcium levels in broilers fed dietary acidifiers. Both of these results contrast with those obtained in the present experiment.

We observed that serum total protein and albumin concentrations were significantly increased with organic acid supplementation. Son *et al*. (2002) reported that adding organic acids to broiler diets slowed the passage of feed through the digestive organs and that the increased digestion time improved nutrient utilization. Several studies demonstrated that the dietary acidification with organic acids enhances nutrient utilization and improves the digestibility coefficient of protein (Abdel-Azeem *et al*., 2000). Kommera *et al*. (2006) mentioned that reduced gastric emptying rate is a possible mechanism to improve the protein digestion in the intestine and this may be an explanation for our findings of increased serum protein and albumin levels.

We did not find similar studies evaluating the interaction between organic acid blends and ZnO, and therefore, its possible effects remain unclear. Further studies are required to elucidate the mechanisms that regulate the digestibility of feed by organic acid blend and ZnO supplements. This study is groundwork for future investigations evaluating zinc levels below broiler requirement for maximum performance in control diets. In summary, this study indicated that the that the addition of zinc oxide and an organic acid blend to broiler diets significantly improved broiler weight gain and feed conversion ratio; however, neither the individual or combined use of zinc oxide and the evaluated organic acid blend statistically affected carcass traits or serum parameters of male broilers.

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