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Impact of Zinc Supplementation and Stocking Density on Performance, Physiological and Immune Responses in Broiler Chickens

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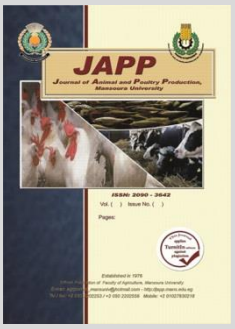

ABSTRACT

The current research was completed to examine the impacts of dietary enrichment with zinc oxide and stocking density on performance, plasma concentration of thyroid hormones, corticosterone(cort) and immune response and antioxidant status in cobb 500 chicks. Experimental treatments were ordered in a 4× 2 factorial plan structure with 4 degrees of included dietary zinc oxide and 2 degrees of stocking density (11.90 chicks/m^2, normal stocking density, or 16.66 chicks/m^2, high stocking density,). Zinc levels were 0.0, 40, 60 and 80 mg/kg diet. Elevated level of zinc (80mg/kg diet) positively affected FCR while LBW, BWG and feed intake were slightly improved compared with the control one. Additionally, Blood plasma concentricity of "total protein (TP), albumin (Alb), total lipids (TL), cholesterol (Chol), high density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL)" did differed significantly between the control and zinc-enhanced groups. Moreover, zinc oxide enhancement in the diet could improve thyroid hormones. Also, plasma Cort levels were significantly inferior at zinc-enhanced groups as contrasted with the control one. Moreover, enhancing zinc had a huge constructive outcome on SOD, MDA and immune response titers against NDV and AFV. The typical stocking density has better effects on performance and plasma levels of Cort, TP, TL, MDA and NDV to those of the high stocking density. Stocking density did not influence "T3, T4, Alb, Chol, LDL, HDL". The results of the present examination show that enhancing zinc oxide to broiler diets has a beneficial effect on productivity, blood parameters and immunity in cob 500 chicks.

Keywords: MDA, SOD, thyroid hormones, corticosterone, cholesterol, zinc, stocking density, broiler chickens.

INTRODUCTION

Zinc is an essential trace element for poultry. It has an important role in their physiology and performance. Enrichment of Zn enhances feed intake and improves growth performance in poultry. Zn is a crucial part of the antioxidant system in animals. Typical metabolic procedures bring about the creation of profoundly oxidative 'responsive oxygen species' (ROS) which induce a high danger of the development of chronic diseases, due to membrane and DNA damage and inhibition of the immune system (Rahman *et al.*, 2014). Zn has never been appeared to apply its antioxidant consequence directly on pro-oxidant production yet appears to indirectly suppress oxidant stress by the incitement of specific substances which have cell reinforcement properties (Powell, 2000). Zn is a most important part of the antioxidant enzyme superoxide dismutase (SOD), which helps preserve the body against ROS by converting superoxide anions into hydrogen peroxide (Niles *et al.*, 2008).Zinc, chromium and selenium act as catalysts in a lot of hormone and enzyme systems (Rama Rao *et al.*, 2016)Supplementation of zinc (Zn) in broiler diet is of exacting attention because it functions mainly a catalyst in several of hormone and enzyme system that are related with growth, immune response and it has antioxidant activity.

Stress in broilers can be caused by different environmental factors (Dohms and Metz, 1991), and stocking density is considered as an vital stress factor in hybrid chicks production. Broilers are housed at dissimilar stocking densities, depending on local system, production system, and target body weight, aiming at minimizing fixed costs and maximizing profitability (Buijs *et al*., 2009; Skomorucha *et al.*, 2009).Our research was to assess the impact of dietary enrichment with zinc oxide on growth performance, some blood metabolites, resistance and lipid peroxidation in cobb500 chicks at dissimilar stocking densities.

MATERIALS AND METHODS

The experimental work of our study was conducted at the Poultry Production Farm; Center of Agricultural Research and Experiments, Faculty of Agriculture, Mansoura, University. The purpose of the current study was to assess the impact of included dietary zinc oxide on growth performance, some blood parameters, immunity and lipid peroxidation in cobb500 chicks at various stocking densities.

Birds and Management:

Cobb 500 chicks (n=192), 3-day-old, were separated into eight treatments groups, every one of which included four replicates (cages). Experiments consisted of a 4x2 factorial course of action with four levels of dietary zinc oxide-supplementation and 2 stocking densities. Zinc oxide(Zn) levels was enhanced to the basal starter diet at 0, 40, 60, and 80, mg/kg diet to get dietary zinc of 22.96, 62.96,82.96 and 102.96 mg/kg of starter diets, respectively. These levels were 21.9; 61.9; 81.9 and 101.9 mg zinc/kg grower diets, respectively. Chicks were raised in battery cages (70 cm length, 60 cm width and 40 cm height of each cage). Thus, the cage floor area was 0.42 m^2 (70 x 60 cm).The number of birds

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placed in each was 5 or 7 birds/cage density. The stocking density was 11.90 birds/m^2 as the normal density (5 birds/cage) and 16.66 birds/m^2as the high density (7 birds/cage). The daily temperature indoors the farm in 1est week was 32ºC, and then slowly reduced to be ranged from 28 to 30 º C in the 2nd week and maintained at 18 to 24º C from 3rd week in anticipation of the end of the experiment. The photoperiod was 23h light: 1h darkness throughout the experiment. Chickens were reared to 42 days of age and fed the starter diets from three to 17 days of age (3127 kcal of ME/kg of diet and 22.51% CP) and grower diets from 17 to 45 days of age (3.141 kcal of ME/kg of diet 19.09 %CP). Diets were formulated to face or exceed the suggested nutrient requirements of broiler chicks according to NRC (1994). Feed in mash form and water (via nipple drinkers) were provided freely. The composition and chemical analysis of the basal starter and grower diets are shown in Table 1.

Table 1. Composition and chemical analysis of the basal diets fed to the experimental

Ingredients (%)	Starter %	Grower %
Yellow corn	64.7	72.23
Soybean meal 44% CP	13	11.5
Corn gluten meal 60.2% CP	18	12.5
Dicalcium phosphate	1.8	1.31
Limestone	1.45	1.49
DL-methionine	0.05	0.0
L-Lysine	0.4	0.37
Sodium chloride	0.3	0.3
Vit.+Min. Premix[1]	0.3	0.3
Total	100	100
Calculated analysis (NRC, 1994)		
ME, kcal/Kg	3127	3141
CP, %	22.51	19.09
Fiber, %	2.6	2.6
Ether extract, %	3.0	3.1
Calcium, %	1.0	0.9
Non-phytate Phosphorus, %	0.45	0.35
Methionine, %	0.52	0.39
Meth.+Cys. (TSAA, %)	0.92	0.73
Lysine, %	1.1	1
Zn, mg/Kg	22.96	21.9

[1]**Premix provided the following per kilogram of diet: "vitamin A (retinyl acetate), 2654 µg; vitamin D_3 (cholecalciferol), 125 µg; vitamin E (dl-α-tocopheryl acetate), 9.9 mg; vitamin K_3(menadionedimethylpyrimidinol), 1.7 mg; vitamin B1 (thiamin mononitrate), 1.6 mg; vitamin B_{12} (cyanocobalamin), 16.7 µg; riboflavin, 5.3 mg; niacin, 36 mg; calcium pantothenate, 13 mg; folic acid, 0.8 mg; biotin, 0.1 mg; choline chloride, 270; BHT, 5.8; Fe (iron sulphate monohydrate), 50 mg; Cu (copper sulphate pentahydrate), 12 mg; I (calcium iodate), 0.9 mg; Zn (zinc oxide), 50 mg; Mn (manganous oxide), 60 mg; Se (sodium selenite), 0.2 mg; and Co (cobalt sulphate), 0.2 mg".**

Performance of cobb 500 chicks:

"Live body weight (LBW), feed intake (FI) and body weight gain (BWG)" were measured weekly during the trial period, then feed conversion ratio (FCR) was calculated (g feed :g gain). Mortality and health status were visually practical and recorded daily all over the experimental period.

Blood sampling and biochemical analysis:

Three birds of each treatment were arbitrarily chosen, slaughtered and blood samples were collected into heparinized tubes; then they were centrifuged at 4000 r.p.m for 15 minute and the plasma obtained was stored at -20º C until analysis. Plasma samples were tested calorimetrically using commercial kits according to the procedures outlined by the manufacturers, for the determination of total protein (Doumas *et al*., 1981), albumin (Doumas *et al*., 1971), total lipids (Frings and Dunn, 1970), cholesterol (Allain *et al*., 1974), high density lipoprotein and low density lipoprotein (Myers *et al.*(1997). Concentrations of thyroid hormones [triiodothyronine (T3) and thyroxine (T4)] and corticosterone (cort) in blood plasma were measured by RIA techniques according to the methods of Britton *et al.* (1975) and Saterlee *et al.* (1980), respectively. Superoxide dismutase (SOD) was determined by available kits according to the methods of (Misra and Fridovich, 1972). Malondialdehyde (MAD) was determined by available kits as described by Mihara and Uchiyama (1978)"."Antibody titers against Newcastle disease virus (NDV) and avian flu virus (AFV) were determined by hem agglutination inhibition technique using U-Bottom microtiter plates (96 wells) as reported by Wegmann, and Smithies (1966), and Van der Zijpp *et al.* (1983).

Statistical analysis:

Arithmetical investigation for the our data was performed by two-way analysis of variance utilizing the method of least square investigation of Co-variance (SAS, 2006). Duncan's various range test was utilized to isolate noteworthy contrasts among implies (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance: -

The results connecting to the influence of dietary zinc oxide levels and stocking density on body weight and feed consumption of broiler chickens at 45 d of age are shown in Table 2. In current study, high level of add zinc oxide(80 mg/kg diet)faintly improved means of LBW, BWG, feed intake and positively affected FCR ($P<0.05$). This result is in harmony with those of Hosseini-Mansoub *et al.*(2010), who establish that feeding on a diet supplemented with Zn(50 mg/kg) resulted in a superior performance in the male broilers. Diets rich in zinc has been shown to decrease the catabolic reaction induced by immune stimulation and might be effectual in promoting growth (Rymer and Givens, 2005). Burrell *et al.(2004)*establish that a diet of maize-soybean meal including 30 p.p.m of zinc was enough to support optimal performance of broilers throughout the first 3 weeks of age. On the other hand, Shyam Sunder *et al.*(2008) showed that supplementation of zinc to the basal diet at graded levels did not significantly influence on body weight, feed intake or feed conversion efficiency, of broiler chickens at 4 weeks of age. This response is in harmony with the conclusion of Rossi *et al.* (2007), who reported that body weight gain of broiler was not affected by dietary addition of rising levels of organic zinc at 0, 15, 30, 45, or 60 p.p.m. Lai *et al.* (2010) found that hybrid chicks fed 40 and 60 mg/kg of Zn had non-significant effect on their feed consumed. Iqbal *et al.* (2011) reviewed that feed intake of broiler chicks was not affected significantly by addition Zn either from organic or inorganic sources. Tang *et al.* (2015) furthermore reviewed that rate of daily feed consumed of broiler chickens were not influenced by enrichment of a variety of sources of zinc. The useful effect of Zn enrichment on feed conversion was recommended to be credited to the enhancement in nutrient digestibility and efficiency of their utilize (Lagana *et al.*, 2007; Sahin *et al.*, 2009).For the reason that, Se and Zn have

a defensive role on pancreatic tissue against oxidative damage, they might assist the pancreas to function appropriately counting secretions of digestive enzymes, thus enhancement digestibility of nutrients and performance.

Chicks at typical stocking density (11.9 birds/m^2) accomplished significantly superior performance contrasted with the high stocking density (16.66 birds/m^2). This demonstrates an essential role of stocking density as a pressure factor on performance. Means of "LBW, BWG and feed intake" of broiler were influenced adversely by high stocking density. Alike, results were found by other researcher (Thomas *et al.* 2004; Muniz *et al.* 2006; Ismail *et al.* 2014) they demonstrated that expanding the number of birds per unit miserable growth rate and feed intake of broilers. On the other hand, Buijs *et al.* (2009) reported that LBW of broiler chicks was not significantly dissimilar amongst birds reared at diverse stocking densities at 39 days of age. Moreover, FCR was not influenced by high stocking density. In the same way, other researcher (Feddes *et al*., 2002; Galobart and Moran, 2005) reviewed that there was no significant effect of stocking density on FCR of hybrid chicks. In difference, El-Gogary and Azzam (2014) showed that through the grower stage, broilers raised at a high stocking density had an lower FCR to that of hybrid chicks housed at a typical density.

Table 2. Effect of zinc oxide enrichment-diets and stocking density on growth performance of hybrid chicks at 45 days of age

Main effects	3-day BW (kg)	45-day BW (kg)	TBWG (kg)	TFI (kg)	TFCR (kg:kg)
Added Zn (A):					
0.0mg/kg diet	0.067	1.782^{ab}	1.714^{ab}	3.490^{ab}	1.955^{a}
40 mg/kg diet	0.067	1.843^{ab}	1.776^{ab}	3.459^{ab}	1.948^{ab}
60 mg/kg diet	0.067	1.771^{b}	1.703^{b}	3.323^{b}	1.951^{ab}
80 mg/kg diet	0.067	1.879^{a}	1.812^{a}	3.495^{a}	1.929^{b}
SEM	0.000	0.031	0.031	0.053	0.007
Significance	NS	*	*	*	*
Cage Density					
5 birds/cage	0.067^{b}	1.918^{a}	1.851^{a}	3.583^{a}	1.937^{b}
7 birds/cage	0.068^{a}	1.720^{b}	1.652^{b}	3.229^{b}	1.955^{a}
SEM	0.000	0.022	0.022	0.038	0.005
Significance	*	*	*	*	*
AB Interaction (Diet*cage Density)					
A1B1	0.067	1.895	1.827	3.543	1.939
A1B2	0.068	1.669	1.601	3.154	1.970
A2B1	0.066	1.962	1.895	3.672	1.938
A2B2	0.068	1.724	1.656	3.246	1.959
A3B1	0.067	1.837	1.770	3.450	1.950
A3B2	0.068	1.705	1.636	3.196	1.953
A4B1	0.067	1.977	1.910	3.668	1.921
A4B2	0.068	1.782	1.713	3.321	1.938
SEM	0.000	0.045	0.045	0.076	0.010
Significance	NS	NS	NS	NS	NS

$^{a-b}$: **"For each of the main effects means in the same Colum with different superscripts differ significantly (P≤ 0.05)".**

Blood plasma parameters: -

The effect of dietary zinc oxide levels and stocking density on blood plasma concentrations of triiodothyronine and thyroxine and corticosteronein broiler chickens are available in Table 3. There was no significant effect of zinc oxide levels on plasma levels of thyroxinein broilers. Nevertheless, plasma triiodothyronine level raised significantly (P≤0.05) in response to supplemented zinc oxide levels and the highest triiodothyronine level occurred when Zn-oxide was additional at 60 mg/kg compared to control one. In contrast, blood plasma corticosterone level decreased significantly in response to added zinc with the control group. Our data are in line with those reported by Sahin *et al.* (2002) that, insulin plasma concentration increased, although corticosterone concentration reduced with rising supplemental dietary zinc. This is a usual metabolic relationship between insulin (anabolic) and corticosterone (catabolic), having reverse effects in metabolism.

Table 3. Effect of zinc oxide including-diets and stocking density on plasma concentrations of T3, T4 and corticosterone (ng/ml) in experimental chickens

Main effects	*T3*	*T4*	*Cort*
Added Zn (A):			
0.0mg/kg diet	2.5700^{b}	14.990	2.368^{a}
40 mg/kg diet	2.9816^{a}	15.291	2.076^{b}
60 mg/kg diet	3.2866^{a}	15.318	1.853^{b}
80 mg/kg diet	3.0900^{a}	15.826	1.930^{b}
SEM	0.128	0.606	0.086
Significance	*	NS	*
Cage Density (B):			
5 birds/cage	3.100	15.260	1.784^{b}
7 birds/cage	2.863	15.452	2.330^{a}
SEM	0.091	0.428	0.061
Significance	NS	NS	*
AB Interaction (Diet*cage Density)			
A1B1	2.610	15.4933	2.013
A1B2	2.530	14.4866	2.723
A2B1	3.090	15.0333	1.856
A2B2	2.873	15.5500	2.296
A3B1	3.553	15.1700	1.600
A3B2	3.020	15.4666	2.106
A4B1	3.150	15.3466	1.666
A4B2	3.030	16.3066	2.193
SEM	0.182	0.857	0.122
Significance	NS	NS	NS

$^{a-b}$: **"For each of the main effects means in the same Colum with different superscripts differ significantly (P≤ 0.05)".**

In contrast, Pathak *et al*., (2011)found that when zinc was administered to ethanol-fed rats in their drinking water, the serum levels of T3, T4, and TSH were brought to quite close to the normal levels. This shows that zinc could play a positive role in thyroid hormone metabolism.Other studies have also demonstrated that zinc supplementation in the diet could improve thyroid function as indicated by the reduction in TSH levels (Bucci *et al.,* 1999). On the other hand, Dönmez *et al.* (2002) revealed that serum T3 and T4 levels and the widths of follicles of thyroid organ were essentially diminished with significant levels (500 and 1000 mg Zn/L) of Zn intake toward the finish of the examination in chickens

Stocking density had no significant effects on plasma thyroid hormones. Our result agrees with those of other researchers who observed that stocking density did not influence plasma levels of T3 and T4in chickens (El-Gogary and Azzam, 2014; Tong *et al.,* 2012). On the other hand, in our study the plasma corticosterone hormone was increased with the high level of stocking density. These results have the same line with these reported by Craig *et al*. (1986) who, found that increasing stocking density increased total plasma corticosteroids in some experiments. Hocking *et al*. (2001) also reported that plasma corticosterone concentration in broiler breeders at 6 wk of age was 0.5

ng/mL under usual stocking density (9 birds/m^2). Cheng and Muri (2004) showed that laying hens showed significantly lesser plasma corticosterone levels in single-bird cages (525 cm^2/bird) than in the 10-bird cages (419 cm^2/bird), indicative of social stressors could be a factor in higher production of corticosterone in hens. Nevertheless, in contrast with the current result, Türkyilmaz (2008) observed that stocking density did not significantly affect on blood corticosterone concentration in hybrid chickens. There was a tendency in the direction of rising corticosterone concentration with higher stocking density, with a statistically significantraise in blood corticosterone concentration in the high stocking density groups.

The effects of dietary enrichment with zinc oxide and stocking density on the plasma concentrations of "total protein, albumin, globulin, total lipids, cholesterol", high density lipoprotein cholesterol and low-density lipoprotein cholesterol (LDL) are found in Table 4. There were significant differences ($P \leq 0.05$) in blood plasma concentrations of "total protein, albumin, globulin, total lipids, cholesterol, high density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL)" between the control and zinc-supplemented groups. Our data are harmony with of those Feng *et al.* (2010), who showed that a zinc-glycinechelate increased serum total protein; however had no effect on albumin, whereas Barman *et al.* (2009) did not get any significant effect of different dietary zinc-addition levels on serum protein concentration. Kucuk *et al.* (2003) showed that additional zinc resulted in increased levels of serum total protein nevertheless reduced chol. concentration. Hosseini-Mansoub *et al.* (2010) reported that triglyceride and cholesterol levels were significantly higher in the groups not provided with antioxidants than those provided with Zn and vitamin E. Sahin *et al.* (2005) observed that Zn enrichment didn't a significantly affect on serum cholesterol concentration and its level linearly increased as dietary Zn supplementation increased from 30 to 60 mg/kg. Tawfeek *et al.* (2014) found that serum cholesterol level insignificant decrease in cholesterol level of Zn + Se-supplemented group of broilers compared with the control one.

Our results observed also that stocking density had a significant effect on plasma "TP, globulin and TL whilst levels of Chol, albumin, LDL and HDL" were not affected.

It appears that plasma lipids were influenced harmfully by increasing stocking density which might be interrelated to the increased production of corticosterone into the circulation. It is well-known that high plasma corticosterone level enhances lipolysis and gluconeogenesis (Sturkie., 2000) which might elucidate the higher levels of TL consistent with our results. Skrbic *et al.* (2009) did not find any effect of stocking density stress on blood cholesterol concentration in broiler chickens.

Table 4. Effect of zinc oxide enrichment-diets and stocking density on some blood plasma constituents in broilers chickens

Main effects	TP g/dl	Alb g/dl	Glob g/dl	TL mg/dl	Cholm g/dl	LDL mg/dl	HDL mg/dl
Added Zn (A):							
0.0mg/kg diet	4.025^{b}	2.220^{b}	1.805^{b}	901.966^{a}	228.116^{a}	62.400^{a}	52.333^{b}
40 mg/kg diet	4.155^{ab}	2.350^{ab}	1.805^{b}	881.600^{a}	194.416^{b}	53.300^{b}	58.500^{ab}
60 mg/kg diet	4.363^{a}	2.415^{a}	1.948^{a}	827.766^{b}	192.450^{b}	53.950^{b}	57.766^{ab}
80 mg/kg diet	4.203^{ab}	2.313^{ab}	1.890^{ab}	786.233^{c}	187.666^{b}	55.283^{b}	60.916^{a}
SEM	0.075	0.058	0.041	12.207	4.373	2.141	2.090
Significance	*	*	*	*	*	*	*
Cage Density (B):							
5 birds/cage	4.337^{a}	2.331	2.005^{a}	817.033^{b}	197.075	56.866	57.916
7 birds/cage	4.035^{b}	2.317	1.718^{b}	881.750^{a}	204.250	55.600	56.841
SEM	0.053	0.041	0.029	8.632	3.092	1.514	1.478
Significance	*	NS	*	*	NS	NS	NS
AB Interaction (Diet*cage Density)							
A1B1	4.156	2.290	1.866	863.100	230.733	63.433	53.0666
A1B2	3.893	2.150	1.743	940.833	225.500	61.366	51.6000
A2B1	4.260	2.306	1.953	852.100	189.600	53.133	58.5666
A2B2	4.050	2.393	1.656	911.100	199.233	53.466	58.4333
A3B1	4.643	2.443	2.200	788.466	188.400	55.166	57.9666
A3B2	4.083	2.386	1.696	867.066	196.500	52.733	57.5666
A4B1	4.290	2.286	2.003	764.466	179.566	55.733	62.0666
A4B2	4.116	2.340	1.776	808.000	195.766	54.833	59.7666
SEM	0.107	0.082	0.058	17.264	6.185	3.028	2.956
Significance	NS	NS	NS	NS	NS	NS	NS

^{a,b}:"For each of the main effects means in the same Colum with different superscripts differ significantly ($P \leq 0.05$)".

The results concerning to the authority of zinc oxide-supplemented diet and stocking density on levels of "superoxide dismutase, malondialdehyde, antibody titers against Newcastle disease virus and avian flu virus" in plasma are found in Table 5. In our study, the addition of zinc oxide significantly increased activity of SOD compared to control one. Although, the chicks fed the Zn oxide-added diets had significantly lesser activity of MDA compared to the control diet. In this consider other changes have been noted in the activity of antioxidant enzymes may also be linked to the level of microelements, such as Fe, Cu, and Zn. Ognik and Krauze (2016) they also stated that zinc and copper are SOD cofactors, while Fe is a catalase cofactor. Activities of Cu–Zn superoxide dismutase were increased with increasing Zn levels in broiler chickens (Bun *et al.*, 2011). Ivanišinová *et al.* (2016) found that activity of SOD measured in erythrocytes was significantly elevated in the Zn-proteinate-supplemented group compared to control

broilers. Zinc can compete with iron and copper to bind to the cell membrane and decrease the production of free radicals, thus exerting a direct antioxidant action (Tate *et al.*, 1999). Hafez *et al*. (2019) revealed that Cu-Zn-SOD and catalase activities were significantly increased while the MDA concentrations were significantly decreased in broiler chickens fed diets supplemented with zinc oxide nanoparticles at either 40 or 80 mg/kg as compared with those fed the control diet. this effect might have attributed to that Zn is an essential component in Cu-Zn-SOD which is involved in cellular scavenging of free radicals and reactive oxygen species (Prasad, 2008). Also, Zn supplementation decreased serum MDA levels in heat stressed birds and this might be attributed to that Zn induces production of metallothionein, which is an effective scavenger for hydroxyl radical (Sahin *et al*., 2009). Kopeć *et al.* (2013) found that feeding broilers on diets enriched with Zn (200 mg/kg) enhanced SOD activity in all tissues (muscles, plasma and erythrocytes).

Table 5. Effect of zinc oxide enrichment-diets and stocking density on plasma concentrations of SOD, MDA and antibody titers against NDV and AFV in broiler chickens

Main effects	SOD (U/ml)	MDA (nmol/ml)	NDV (log^2)	AFN (log^2)
Added Zn (A):				
0.0mg/kg diet	11.518^b	53.866^a	5.866^b	4.033^b
40 mg/kg diet	16.99^a	47.616^b	6.750a	4.900^a
60 mg/kg diet	18.00^a	43.616^b	6.816^a	4.833^a
80 mg/kg diet	18.77^a	44.583^b	6.700^a	4.950^a
SEM	0.633	1.713	0.215	0.208
Significance	*	*	*	*
Cage Density (B):				
5 birds/cage	16.59	41.175^b	6.941^a	4.808
7 birds/cage	16.05	53.666^a	6.125^b	4.550
SEM	0.364	1.211	0.152	0.147
Significance	NS	*	*	NS
AB Interaction (Diet*cage Density)				
A1B1	11.750	47.0333	6.100	4.266
A1B2	11.286	60.7000	5.633	3.800
A2B1	17.846	41.3000	7.266	4.733
A2B2	16.143	53.9333	6.233	5.066
A3B1	17.420	36.5333	7.233	5.266
A3B2	18.586	50.7000	6.400	4.400
A4B1	19.380	39.8333	7.166	4.966
A4B2	18.193	49.3333	6.233	4.933
SEM	0.895	2.423	0.304	0.295
Significance	NS	NS	NS	NS

$^{a-b}$: **"For each of the main effects means in the same Colum with different superscripts differ significantly (P≤ 0.05)".**

The current results reported that the immune response titers against NDV and AFV in blood plasma of zinc oxide- reinforced treated hybrid chickens were significantly superior to that of the control one. On the contrary Rama Rao *et al*. (2016) where supplementation of organic Zn, Se and Cr did not significantly influence the immune response titer of New castle disease vaccine. Sajadifar *et al*. (2013) also reported higher requirement of Zn (120 and 200 mg/kg) for augmentation of humoral immune response in chickens. Similarly, Zn supplementation of broiler diets or litter increases the immune response of chickens (Park *et al*., 2004).Previous studies reviewed by Das (2002) also reported higher requirement of Zn (>80 mg/kg) to influence humoral immune response in chicken. It was also observed by Bartlett and Smith(2003) that higher antibody titers to sheeperd blood cells (SRBC) with 181 ppm of Zn compared with lower levels (34 to 68 ppm) of supplementation. Similar significant improvement in anti-body titer against specific antigen (SRBC/NDV virus) was reported in the literature (Bartlett and Smith 2002; Abdallah*et al.,* 2009) in commercial broilers fed organic form of Zn and Cu. Shyam Sunder *et al*.(2008) found that the antibody titers against SRBC inoculation, a measure of humoral immune response, varied significantly with the level of supplemental Zn at 4 wk of age. Highest antibody titers were observed at 80 ppm, above which no further improvement in antibody titers was noticed, implying that 80 ppm was adequate to provide optimum response.

There was a critical impact of stocking density on plasma activity of SOD however levels of MDA expanded by increasing stocking density. Physiological or environmental stress (because of increasing stocking density) if the stocking density is excessively high, the temperature may emerge which may be harmfully influences the physiological status of the chick.

It is notable that warmth causes an expanded production of MDA (Shain *et al*., 2001; Naziroğlu *et al*., 2000). In this research, there was significant lowering effect of stocking density on immune response titers against NDV even though, stocking density had no impact on AFV. Eriflir and Eriflir (2002) revealed that there was a significant decrease in resistant reaction with an expansion in stocking density in Japanese quails. Tufft and Nockels (1991) additionally detailed that lessening in space grant made broilers further susceptible to infections. on the other hand, (Türkyilmaz, 2008) confirmed that stocking density had no impact on safe reaction in broilers, which is alike to those observed by Hocking *et al*., (2002), who found that resistant capacity was not influenced by nourishment limitation in broiler breeders.

CONCLUSION

The conclusion of our study propose that dietary enrichment of zinc oxide at levels of 40, 60 or 80 ppm has helpful effects on productive performance, antioxidant status and immune responses of hybrid broiler.

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تأثير إضافة عنصر الزنك وكثافة الإسكان على الأداء الإنتاجى ، الفسيولوجى و الخواص المناعية لكتاكيت اللحم

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أجريت هذه الدراسة بهدف تقييم آثر تدعيم العلائق بعنصر الزنك مع كثافتى إسكان على الأداء الإنتاجى ونشاط الغدة الدرقية وتركيز الكورتيكوستيرون وبعض قياسات الدم (الأجسام المضادة لفيروس النيوكاسيل وفيروس أنفلونزا الطيور) وكذالك نشاط مضادات الأكسدة فى بلازما الدم لكتاكيت التسمين.قسم عدد ١٩٢ كتكوت عمر ثلاث أيام من سلالة كوب ٥٠٠ إلى ثمان معاملات تجريبية بكل معاملة أربع مكررات، صممت التجربة فى تصميم عاملى (٤ X ٢) أربع مستويات من عنصر الزنك (٠،٠ ، ٤٠ ، ٦٠ و٨٠ مجم/كجم علف) مع كثافتي إسكان (١١,٩ طائر/م٢ أو ١٦,٦٦ طائر/م٢).وقد أوضحت النتائج أن تدعيم العلائق بمستوى عالي من الزنك (٨٠ مجم /كجم علف) قد أدى إلى تحسن معنوى فى صفات الأداء الإنتاجى لكتاكيت اللحم (وزن الجسم ، الزيادة فى وزن الجسم، معدل استهلاك العلف و معامل التحويل الغذائي).كما أظهرت النتائج أيضا زيادة معنوية فى تركيزات بلازما الدم من البروتين الكلى، الألبيومين، الدهون الكلية، الكوليستيرول، الليبوبروتينات عالية الكثافة، مع انخفاض تركيز الليبوبروتينات منخفضة الكثافة فى بلازما الدم للمجموعات المغذاه على العلائق المدعمة بعنصر الزنك بمستوياته المختلفة مقارنة بمجموعة الكنترول. كما أدت التغذية على العلائق المدعمة بعنصر الزنك بمستوياته التجريبية إلى انخفاض تركيز الكورتيكوستيرون فى بلازما الدم مقارنة بالمجموعة الضابطة إلا أنه كان مصحوبا بتحسن فى نشاط الغدة الدرقية.كان لإضافة عنصر الزنك بمستوياته التجريبية تأثير إيجابى المعنوية على نشاط السوبر أكسيد ديسميوتيز ، الأجسام المضادة لكل من فيروس النيوكاسيل و فيروس أنفلونزا الطيور، بينما كان تأثير هذه الإضافة منخفضا معنويا على نشاط إنزيم المالون داى ألدهيد مقارنة بمجموعة الكنترول.وقد حققت كثافة الإسكان المنخفضة تأثيرا إيجابيا على الأداء الإنتاجى لكتاكيت اللحم، مع انخفاض مستوى الكورت، الدهون الكلية والمالون داى ألدهيد فى بلازما الدم للكتاكيت مقارنة بكثافة الإسكان العالية ، كما ارتفع مستور البروتين الكلى والأجسام المضادة لكل من فيروس النيوكاسيل و فيروس أنفلونزا الطيور فى بلازما الدم للمجموعة التجريبية منخفضة كثافة التسكين مقارنة بالمجموعة ذات كثافة الإسكان العالية. بينما لم يتأثر نشاط هرمونات الغدة الدرقية، نشاط السوبر أكسيد ديسميوتيز وتركيز الألبيومين، الكوليستيرول، الليبوبروتينات عالية الكثافة و الليبوبروتينات منخفضة الكثافة فى بلازما الدم للمجموعات التجريبية تحت كثافتى الإسكان.وخلصت هذه الدراسة إلى أن تدعيم العلائق بالزنك له تأثير إيجابي على الصفات الإنتاجية والمناعية وقياسات الدم فى كتاكيت التسمين مع مراعاة كثافة الإسكان المنخفضة (١١،٩ طائر/م٢)