

# Effects of Zinc Nanoparticles on Growth Performance, Carcass Characteristics, Immunity, and Meat Quality of Broiler Chickens

## Research Article

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## ABSTRACT

Zinc (Zn) supplementation of food can be used in broilers to overcome Zn deficiencies in human and also to improve the growth rate and immune system of birds. Due to the lack of information about the impact of Zn nanoparticles (Zn-NPs) on meat quality, this study was conducted to evaluate the differences between dietary Zn-NPs and other Zn sources on broiler performance, carcass characteristics, humoral immunity, meat quality, meat Zn content, as well as fresh and frozen meat oxidative values. Day-old broiler chicks (Ross 308, n=240) were randomly divided into six dietary treatments with four replicates, based on a completely randomized design (d 1-42). Dietary treatments composed of different Zn sources supplemented per kg of basal diet (30 mg/kg Zn) included: control (70 mg from ZnSO<sub>4</sub>), 70ZAAC (70 mg from Zn amino acid complex), 30BZMO (30 mg Zn from Zn-NPs), 50BZMO (50 mg Zn from Zn-NPs), 70BZMO (70 mg Zn from Zn-NPs), and 90BZMO (90 mg Zn from Zn-NPs). Results showed a higher amount of IgM in chickens fed with Zn-NPs and 70ZAAC, though no effect was found on performance, carcass characteristics, and also the relative weight of lymphatic organs. Breast meat shear force, redness and lightness indexes, malondialdehyde (MDA) content, and Zn content were significantly affected by experimental diets, though no effect of treatments was observed on MDA and Zn content of thigh muscle. Lower pH value of breast meat was found in chickens fed with Zn-NPs and 70ZAAC. The present results showed that 30BZMO, as a recommended Zn-NPs treatment, supported growth performance, improved humoral immunity, Zn content of breast meat, and meat quality, while decreasing MDA content of breast meat.

**KEY WORDS** broiler, immune system, meat quality, nanochelating technology, performance, zinc nanoparticles.

## INTRODUCTION

Based on the world health organization (WHO), zinc (Zn) deficiency is an important cause of diseases in developing countries (Shrimpton *et al.* 2005). As the second trace element in the body of animals, Zn needs to be regularly fed to compensate the lack of its body storage (Swain *et al.* 2016). Also, Zn is the cofactor of more than 300 enzymes and plays a vital role in numerous biological processes (Cao *et*

*al.* 2000; Ao *et al.* 2011). Moreover, Zn is crucial for cell growth, nucleic acid synthesis, cell division, protein synthesis, growth, skeletal development, and immune system (Sunder *et al.* 2008; Sahoo *et al.* 2014). Food supplementation is the best and the safest method to eliminate nutritional deficiencies (Salgueiro *et al.* 2002). Despite the high consumption of poultry meat, a supply of Zn from this product is very low (Houshiar-Rad *et al.* 2013). Therefore, zinc supplementation for chicken meat can play a signifi-

cant role in the supply of Zn to societies with Zn deficiency like Iran and Egypt (Houshiar-Rad *et al.* 2013). Many studies have indicated a linear increase in Zn content of chicken tissues by increasing dietary Zn levels (Sunder *et al.* 2008; Salim *et al.* 2012b; Wen-Xiang *et al.* 2015; Wen *et al.* 2019). Zinc supplementation of broiler's diet improved their performance, immune system, organs' Zn fortification, and bone ossification (Akbari Moghaddam Kakhki *et al.* 2016; Kwiecień *et al.* 2016; Akhavan-Salamat and Ghasemi, 2019). It was also indicated that dietary Zn supplementation of hens did not affect their chick's performance (Hudson *et al.* 2005). Among different Zn sources, inorganic Zn has the lowest bioavailability; then, feeding the inorganic form of Zn would cause environmental pollution due to the low level of absorption and high level of excretion (Salim *et al.* 2012a; Salim *et al.* 2012b; Bratz *et al.* 2013). Organic Zn (e.g. zinc-methionine and Zn amino acid complex) has higher bioavailability than inorganic sources and is more effective in stimulating growth than inorganic sources (Rahman *et al.* 2002; Jankowski *et al.* 2019). In the last decade, nanotechnology has been widely used to improve absorption efficiency and increase the bioavailability of trace minerals such as Zn in the livestock industry (Zhao *et al.* 2014). Nanomaterials exhibit novel properties, including high specific surface areas, surface activities, surface-active centers, and catalytic efficiencies (Gao and Matsui, 2005). Zn nanoparticles (Zn-NPs) have high bioavailability and have positive effects on feed intake, growth, immune system, production performance, and dressing performance, as well as lower environmental pollution (Singh *et al.* 2016; Swain *et al.* 2016; Jarosz *et al.* 2017; Jankowski *et al.* 2019). Therefore, using high bioavailable minerals such as Zn-NPs in broiler diets not only may enhance the Zn content of broiler chicken's meat, but also may eliminate the disposal of Zn in the environment. It was indicated that supplementing broiler chickens' diet with Zn-NPs improved average daily weight gain, antibody titers, relative spleen weight, and serum superoxide dismutase activity, though reducing overall FCR and serum malondialdehyde concentration (Akhavan-Salamat and Ghasemi, 2019). On the other hand, high levels of Zn-NPs may have mutagenic and cytotoxic effects and cause liver and renal toxicity, and growth retardation (Sadoval *et al.* 1999; Swain *et al.* 2016; De Andrade Vieira *et al.* 2019). Improving the preservability and quality of broiler chicken's meat are now major goals for the poultry industry (Salim *et al.* 2012a). One of the particular Zn functions is its role in the antioxidant defense system (Saleh *et al.* 2018). So, it seems that Zn can play an essential role in the fight against oxidative corruption during preservation and, it can lead to an increase in meat quality and its shelf life. Based on our knowledge, no information is available regarding the effects of dietary Zn-

NPs on meat quality of chickens. Therefore, the aim of this study was to assess the differences between dietary Zn-NPs and other Zn sources on growth performance, carcass characteristics, immune system, meat quality and also, Zn content of meat and oxidative stability in broiler chickens.

## MATERIALS AND METHODS

### Zinc sources

Three sources of supplemental Zn were used in this study to formulate dietary treatments of broilers. Treatments included: 1) feed-grade ZnSO<sub>4</sub> (Sigma-Aldrich, ≥98% Zn, Cat. No. Z0501) as an inorganic form of Zn; 2) Zn amino acid complex (Sigma-Aldrich, ≥62.8% Zn, Cat. No. Z0501) as an organic form of Zn; 3) bonza Zn metabolism optimizer (BZMO) as a Zn-NPs source (Bonza, Sodour Ahrar Shargh Company, Tehran, Iran, ≥14% Zn). BZMO was designed and synthesized using nanochelating technology, which registered at the United States Patent and Trademark Office (US8288587 B2) and also European Patent Office (EP 2444096 A1). Moreover, BZMO composition and preparation methods have been presented by Mohammadi *et al.* (2015). Furthermore, high-resolution transmission electron microscopy (HRTEM) were used for imaging and analytical characterization of BZMO (Central Laboratory of University of Tabriz), and it was indicated that the size of Zn-NPs was smaller than 50 nm.

### Birds and experimental design

This study was conducted from September to October, 2015 in Poultry Research Unit, located in Khalat-Pushan Agricultural Research Station, University of Tabriz, East Azarbaijan province, Iran (38° 01'47.6"N 46° 23'45.8"E). All the procedures of this experiment were approved by the Animal Care and Use Committee at the University of Tabriz, Iran. For this reason, 240 Ross-308 mixed-sex broiler chicks were randomly assigned into six treatment groups, based on a completely randomized design with four replicates and ten broiler chickens in each replicate (pen) from d 1 to 42. The basal diet was based on a corn-soybean meal and contained 30 mg Zn /kg basal diet (starter, grower, and finisher). Experimental diets composed of adding different Zn sources per kg of basal diet based on the following treatments, included: 1) 70 mg from feed-grade ZnSO<sub>4</sub> (control); 2) 70 mg from Zn amino acid complex (70ZAAC); 3) 30 mg from Zn-NPs (30BZMO); 4) 50 mg from Zn-NPs (50BZMO); 5) 70 mg from Zn-NPs (70BZMO); 6) 90 mg from Zn-NPs (90BZMO). All diets were formulated based on Ross 308 recommendations to provide all nutrient requirements (except for Zn) of broiler chickens during the growth period (Table 1). In this experiment, Zn-free mineral premix was used. Afterward, graded levels of Zn with different sources were added to make dietary treatments.

**Table 1** Ingredients and nutrients composition of the basal diet (as fed)

Ingredients (g/kg)	Starter (d 1-10)	Grower (d 11-24)	Finisher (d 25-42)
Corn	529.70	570.00	570
Soybean meal	408.80	349.10	321.1
Whole grain barley	0	29.30	53.9
Fatty acid	13.70	10.60	16
Oyster shell	16.00	13.30	12.6
Dicalcium phosphate	18.90	15.90	14.7
Sodium chloride	3	1.80	1.8
Sodium bicarbonate	0.10	2.20	3.2
L-lysine	1.10	0.30	0
DL-methionine	3.00	2.20	1.6
L-threonine	0.70	0.30	0
Zn-free mineral premix <sup>1</sup>	2.50	2.50	2.5
Vitamin premix <sup>2</sup>	2.50	2.50	2.5
<b>Nutrients concentration of basal diet (as calculated)</b>			
Apparent metabolizable energy corrected for nitrogen (AME <sub>n</sub> ) (MJ/kg)	11.93	12.14	12.35
Crude protein (g/kg)	220.00	200.00	190.00
Arginine (g/kg)	13.60	11.70	10.40
Lysine (g/kg)	13.40	11.40	10.00
Threonine (g/kg)	8.80	7.70	6.80
Methionine (g/kg)	4.80	4.10	3.80
Methionine + cysteine (g/kg)	10.00	8.70	7.90
Calcium (g/kg)	9.90	8.30	7.80
Available phosphate (g/kg)	4.70	4.10	3.80
Zn (g/kg)	0.03	0.03	0.03

<sup>1</sup> Supplied per kilogram of Zn-free mineral premix: Iron: 44000 mg/kg; Manganese: 64000 mg/kg; Copper: 10000 mg/kg; Iodine: 3500 mg/kg and Selenium: 150 mg/kg.

<sup>2</sup> Supplied per kilogram of vitamin premix: vitamin A (retinyl acetate): 7200 mg/kg; vitamin D<sub>3</sub> (cholecalciferol): 600 mg/kg; vitamin E (DL- $\alpha$ -tocopheryl acetate): 14400 mg/kg; vitamin B<sub>6</sub> (pyridoxine hydrochloride): 1200 mg/kg; vitamin B<sub>12</sub> (cyanocobalamin): 600 mg/kg; Thiamin mononitrate: 720 mg/kg; Riboflavin: 6600 mg; Niacin: 10000 mg/kg; Pantothenic acid: 30000 mg/kg; Menadione: 1600 mg/kg; Folic acid: 500 mg/kg and Choline chloride: 400000 mg/kg.

Chickens had *ad libitum* access to feed and water during the experiment and were bred based on 2014 Ross-308 breeding guide.

### Growth performance

Body weight (weighing after 6 h of fasting) and feed intake were recorded on a pen base. Then, feed conversion ratio (FCR) was calculated based on feed: gain ratio. Moreover, livability was recorded daily and reported as a percentage in each pen. Feed intake, body weight gain, and feed conversion ratio were corrected for dead broiler chickens in each pen. Finally, for comparing the production of experimental units, European production efficiency index formula was used, which is presented as follows:

Production efficiency index = [(percentage of survival  $\times$  average body weight) / (FCR  $\times$  age (d))] / 100

### Carcass characteristic

On the 42<sup>nd</sup> day of the experiment, four broiler chickens in each replicate of different treatments (after 12 h of feed deprivation) were randomly selected and slaughtered to evaluate carcass characteristics.

Then, carcass parameters were weighed, which included: carcass, breast, thighs, abdominal fat pad, heart, liver, gizzard, pancreas, small intestine segments (duodenum, jejunum, and ileum) and lymphoid organs (spleen, thymus, and bursa of fabricius).

Afterward, the relative weight of carcass parameters to live body weight was calculated and reported. Furthermore, deboned breast and thigh fillets were individually packed in plastic bags and stored in two forms, including fresh (at 4 °C) for 24 h and frozen (at -20 °C) for two months in order to evaluate meat lipid oxidation.

### Meat quality measurements

After 24 h of chilling the breast meat samples at 4 °C, meat quality characteristics were determined in triplicates per each sample.

Breast meat pH was evaluated based on AOAC (1990) using a pH meter (Metrohm 827, Sweden). Drip loss of breast meat samples was also determined based on Saenmahayak *et al.* (2012) method.

Breast meat color indexes (L\*: lightness, a\*: redness, and b\*: yellowness values) were measured using Minolta Chroma Meter CR-400 (Osaka, Japan).

Furthermore, the shear force of breast muscle was measured by a texture analyzer instrument (Instron, M350-10CT, England) as described by Warner *et al.* (2010).

#### Measuring meat lipid oxidation

The oxidative stability of fresh and frozen breast meat samples was determined by measuring thiobarbituric acid reactive substances (TBARSs) using a colorimetric method.

The method of Monin *et al.* (2003) was used to measure TBARSs. The results were also presented as micrograms of malondialdehyde (MDA) per gram of breast meat (He *et al.* 2015).

#### Measuring meat Zn content

Zinc content of broiler breast and thigh meat was measured based on AOAC (1990) and using atomic absorption spectrophotometer instrument (Shimadzu, AA-6300, Japan).

#### Humoral immunity response

To evaluate antibody titers (total Ig) against sheep red blood cell (SRBC), a nonpathogenic antigen (Sunder *et al.* 2008), two birds per each replicate (8 birds/treatment) were randomly selected. Then, 1 ml of phosphate-buffered saline solution (PBS) containing 10% SRBC suspension was injected into the left breast muscle at d 28 and 35. Next, seven days after injections (d 35 and 42), blood samples were collected from a wing vein into tubes. Thereafter, samples were let to clot for about 60 min at room temperature and centrifuged at 3000 rpm for 15 min to separate sera.

Afterward, samples were stored at -20 °C until analysis. Primary and secondary antibody titers to sheep red blood cells (SRBC) were evaluated based on haemagglutination assay.

Accordingly, antibody titers were expressed as the log<sub>2</sub> of the highest dilution, which had 50% agglutination (Sunder *et al.* 2008). Furthermore, IgG (mercaptoethanol-resistant antibodies) and IgM (mercaptoethanol-sensitive antibodies) were determined (Boa-Amponsem *et al.* 2000).

#### Statistical analyses

The obtained data on growth performance parameters, carcass characteristics, meat quality factors, and antibody titers to SRBC were statistically analyzed. For this reason, after doing proper normality tests, data were analyzed based on a completely randomized design using generalized linear model (GLM) procedure of SAS software (SAS, 2003). For comparing treatments, Duncan's multiple range test was used, and  $P < 0.05$  was assumed significant.

#### Growth performance and carcass characteristics

Dietary Zn treatments in the present study had no significant effect on average daily weight gain (ADG), average daily feed intake (ADFI), and FCR during rearing periods ( $P > 0.05$ ), (Table 2). On the other hand, the European production efficiency index (EPEI) was significantly affected by experimental diets during starter ( $P < 0.05$ ), though no effect was found during grower, finisher, or the overall rearing periods ( $P > 0.05$ ), (Table 2).

Also, the highest EPEI index was observed in 70BZMO and 90BZMO (two Zn-NPs treatments), but the lowest EPEI index was observed in 70ZAAC (Table 2).

Similar to growth performance, dietary Zn treatments did not influence the relative weight of carcass characteristics, including: dressing percentage, thigh, breast, liver, pancreas, gizzard, and abdominal fat pad on the 42<sup>nd</sup> day of the experiment ( $P > 0.05$ ), (Table 2). On the other hand, the relative weight of heart was significantly affected by Zn treatments ( $P < 0.05$ ) and the highest amount was observed in 50BZMO and control groups, but the lowest amount was found in 30BZM and 90BZM treatments (Table 2). Also, the relative weight of duodenum, jejunum, ileum, and small intestine were not affected by dietary Zn treatments ( $P > 0.05$ ), (Table 2).

#### Zn and MDA content of meat

Zn content of breast muscle was significantly affected by dietary Zn treatments ( $P < 0.01$ ), and the highest amount was observed in 30BZMO treatment, but the lowest amount was found in 70ZAAC treatment (Table 3). Zinc content of thigh meat was not affected by Zn treatments ( $P > 0.05$ ), (Table 3). As an interesting result, the lowest level of Zn-NPs (30 mg/kg) fortified Zn content of breast meat 60% higher than the control group, which may be due to the high bioavailability of Zn in this group.

Dietary Zn treatments significantly influenced the MDA content of breast muscle preserved 48 h at 4 °C ( $P < 0.01$ ). Also, the lowest amount of breast muscle MDA was observed in 70BZMO and 30BZMO treatments, but the highest amount was observed in 50BZMO and control groups (Table 3).

Moreover, MDA content of breast muscle after 60 days of preservation at -20 °C was affected by different dietary Zn treatments ( $P < 0.05$ ), and the lowest amount of MDA was observed in 30BZMO treatment, though 70BZMO and 50BZMO treatments showed the highest values (Table 3). However, MDA content of thigh muscle was not affected by Zn treatments ( $P > 0.05$ ), (Table 3).

## RESULTS AND DISCUSSION

**Table 2** The effect of different dietary Zn sources on performance, carcass characteristics, and small intestine of broiler chickens

Item <sup>1</sup>	Different dietary zinc treatments						SEM	P-value
	Control	70ZAAC	30BZMO	50BZMO	70BZMO	90BZMO		
<b>ADFI (g/d)</b>								
Starter	26.38	26.63	26.54	27.90	27.34	26.10	0.61	0.35
Grower	82.54	78.25	81.50	82.02	82.02	86.56	3.06	0.58
Finisher	172.15	175.97	167.66	170.97	172.92	174.46	5.14	0.83
Total	105.93	107.46	105.13	106.97	107.38	109.96	2.77	0.87
<b>ADG (g/d)</b>								
Starter	17.50	17.56	17.78	18.58	18.79	18.35	0.55	0.44
Grower	50.03	48.69	51.15	50.85	53.10	54.41	2.00	0.40
Finisher	88.73	93.10	89.29	89.12	88.43	90.58	2.68	0.82
Total	58.87	60.31	59.55	59.57	60.07	61.32	1.55	0.91
<b>FCR</b>								
Starter	1.51	1.52	1.49	1.50	1.45	1.42	0.02	0.18
Grower	1.65	1.61	1.59	1.61	1.53	1.59	0.03	0.42
Finisher	1.94	1.89	1.87	1.91	1.95	1.95	0.03	0.61
Total	1.80	1.78	1.76	1.79	1.78	1.78	0.02	0.89
<b>EPEI</b>								
Starter	144.95 <sup>abc</sup>	140.32 <sup>c</sup>	144.26 <sup>bc</sup>	145.91 <sup>abc</sup>	158.24 <sup>a</sup>	155.62 <sup>ab</sup>	4.20	0.04
Grower	361.74	724.12	410.41	407.95	422.99	415.44	17.19	0.18
Finisher	705.67	724.12	753.09	721.56	680.68	712.81	29.26	0.65
Total	295.24	313.21	327.55	307.98	295.17	300.14	19.78	0.84
<b>Carcass traits (%)<sup>2</sup></b>								
Dressing percentage	63.96	60.83	66.13	65.98	66.46	65.04	1.8	0.28
Thigh	18.06	17.45	17.42	17.39	17.26	18.26	0.33	0.22
Breast	26.27	26.16	28.54	28.18	28.22	27.07	0.99	0.39
Liver	1.78	2.03	2.17	1.94	1.95	1.85	0.12	0.34
Heart	0.59 <sup>a</sup>	0.51 <sup>b</sup>	0.46 <sup>b</sup>	0.60 <sup>a</sup>	0.55 <sup>ab</sup>	0.46 <sup>b</sup>	0.32	0.02
Pancreas	0.22	0.21	0.21	0.22	0.22	0.21	0.01	0.98
Gizzard	1.43	1.47	1.36	1.42	1.52	1.40	0.10	1.43
Abdominal fat pad	0.71	0.85	0.60	0.45	0.41	0.69	0.13	0.62
<b>Small intestine (%)<sup>2</sup></b>								
Duodenum	0.56	0.59	0.49	0.46	0.54	0.48	0.02	0.06
Jejunum	1.48	1.22	1.34	1.04	1.21	1.14	0.11	0.21
Ileum	1.21	0.97	1.10	0.86	0.84	0.88	0.1	0.14
Total	3.25	2.78	2.93	2.36	2.59	2.50	0.23	0.06

<sup>1</sup> Starter stands for the duration from d 1 to 10, Grower stands for the duration from d 11 to 24, Finisher stands for the duration from d 25 to 42, and Total stands for the duration from d 1 to 42.

<sup>2</sup> Relative weight of the trait to live body weight.

Control: 70 mg ZnSO<sub>4</sub>; 70ZAAC: 70 mg zinc amino acid complex; 30BZMO: 30 mg zinc nanoparticles; 50BZMO: 50 mg zinc nanoparticles; 70BZMO: 70 mg zinc nanoparticles and 90BZMO: 90 mg zinc nanoparticles.

ADFI: average daily feed intake; ADG: average daily weight gain; FCR: stands for feed conversion ratio; EPEI: European production efficiency index.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

**Table 3** The effect of different dietary Zn sources on the amount of malondialdehyde (MDA) and zinc content in breast meat of broiler chickens

Item <sup>1</sup>	MDA in breast meat <sup>1</sup>		MDA in thigh meat <sup>1</sup>		Zinc content <sup>2</sup>	
	48 h	day 60	48 h	day 60	Breast meat	Thigh meat
Control	4.00 <sup>a</sup>	3.49 <sup>ab</sup>	1.77	4.11	2.90 <sup>bc</sup>	1.38
70ZAAC	2.49 <sup>bc</sup>	3.97 <sup>ab</sup>	1.42	4.34	1.23 <sup>d</sup>	1.00
30BZMO	1.95 <sup>c</sup>	2.83 <sup>b</sup>	1.68	4.12	4.61 <sup>a</sup>	1.59
50BZMO	4.56 <sup>a</sup>	4.77 <sup>a</sup>	1.41	4.67	1.81 <sup>cd</sup>	0.96
70BZMO	2.62 <sup>c</sup>	4.89 <sup>a</sup>	1.78	4.32	2.13 <sup>cd</sup>	1.38
90BZMO	3.47 <sup>ab</sup>	3.74 <sup>ab</sup>	1.19	3.60	3.87 <sup>ab</sup>	0.80
SEM	0.37	0.43	0.17	0.42	0.35	0.29
P-value	< 0.01	0.02	0.14	0.62	< 0.01	0.39

<sup>1</sup> µg MDA/100 g of thigh or breast meat.

<sup>2</sup> mg/100 g of thigh or breast meat.

Control: 70 mg ZnSO<sub>4</sub>; 70ZAAC: 70 mg zinc amino acid complex; 30BZMO: 30 mg zinc nanoparticles; 50BZMO: 50 mg zinc nanoparticles; 70BZMO: 70 mg zinc nanoparticles and 90BZMO: 90 mg zinc nanoparticles.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

### Meat quality characteristics

Breast meat lightness index ( $L^*$ ) was affected by dietary Zn treatments, and the control group had the lowest  $L^*$  index compared with other treatment groups ( $P < 0.05$ ), (Table 4). Breast meat redness index ( $a^*$ ) was higher in 70ZAAC and Zn-NPs treatments compared with the control group ( $P < 0.01$ ), (Table 4).

Shear force of breast meat was significantly affected by Zn treatments ( $P < 0.05$ ), and 50BZMO group had 121% lower shear force than the control group (Table 4). Different Zn treatments did not have any influence on breast meat yellowness index ( $b^*$ ) and drip loss ( $P > 0.05$ ), (Table 4).

Breast meat pH (24 hours after slaughter) was significantly higher in the control group compared with other Zn treatment groups ( $P < 0.05$ ), (Table 4).

### Antibody titers and lymphatic organs

Results of the present study indicated no effect of different Zn levels and sources on primary antibody titers against SRBC (total Ig, IgG, and IgM) at day 32 and also IgG titer at day 45 of the experiment ( $P > 0.05$ ), (Table 5). On the other hand, results of secondary antibody titers at d 45 indicated that IgM titer significantly increased ( $P < 0.05$ ) in 70ZAAC, 30BZMO, and 90BZMO treatments and total Ig tended to be higher ( $P = 0.06$ ) in 70ZAAC, 30BZMO, 70BZMO, and 90BZMO treatments than 50BZMO and control groups (Table 5). Furthermore, no effect of treatments on the relative weight of lymphatic organs (bursa of Fabricius, spleen, and thymus) was observed ( $P > 0.05$ ), (Table 5).

Results of the present study indicated no effect of Zn treatments on growth performance (except for EPEI index) or carcass characteristics (except for heart weight) of broilers. Resemble the present results, other researches indicated no effect of different Zn sources on growth performance of broiler chickens (Salim *et al.* 2010; Salim *et al.* 2012a; Salim *et al.* 2012b). Though, some previous works reported the significant and improving effects of nano-Zn on feed intake, FCR, and live body weight of broiler chickens (Ahmadi *et al.* 2013; Mishra *et al.* 2014; Zhao *et al.* 2014; Mohammadi *et al.* 2015). In a study, 40 mg/kg zinc-methionine and Zn oxide-nanoparticles improved average daily weight gain and overall FCR, though Zn sources and levels had no effect on carcass characteristics of broiler chickens (Akhavan-Salamat and Ghasemi, 2019). Also, dietary increasing of zinc-methionine levels (0, 25, 50, and 100 mg/kg) improved weight gain, FCR, and breast muscle (Saleh *et al.* 2018). Jankowski *et al.* (2019) with feeding three sources of Zn (zinc oxide, zinc nanoparticles, and zinc-methionine) in turkey hens reported higher ADG, but lower FCR in zinc nanoparticles and zinc-methionine

treatments as compared with zinc oxide treatment; however, no effect was observed on carcass characteristics. On the other hand, other reports showed no significant effects of the different levels of Zn-NPs on carcass characteristics of broiler chickens (Ahmadi *et al.* 2013; Mohammadi *et al.* 2015; Akbari Moghaddam Kakhki *et al.* 2017). The differences in the bioavailability and physicochemical properties of the Zn sources, especially Zn-NPs may explain inconsistent results observed in different studies. It was indicated that organic Zn sources (proteinated and amino acid chelated forms), as well as Zn-NPs sources, had higher bioavailability than inorganic Zn sources (oxide and sulfate forms), (Ao *et al.* 2006; Ao *et al.* 2009; Mohammadi *et al.* 2015).

Also, higher solubility and lower interactions of the chelated Zn with other nutrients (during absorption at the gut level) improve the bioavailability of Zn (Ao *et al.* 2009). Furthermore, it was reported that Zn has a vital role in maintaining the structure of proteins; so, Zn deficiency could affect protein metabolism in fast-growing animals (Swinkels *et al.* 1994). Furthermore, low dietary Zn content caused a reduction in appetite and then, growth performance (Swinkels *et al.* 1994). On the other hand, high dietary Zn content had a negative impact on body weight gain (Sadoval *et al.* 1999). Because of observing the insignificant impact of different doses and different sources of Zn on growth performance and carcass characteristics, it can be concluded that different types of Zn sources provided sufficient Zn needed for the growth of chickens. Accordingly, even lower levels of Zn-NPs (30BZMO and 50BZMO) were able to provide enough Zn to support the growth of broilers.

The results of the present study indicated the significant effect of Zn treatments on Zn content of breast meat. Although it has been reported that organic Zn and Zn-NPs supplementations increased Zn content of broiler meat (Salim *et al.* 2012b; Selim *et al.* 2014), in the present study, the lowest Zn content of breast meat was observed in Zn amino acid complex group (70ZAAC). Among the dietary Zn treatments of the present study, two treatments of Zn-NPs (30BZMO and 90BZMO) had the highest Zn content of breast meat. In a study, it was indicated that an increase in organic Zn supplementation increased Zn content of organs, in which the highest amount was observed in liver, thigh muscle, and breast muscle, respectively (Akbari Moghaddam Kakhki *et al.* 2016). Furthermore, another study indicated increasing in Zn content of some organs (pancreas Zn, plasma Zn, and tibia ash Zn) with increasing in dietary Zn consumption (Wen-Xiang *et al.* 2015). However, another study indicated no effect of increasing dietary Zn on Zn content of organs (gizzard, pancreas, thyroid, and liver), (Kaya *et al.* 2002).

**Table 4** The effect of different dietary Zn sources on breast meat quality characteristics

Items	Breast meat color indexes			Shear force (N)	Drip loss (%)	pH
	L*	a*	b*			
Control	47.87 <sup>b</sup>	6.39 <sup>b</sup>	18.91	2.66 <sup>a</sup>	0.95	6.68 <sup>a</sup>
70ZAAC	57.52 <sup>a</sup>	13.52 <sup>a</sup>	25.14	2.68 <sup>a</sup>	1.60	5.89 <sup>b</sup>
30BZMO	54.85 <sup>a</sup>	11.06 <sup>a</sup>	23.12	2.68 <sup>a</sup>	1.39	5.83 <sup>b</sup>
50BZMO	57.97 <sup>a</sup>	14.43 <sup>a</sup>	25.04	1.20 <sup>b</sup>	1.24	6.06 <sup>b</sup>
70BZMO	56.80 <sup>a</sup>	13.34 <sup>a</sup>	25.51	2.11 <sup>ab</sup>	1.22	5.99 <sup>b</sup>
90BZMO	57.33 <sup>a</sup>	14.27 <sup>a</sup>	25.00	3.38 <sup>a</sup>	1.31	6.00 <sup>b</sup>
SEM	2.02	1.18	2.09	0.43	0.20	0.16
P-value	0.02	< 0.01	0.25	0.04	0.41	0.02

Control: 70 mg ZnSO<sub>4</sub>; 70ZAAC: 70 mg zinc amino acid complex; 30BZMO: 30 mg zinc nanoparticles; 50BZMO: 50 mg zinc nanoparticles; 70BZMO: 70 mg zinc nanoparticles and 90BZMO: 90 mg zinc nanoparticles.

L\*: meat lightness index; a\*: meat redness index and b\*: meat yellowness index.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

**Table 5** The effect of different dietary Zn sources on immune system of broiler chickens

Items	Antibody titers at d 35 (log2)			Antibody titers at d 42 (log2)			Lymphatic organs (%)		
	Total Ig	IgG	IgM	Total Ig	IgG	IgM	Bursa of fabricius	Spleen	Thymus
Control	3.87	1.50	2.37	3.37	1.12	2.25 <sup>ab</sup>	0.16	0.10	0.40
70ZAAC	4.25	1.25	3.00	4.62	1.25	3.75 <sup>a</sup>	0.15	0.08	0.30
30BZMO	4.50	1.25	3.25	4.50	1.12	3.37 <sup>a</sup>	0.19	0.11	0.34
50BZMO	3.87	1.12	2.75	3.12	1.12	2.00 <sup>b</sup>	0.14	0.09	0.24
70BZMO	4.75	1.37	3.37	4.25	1.12	3.12 <sup>ab</sup>	0.18	0.09	0.32
90BZMO	3.87	1.12	2.75	4.50	1.12	3.37 <sup>a</sup>	0.18	0.12	0.32
SEM	0.51	0.16	0.45	0.42	0.13	0.37	0.02	0.01	0.05
P-value	0.75	0.35	0.52	0.06	0.42	0.03	0.74	0.31	0.44

Control: 70 mg ZnSO<sub>4</sub>; 70ZAAC: 70 mg zinc amino acid complex; 30BZMO: 30 mg zinc nanoparticles; 50BZMO: 50 mg zinc nanoparticles; 70BZMO: 70 mg zinc nanoparticles and 90BZMO: 90 mg zinc nanoparticles.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Higher Zn preservation in body organs indicates for higher cellular uptake of Zn-NPs and muscle enrichment, but lower disposal of Zn in the environment (Yu *et al.* 2011). However, it should be considered that low amount of Zn-binding proteins (metallothionein) in muscles can restrict the amount of Zn fortification (Lee *et al.* 2010). Considering high demand for broiler meat by consumers in the developing countries and high bioavailability of Zn in meat of broilers (Subar *et al.* 1998; Bou *et al.* 2004), it can be concluded that 30BZMO treatment can be an advisable level for poultry industry to overcome Zn deficiency in the societies.

Results of the present study indicated that MDA content of breast meat was affected by Zn treatments, and the lowest amount was observed in 30BZMO treatment group. However, no effect of treatments on MDA content of thigh was observed. Selim *et al.* (2014), Mohammadi *et al.* (2015), and Saleh *et al.* (2018) demonstrated a decrease in MDA content of breast meat with increasing dietary Zn supplementation of broiler chickens.

Also, Mohammadi *et al.* (2015) indicated that supplementing Zn-NPs in the diet of broilers decreased MDA content of thigh muscle, which was in the opposite with the results of the present study.

Furthermore, broiler chickens fed Zn-NPs and zinc-methionine showed lower serum MDA concentration, but higher serum superoxide dismutase activity (Akhavan-Salamat and Ghasemi, 2019). Also, feeding higher levels of zinc sulfate in Pekin ducks decreased MDA level in breast muscle, but increased the activity of superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase (Wen *et al.* 2019). In another similar study, dietary supplementation with Zn-NPs and zinc-methionine increased serum superoxide dismutase, but decreased serum MDA in comparison with serum of turkeys fed zinc oxide (Jankowski *et al.* 2019). However, some studies reported no effect of Zn supplementation of broiler's diet on oxidative status of meat (Bou *et al.* 2004; Bou *et al.* 2005; Saenmahayak *et al.* 2012). Normal metabolism of the body produces free radicals, which should be eliminated by antioxidant system of the body (Bou *et al.* 2009). Previous studies indicated antioxidant properties of Zn, which decreases the production of free radicals during meat lipid peroxidation (Vakili and Rashidi, 2011; Saleh *et al.* 2018).

Also, part of improving effect of Zn on reducing MDA may be mediated through the role of Zn on improving intestinal vitamin E absorption (enhancing the formation of chylomicrons), (Akbari Moghaddam Kakhki *et al.* 2016).

Superoxide dismutase (one of the enzymes in the anti-oxidant system) contains Zn in its structure as its cofactor (Bou *et al.* 2009). Accordingly, feeding Zn by improving the activity of this enzyme can reduce MDA production (Akhavan-Salamat and Ghasemi, 2019). Also, Zn stimulates metallothionein synthesis, which protects cells against lipid peroxidation and free radicals (Powell, 2000; Prasad, 2008). Accordingly, the positive effect of Zn supplementation on reducing breast meat MDA, especially in 30BZMO treatment, may refer to lower oxidative damages and lipid peroxidation by improving antioxidant status of the broilers' breast meat (Pamoka *et al.* 2009; Akbari Moghaddam Kakhi *et al.* 2016; Akhavan-Salamat and Ghasemi, 2019).

Present results indicated higher breast meat L\* index in treatment groups (Zn-NPs treatments and zinc amino acid) than the control group. On the opposite, feeding zinc sulfate (0, 15, 30, 60, 120, 240 mg zinc/kg) for 35 days in Pekin ducks decreased the lightness value in their breast meat (Wen *et al.* 2019). In another study, three zinc sources (Zn lactate, Zn chelate, and Zn oxide) had no effect on L\* index of goat meat (Saláková *et al.* 2011). It is generally accepted that a higher growth rate results in a higher meat L\* index (Fletcher, 1999). The results of the present study indicated numerically higher total ADG in treatment groups than the control group; then, this non-significant higher growth rate may be the reason of higher breast meat L\* index in treatment groups. Also, it was indicated that decreasing pH of meat results in a higher meat lightness index (Berri *et al.* 2001), which is in accordance with the present results showed lower pH in treatment groups compared with Control group. Furthermore, Chartrin *et al.* (2006) reported that an increase in the fat content of muscle causes an increase in both L\* and b\* indexes of meat. Previous studies indicated improving effect of Zn levels and Zn sources on improving intramuscular fat (Rodríguez-Maya *et al.* 2019; Wen *et al.* 2019). Similarly, results of the present study indicated a non-significant increase in b\* index of treatment groups compared with the control group, which accompanied by increasing in breast meat L\* index in these treatments.

Breast meat a\* index was higher in treatments (Zn-NPs treatments and zinc amino acid) than the control group. Resemble the present results, Liu *et al.* (2011) reported the increasing effect of dietary Zn supplementation on breast muscle redness index. However, it was reported that feeding three organic zinc sources (Zn lactate, Zn chelate, and Zn oxide) had no effect on goat meat a\* index (Saláková *et al.* 2011). D'Agata *et al.* (2009) reported that improving antioxidant status increased meat redness. As many studies (Akhavan-Salamat and Ghasemi, 2019; Jankowski *et al.* 2019; Wen *et al.* 2019), as well as the results of the present study, indicated the improvement of antioxidant status with

feeding Zn with higher bioavailability; accordingly, Zn from 70ZAAC and Zn-NPs treatments may be more effective in improving antioxidant status and then, in decreasing meat discoloration during 24 h of preservation.

In the present study, the lowest shear force of breast meat was observed in 50BZMO treatment. A previous study indicated that Zn supplementation improved hepatic fatty acid synthesis, marbling, and meat b\* value, while decreasing shear force (Liu *et al.* 2011). Also, Wen *et al.* (2019) reported higher intramuscular fat, but lower shear force in the breast meat of ducks fed higher levels of zinc sulfate. Also, another study indicated higher intramuscular fat and marbling of lamb meat with feeding Zn methionine + Zn oxide (Rodríguez-Maya *et al.* 2019). It was indicated that increasing in intramuscular fat with increasing connective tissue distances decreases collagen concentration and then, indirectly increases meat tenderness (Warner *et al.* 2010). Accordingly, observing lower shear force in 50BZMO treatment may be related to higher intramuscular fat in this group.

The present results indicated lower breast meat pH in Zn treatments (Zn-NPs treatments and zinc amino acid) than the control group. Consistent with the results, Selim *et al.* (2014) reported lower pH of breast and thigh meat in Cobb 500 broiler chicks fed zinc methionine and nano-zinc oxide compared with zinc sulphate group. On the other hand, Liu *et al.* (2011) reported that dietary Zn supplementation increased thigh muscle pH in broilers. Also, an increase in feeding levels of zinc sulfate in Pekin ducks increased the pH of breast meat at 24-h postmortem (Wen *et al.* 2019).

However, Saláková *et al.* (2011) reported no significant effect of zinc sources (Zn lactate, Zn chelate, and Zn oxide) on pH of goats' meat. It has also been proved that protein deficiency results in pH increases (Schreurs *et al.* 1995). Therefore, in the present study, Zn supplementations may reduce pH by increasing muscle protein synthesis. As high muscle pH increases the rate of microbial spoilage and reduces the shelf-life of broiler meat (Allen *et al.* 1997); then, 70ZAAC and Zn-NPs treatments, which had lower pH, are more capable of increasing the shelf-life of broiler meat than the control group. On the other hand, a fast decrease in pH may lead to a reduction of tenderness along with lower water-holding capacity (WHC) of meat (Dransfeld, 1994; Qiao *et al.* 2001). Based on the present results, decreasing in pH of breast meat did not have a negative impact on its drip loss and shear force.

Results of the present study indicated higher IgM titer (non-significantly higher total Ig) at day 42 of the experiment in 70ZAAC, 30BZMO, and 90BZMO treatments than 50BZMO and control groups. However, no effect of Zn treatments was observed on the lymphatic organs (bursa of fabricius, spleen, and thymus), total Ig, and IgG. In a study,

supplementation of broiler chickens' diet with Zn oxide-nanoparticles and zinc-methionine increased relative weight of spleen, primary total antibody titers, and IgG antibody titers against SRBC (Akhavan-Salamat and Ghasemi, 2019). In this study, only high levels of zinc-methionine improved antibody titers, but all levels of Zn oxide-nanoparticles were able to improve antibody response of broiler chickens (Akhavan-Salamat and Ghasemi, 2019).

Dietary supplementation of turkey's diet with zinc nanoparticles and zinc-methionine also improved spleen weight, IgG, and total antibody in comparison with zinc oxide treatment (Jankowski *et al.* 2019). Saleh *et al.* (2018) reported higher antibody titers against Newcastle disease and Proflok infectious bursal disease by feeding 50 and 100 mg zinc-methionine/kg compared with those of 0 or 25 mg zinc-methionine/kg. Mohammadi *et al.* (2015) reported that adding 80 mg/kg Zn-nano complex (Bonza Zn Metabolism Optimizer) to the diet increased total antibody titer against Newcastle and weight of bursa of fabricius and spleen. Furthermore, Sahoo *et al.* (2014) reported that low levels of nano Zn (0.06 mg/kg) were able to improve the immune system similar with high levels of Zn from organic and inorganic sources (15 mg/kg). Also, Tian *et al.* (2010) reported that levels of 40-80 mg/kg of nano Zn increased antibody titer against Newcastle virus. Generally, it has been shown that increasing dietary Zn increases Zn absorption and improves the immune system function (Rajendran, 2013; Yogesh *et al.* 2013). Also, it was indicated that Zn deficiency increased blood glucocorticoid concentrations (Shankar and Prasad, 1998), which further suppressed immune function. Akbari Moghaddam Kakhki *et al.* (2016) reported that higher dietary Zn supplementation increased humoral immunity. A study also indicated that higher Zn levels are more efficient in improving immune system of broilers (Smith, 2003). Jarosz *et al.* (2017) reported that dietary supplementation with zinc chelates (Zn with glycine) improved cellular and humoral immunity of broilers, though supplementing inorganic Zn (ZnSO<sub>4</sub>) showed no immunomodulatory responses. Moreover, it was indicated that Zn absorption from organic Zn or nano-Zn sources is higher (because of higher bioavailability), which leads to a larger Zn pool and plasma Zn level; then, this condition improves T and B lymphocytes, as well as neutrophil function (Kidd *et al.* 1996; Hudson *et al.* 2004). It was proved that Zn is necessary for many functions of immune system, including: 1- the activity of thymulin (a Zn-containing hormone, which is necessary for modulating release of cytokines and inducing T-cell proliferation and differentiation in thymus); 2- the total counts of thymocytes and peripheral T-cells; 3- the release of interferon - $\alpha$ , cytokines, and tumor necrosis factors; 4- proper function of phagocytes, heterophils, and T lymphocytes (Driessen *et al.* 1994; Saha *et al.*

1995; Maggini *et al.* 2007; Akbari Moghaddam Kakhki *et al.* 2016; Jarosz *et al.* 2017). Furthermore, part of the Zn impact on immune function may be mediated through the protection against oxidative damages and / or by improving antioxidative status of the chicken (Osaretin and Gabriel, 2009; Akhavan-Salamat and Ghasemi, 2019). Then, improving impact of Zn sources on IgM titer in the present study may be mediated through the mentioned mechanisms. Based on the present results, 70 mg Zn amino acid complex (70ZAAC) and all Zn-NPs levels (except for 50BZMO treatment) improved immune system of broiler chickens, which was possibly caused through higher Zn bioavailability of these sources of Zn (Ao *et al.* 2009).

## CONCLUSION

Based on the results of the present study, dietary Zn treatments did not have a significant effect on average daily weight gain, FCR, most of carcass characteristics, small intestine, lymphatic organs, the Zn and MDA content of thigh muscle, and antibody titers (except for IgM at d 42 of the experiment). However, dietary Zn treatments significantly influenced IgM titer at day 42 of the experiment, Zn content of breast muscle, breast meat lightness and redness indexes, as well as breast meat shear force, pH, and MDA content. Results of the present study, for the first time, indicated an improving effect of Zn-NPs on breast meat quality characteristics (L\* and a\* indexes, pH, and shear force). Also, the overall results of this study indicated that 30BZMO treatment, as the lowest Zn-NPs level, supported humoral immunity (IgM), improved breast muscle Zn content, and improved meat quality characteristics, while decreasing breast meat MDA content; accordingly, 30BZMO is an advisable Zn-NPs level in this experiment.

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