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THE EFFECT OF ADDING DIFFERENT LEVELS OF NANO AND NON-NANO ZINC OXIDE TO THE DIET ON PHYSIOLOGICAL TRAITS OF SOME BROILERS

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ABSTRACT

This study was conducted at the Poultry Research Station / Agricultural Research Office/Ministry of Agriculture, for the period from 3/8/2019 to 13/10/2019 to study the effect of adding different levels of Nano and non-Nano zinc oxide to broiler diets on production performance and some physiological traits. 420 unsexed one-day-old chicks (Ross308) were used in the experiment with an average weight of 36 g, the chicks were distributed randomly into 7 treatments, each one included 4 replicates by 15 chicks per replicate. The experimental treatments were as follows: T_1 control treatment without adding zinc, T_2, T_3, and T_4 adds Nano zinc oxide at a concentration of 10, 30, and 50 mg/kg feed for the treatments, respectively, and T_5, T_6, and T_7 adding Non-Nano zinc oxide at a concentration of 10, 30 and 50. mg/kg feed for treatments, respectively. The experimental results indicated that there was a significant superiority ($P < 0.05$) was occurring in blood zinc concentration at 42 days of treatment T_6 compared with T_1, T_3, and T_7. As well as, treatment T_2 and T_6 were superior compared to treatment T_7 in the triglyceride level at 42 days, moreover a significant superiority ($P < 0.05$) was observed in infectious bronchitis (IB) titer at treatment T_4 compared to the rest of the treatments. Finally, a significant superiority ($P < 0.05$) was observed at treatment T_2 and T_3 compared to T_4 in zinc concentration in the thigh as well as its increase in the kidneys at treatment T_3 compared to T_2, T_4, T_5, and T_7.

Keywords : Zinc Oxide, Broilers, Physiological Traits

Introduction

The poultry industry is one of the food security cornerstones in the world, as it relies on providing meat and eggs to come across the growing human need. Generally, one of the most important new challenges in the poultry industry is the use of nanotechnology, which is one of the anticipated technologies that occupy the forefront of scientific and research interests. Besides, it will have enormous potentials capable of changing the science in the near future, and as a result of the ban against antibiotics as growth stimuli, researchers have resorted to using environmentally friendly and consumer alternatives that carry positive properties without negative effects (Liji *et al*., 2001). In recent years, there have been many attempts to introduce nanotechnology to the poultry diet, and this technology has helped improve the efficiency of the feed provided. As well, as, reduce the cost of feed by reducing the amount of mineral supplements (Mohapatra *et al*., 2014) due to the increased properties of nanoparticles such as its small size that ranges from (1-100) nm, a large surface area and thus an increase in reactivity and absorption strength (Liao *et al*., 2010 and Al-Mutairi, 2012). Among the most important additives is Nano zinc oxide, where the application of nanotechnology has led to the emergence of nanoparticles of zinc known as Nano zinc oxide that can replace other forms of zinc given its new and distinct qualities (Wijnhoven *et al*., 2009). Furthermore, (Zhao *et al*., 2014) stated that the comparison of zinc oxide to Nano zinc oxide showed that the Nano zinc oxide has stronger chemical activity and participates in oxidative stress reactions with a variety of organic compounds. Additionally, the permeability of Nano zinc oxide can help prevent harmful intestinal reactions and improve absorption, where (Sahoo *et al*., 2014) indicated that the addition of Nano zinc oxide had better results in bioavailability when compared to other zinc sources as well as being less toxic to broilers. Zinc is a vital mineral for birds and has a major role in the immune system and some reproductive hormones (Cousins and Hempe, 1990). In addition, it is involved in RNA and DNA synthesis, deposition of minerals in bones, tissue growth and restoration (Salim *et al*., 2008). In addition to being an effective antioxidant that works to eliminate the damage of free radical, resulting from normal metabolism processes (Rahrman *et al*., 2014), as well as it contributes to the metabolism of proteins, carbohydrates and fats (MacDonald, 2000). The bioavailability of organic zinc is higher than that of inorganic zinc, but the application of organic zinc is limited due to its high cost in animal production (Brat *et al*., 2013). Moreover, high zinc content can lead to an increase in waste products, causing environmental pollution, and the presence of zinc in large quantities may effect on the balance of other trace elements in the body and reduces the stability of vitamins and other elements (Zhao *et al*., 2014). Based on the previously mentioned data, the aim of this study is to determine the effect of adding Nano and non-Nano zinc oxide to the broiler diet and its effect on the physiological performance while determining the optimal concentration.

Materials and Methods

This study was conducted at the Poultry Research Station / Agricultural Research Office / Ministry of Agriculture, for the period from 3/8/2019 to 13/10/2019 to study the effect of adding different levels of Nano and non-Nano zinc oxide to broiler diets in concentrations of (10.30,

50 mg/kg feed). 420 unsexed one-day-old chicks (Ross308) were used in the experiment was prepared for one of the private hatcheries contracted with the Agricultural Research Office of the Ministry of Agriculture. The chicks were distributed randomly into 7 treatments; each treatment included 4 replicates of 15 birds per replicate, and was distributed with an average initial weight (36 g / chick) into 28 rooms with dimensions (2.5 x 3 m). The chicks were raised on a ground with a thickness of 2-3 cm in a closed hall in the poultry field affiliated to the Agricultural Research Office/Ministry of Agriculture, and all the conditions for raising broilers were available in it. A continuous lighting system was used for 23 hours a day with a daily dark space, all chicks were fed free feeding (ad-libitum) on a starter diet from 1-10 days of age, and then gradually replaced by a grower diet from 11-22 days of age, after that replaced by a finisher diet from 23-42 days of age. The feed materials were prepared, crushed, and mixed according to the required proportions, and the Nano and non-Nano zinc oxide was added according to their proportions in the diets of different treatments using a sensitive balance. Besides, the mixing was done by dilution through mixing a small amount of the additive and the feed in a beaker and then mixed with a larger quantity of feed. This method was continued several times to ensure obtaining a consistent diet, the diets were prepared in the feed laboratory located in the Poultry Research Station/Livestock Department/ Agricultural Research Office / Ministry of Agriculture. Table 1 showed the percentage of primary feed materials used in the diet composition and the calculated chemical composition, and the nutritional treatments were divided as follows; the first treatment - a control treatment without addition, the second treatment- adding Nano zinc oxide at a concentration of 10 mg/kg feed. The third treatment - adding Nano zinc oxide at a concentration of 30 mg/kg feed, the fourth treatment - adding Nano zinc oxide at a concentration of 50 mg/kg feed and the fifth treatment- adding non-Nano zinc oxide at a concentration of 10 mg/kg feed. Finally, the sixth treatment - adding non-Nano zinc oxide at a concentration of 30 mg/kg feed, the seventh treatment - adding non-Nano zinc oxide at a concentration of 50 mg/kg feed. The cholesterol test was performed using a kit produced by a Spanish company Linear Chemicals. S.L. Serial number is REF118005. Triglycerides were estimated using a kit tool produced by a Spanish company Linear Chemicals. S.L. Serial number is REF1155005. The antibody titer was measured in the blood serum of Newcastle and bronchitis by the test of (ELISA) enzyme-linked immunosorbent assay in the laboratory of the group specializing in veterinary immune tests in Al-Sinak / Baghdad. The ELISA test is an immunological test used for titration of any antigen with high accuracy to 0.0005 microns. The basis for this test work is the binding of antibodies against a specific antigen present in the blood sample to be tested with special antigens affixed to a (Microtiter plate) equipped with several standard solutions (Kit). After washing, the conjugate is added to the antibody directed against the first antibody to bind to it and it is marked with Horseradish peroxidase (HRP). Similarly, after washing for the second time, the substrate is added, which is a colorless substance that is transformed by the aforementioned enzyme into a bluish-green substance that can be measured by the spectrophotometer in the ELISA test after stopping the reaction, according to (Synder *et al*., 1984). The zinc was estimated in the organs (thigh, breast, liver, kidneys) by taking (5-3g) of the sample and placing it in a ceramic jar and entering the oven at a temperature of (800 °C) for 45-30 minutes. Then, the model is taken out to cool it as (10-5ml) of Aqua regia is added to it to dissolve the remainder of the and transfer it to a glass baker and place it on an electric heater at a temperature of (100 °C) for 15 minutes, after which it is transferred to a (25ml) volumetric bottle. Furthermore, the volume is completed to the mark with demineralized water and thus the model is ready for measurement according to the (Cuparigova and Stafilov 2011) method, it was also determined in the blood by preparing a solvent by adding 10% Titronx 100 in a (500ml) volumetric flask. As well as, the addition of (1 ml) nitric acid HNO_3 and then adding (20%) of a solution of ammonium dihydrogen phosphate with the adding (300ml) of demineralized water and put all of them on a magnetic stirrer to mix them well.

Table 1 : The percentages of diet components used in the experiment and their chemical composition

Feed material	Starter diet (1-10) day	Grower diet (11-22) day	Finisher diet (23-42) day
Maize	47.52	50.85	54.84
wheat	10	10	10
Soybean meal 48%	32	28	24
Protein Concentrate[(1)]	5	5	5
Vegetable fat	3	4.15	4.3
$CaHPO_4$	0.7	0.5	0.4
Limestone	1.2	1.14	1.1
Salt	0.1	0.1	0.1
Methionine	0.24	0.13	0.13
Lysine	0.24	0.13	0.13
Total	100	100	100
Calculated Chemical Analysis[(2)]			
Crude protein (%)	22.55	20.91	19.33
Dietary energy Kcal/kg	3060	3178	3227
Lysine (%)	1.48	1.29	1.18
Methionine+ Cysteine (%)	1.10	0.95	0.90
Ca (%)	0.974	0.8990	0.844
Available P (%)	0.481	0.439	0.416

[(1)]BROCON protein concentrate- 5 Special W: Chinese origin. Each kg of it contains: 40% crude protein, 3.5% fat, 1% fiber, 6% Ca, 3% available P, 3.25% lysine, 3.5% methionine, 3.90% methionine + cysteine, 2.2% sodium, 2100 kcal / kg Dietary energy. 200,000 IU vitamin A, 40,000 IU vitamin D_3, 500 mg vitamin E, 30 mg vitamin K_3, 15 mg B_1, vitamin B_2, 150 mg B_3, 20 mg B_6, 300 mg B_{12}, 10 mg folic acid, 100 µg biotin. 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT).

[(2)]The chemical analysis of the diet according to NRC (1994).

Blood was collected from birds for each treatment (4 of each sex) aged 21 and 42 days from the wing or brachial vein, and it was evacuated directly into two types of tubes, the first tubes containing the Potassium-Ethylene Diamine Tetra Acid K-EDTA. It was used to perform tests for the packed cell volume PCV and hemoglobin Hb, and the second tubes containing 6 ml gel tubes and these tubes are placed in a centrifuge at a speed of 4000 RPM for 10 minutes to separate the serum. Besides, it shall be kept and frozen at a temperature (- 20 °C) until the tests are carried out. The PCV was estimated as explained by (Archer, 1965), and (Hb) was calculated based on the equation mentioned by (Campbell, 1995).

0.5 ml of blood plasma were taken and placed in a 5ml of the volumetric bottle and the volume is completed to the mark utilizing the previously prepared solvent, and then the model is measured in the same way above. A Kit tool by Randox was used to estimate the calcium concentration in blood serum, and the steps attached to the kit were followed, the results were read using spectrophotometry at a wavelength of 570 nm. The phosphorus concentration in blood serum was also measured by following the instructions attached to the kit produced by Randox, as the absorbance is read at a wavelength of 740 nm, using a Spectrophotometer. Finally, the experimental data were analyzed by using the Complete Randomized Design (CRD) to determine the effect of the treatments on the studied traits. The significant differences between the averages were compared with (Duncan's New Multiple Range Test 1955), and the statistical analysis software SAS program (2012) was used in the statistical analysis according to the following mathematical model:

$$Yij = \mu + Ti + eij$$

Results and Discussion

It was observed from Table 2 that there were no significant differences between the treatments in the concentration of calcium and phosphorus at the ages of 21 and 42 days, and it was observed that there were no significant differences in the concentrations of zinc in the blood serum at the age of 21 days. Whereas, there were significant differences between the treatments ($P< 0.05$) at the age of 42 days, as the treatment T_6 was superior over the treatments T_1, T_3, and T_7, which recorded 12.63 mg / L, while it did not differ significantly with the treatments T_2, T_4 and T_5. The blood zinc concentration correlated positively with the amount of zinc in the diet (Arlette and Darlen, 1979). In contrast, (Smith and Barlett, 2003) reported that blood zinc levels were not affected by its amount in the diet in broiler birds exposed to heat stress. Table 3 showed that there were no significant differences in the packed cell volume and hemoglobin concentration in the blood when adding Nano and non-Nano zinc oxide to broiler diets at 21 and 42 days of age

Table 2 : The effect of adding different levels of Nano and non-Nano zinc oxide to broiler (Ross 308) diets on calcium, phosphorus and zinc concentrations (mg / L) in blood serum at age 21 and 42 days

Treatments	Traits					
	21			42		
	Ca	P	Zn	Ca	P	Zn
T_1	0.18±10.50	0.52±7.85	0.33±12.80	0.23±9.55	0.45±7.28	^{B} 0.17±10.75
T_2	0.62±10.95	0.72±7.10	0.34±13.13	1.06±10.85	0.71±7.57	^{AB} 0.76±11.68
T_3	0.45±10.73	0.36±7.23	0.78±12.45	0.31±10.23	0.88±7.75	^{B} 0.32±11.13
T_4	0.31±10.95	0.29±6.90	1.29±13.75	0.09±10.25	0.25±7.35	^{AB} 0.09±12.08
T_5	0.20±10.98	0.29±7.43	0.41±13.00	0.23±9.68	0.65±7.35	^{AB} 0.42±11.48
T_6	0.24±10.90	0.47±6.80	1.68±12.90	0.23±10.13	0.74±7.83	^{A} 0.36±12.63
T_7	0.19±11.13	0.65±7.98	0.14±13.03	0.17±9.85	0.22±7.48	^{B} 0.54±11.10
Significant level	N.S	N.S	N.S	N.S	N.S	*

* T_1 control treatment without addition, T_2, T_3, T_4 adding Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for the treatments respectively, T_5, T_6, T_7 adding non-Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for treatments respectively.
* The averages that have different letters indicate the presence of significant differences between the average treatments at ($P \leq 0.05$).
* N.S There were no significant differences between the average treatments.

Table 3 : The effect of adding different levels of Nano and non-Nano zinc oxide to broiler (Ross 308) diets on packed cell volume (%) and hemoglobin (mg / 100 ml) at age 21 and 42 days

Treatments	Traits			
	21		42	
	Hb	PCV	Hb	PCV
T_1	0.28±7.42	0.85±22.25	0.52±8.25	1.55±24.75
T_2	0.53±8.58	1.60±25.75	0.73±8.17	2.18±24.50
T_3	0.87±8.83	2.60±26.50	0.29±7.50	0.87±22.50
T_4	0.28±7.13	0.85±21.38	1.77±9.50	5.32±28.50
T_5	0.45±8.17	1.34±24.50	0.28±7.08	0.85±21.25
T_6	0.65±9.08	1.96±27.25	0.65±8.83	1.94±26.50
T_7	0.32±7.42	0.95±22.25	0.27±7.67	0.82±23.00
Significant level	N.S	N.S	N.S	N.S

* T_1 control treatment without addition, T_2, T_3, T_4 adding Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for the treatments respectively, T_5, T_6, T_7 adding non-Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for treatments respectively.
* N.S There were no significant differences between the average treatments.

The data are shown in Table 4 indicating that there were no significant differences between the treatments in cholesterol concentration at the age of 21 and 42 days and the concentration of triglycerides at the age of 21 days, whereas there were significant differences in the concentration of triglycerides at the age of 42 days. The two treatments T_2 and T_6 were superior over the treatment T_7, as recorded 144.25, 149.00 mg / 100 ml, while it did not differ with the treatments T_1, T_3, T_4, and T_5. Zinc participates in the synthesis of many enzymes, especially those that increase the release of triglycerides as energy alternates instead of accumulating in the blood plasma (Park *et al*., 2004) between zinc and the thyroxine hormone, (Young, 1968; Sturkie, 1986; Kuhn *et al*., 1993 and Gadir, 1996), the result showed that the thyroid gland can control lipid metabolism. Thyroid hormones increase cholesterol formation and increase the ability of the liver to secrete cholesterol in the bile as mentioned by (Al-Daraji *et al*., 2012). Otherwise, a zinc's role in the work of the enzyme, as it is an integral part of many mineral enzymes in the body that play a vital role in fat metabolism (Al-Daraji and Amen, 2011). In contrast to the results (Al-Shemmari and Fahad, 2019) obtained a decrease in the concentration of triglycerides in the blood plasma when a mixture of zinc and folic acid is added. (Ahmadi *et al*., 2013) found a decrease in triglycerides and low-density lipoprotein LDL during the starter period when adding Nano-zinc oxide at level 60 and 90 mg/kg to broiler diets, (Paul *et al*., 2001) found that zinc supplementation reduced triglycerides in mice. Whereas (Eder and Kirchgessner 1995) reported that zinc deficiency in mice increased triglyceride and LDL concentrations, (Zyla *et al*., 2013) found an increase in serum triglyceride concentrations when feeding broilers on a diet supplemented with zinc, vitamin E, and phytase, which affect lipid metabolism(Aksu *et al*., 2010 and Zyla *et al*., 2013). (Zaghari *et al*., 2013) confirmed a decrease in triglyceride concentration and an increase of the LDL in birds serum fed a diet supplemented with zinc oxide of 100 mg.

Table 4 : Effect of adding different levels of Nano and non-Nano zinc oxide to broiler (Ross 308) diets on cholesterol and triglyceride concentrations (mg / 100 ml) at age 21 and 42 days.

Treatments	Traits			
	21		42	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride
T_1	4.64±137.00	5.95±109.75	3.42±126.25	^{AB} 4.87±117.25
T_2	9.31±146.75	12.92±125.00	6.36±133.50	^{A} 17.01±144.25
T_3	14.38±148.75	13.89±126.25	10.89±125.25	^{AB} 11.30±111.75
T_4	2.75±138.50	14.70±131.00	3.57±123.50	^{AB} 21.19±128.00
T_5	5.89±147.75	21.50±126.50	7.64±123.50	^{AB} 8.57±114.25
T_6	6.10±143.00	10.23±118.25	10.21±135.00	^{A} 13.04±149.00
T_7	8.89±137.75	10.50±117.00	19.09±129.00	^{B} 5.66±87.00
Significant level	N.S	N.S	N.S	*

* T_1 control treatment without addition, T_2, T_3, T_4 adding Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for the treatments respectively, T_5, T_6, T_7 adding non-Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for treatments respectively.
* The averages that have different letters indicate the presence of significant differences between the average treatments at ($P \leq 0.05$).
* N.S There were no significant differences between the average treatments.

The results of Table 5 indicated that there were no significant differences between the treatments in the zinc concentration of breast and liver tissues, while significant differences ($P < 0.05$) were observed between the treatments in zinc concentration of thigh and kidney tissues. The two treatments T_2 and T_3 were superior over treatment T_4, which recorded 51.96, 50.32 mg/kg, respectively, while it did not differ with treatment T_1, T_5, T_6, and T_7 for thigh zinc concentration. As for the concentration of zinc in the kidneys, it was observed that the treatment T_3 exceeded the treatments T_2, T_4, T_5, and T_7, as it recorded 146.94 mg/kg, while it did not differ with the two treatments T_1 and T_6. The using Nano zinc supplements in animal feed can change the mineral precipitation in tissues due to the appropriate bioavailability when compared to inorganic sources, the ability of Nano minerals to pass through the small intestine and distributed in the body is much greater than that of inorganic and organic minerals (Hillyer and Albrecht, 2001). The biological distribution of mineral tissues can also be used as an indicator of mineral storage in the body (Wedekind *et al*., 1992). The concentration of zinc varies in tissues and the reason for this difference is due to the morphology, biochemical and functional state of the tissues (Ramiah *et al*., 2019), (Akbari Moghaddam Kakhki *et al*., 2017) indicated that an increase in the concentration of zinc in the thigh, breast, and liver is through adding zinc to the diet, regardless of its source. (Ramiah *et al*., 2019) obtained results similar to this study that the zinc concentration increases in the thigh muscle of broilers that were fed with Nano zinc oxide under normal temperatures compared to heat stress conditions. (Pimentel *et al*., 1991) proved in an experiment consisting of two groups, the first group was fed on zinc methionine and the second on zinc oxide that the content of zinc in the bones, thigh, and the liver does not depend on the form of chemical bonds of zinc, but on the level of this element in the diet. (Tronina *et al*., 2007) obtained similar results to this experiment, using zinc methionine and zinc oxide for broiler and at the same level, as zinc concentration in the thigh, kidney, and serum in the two groups. Similarly, (Sahoo *et al*., 2014) noted an increase in zinc concentration in blood serum of birds treated with Nano-zinc 0.06 ppm and this is close to the results obtained, that the explanation for this condition may be due to the high level of zinc in the treatment or it may be due to the bioavailability of nanoparticles. (Sunder *et al*., 2008) observed zinc deposition in the kidney when feeding broilers with a level of 88-113 ppm in the fourth week of life using zinc sulfate.

Table 5 : Effect of adding different levels of Nano and non-Nano zinc oxide to broiler (Ross 308) diets on the level of zinc (mg / kg) in thigh, breast, liver, and kidneys.

Treatments	Zn concentration			
	Thigh	Breast	Liver	Kidneys
T_1	^{AB} 39.33 ±0.35	48.80±0.16	60.80±1.19	^{AB} 141.28±1.26
T_2	^{A} 51.96±9.52	51.67±1.28	49.95±1.41	^{B} 123.48±5.86
T_3	^{A} 50.32±0.34	51.28±1.11	52.93±24.15	^{A} 146.94±2.04
T_4	^{B} 33.38±1.90	48.11±2.30	52.22±4.67	^{B} 123.53±7.78
T_5	^{AB} 40.28±6.02	45.45±2.48	79.29±13.98	^{B} 123.39±4.37
T_6	^{AB} 44.33±5.28	49.17±2.5	68.72±8.74	^{AB} 136.22±5.36
T_7	^{AB} 42.46±3.83	46.89±1.70	61.82±3.08	^{B} 126.74±7.01
Significant level	*	N.S	N.S	*

* T_1 control treatment without addition, T_2, T_3, T_4 adding Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for the treatments respectively, T_5, T_6, T_7 adding non-Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for treatments respectively.
* The averages that have different letters indicate the presence of significant differences between the average treatments at ($P \leq 0.05$).
* N.S There were no significant differences between the average treatments.

Table 6 showed that there were no significant differences between the treatments in the for Newcastle disease, while significant differences ($P < 0.05$) were observed between the treatments in the infectious bronchitis titer, as it is evident that treatment T_4 exceeded the treatments T_1, T_2, T_3, T_5, T_6 and T_7, which recorded 4385.50. Zinc is an important element necessary for the growth and development of the immune system in birds.

Table 6 : Effect of adding different levels of Nano and non-Nano zinc oxide to broiler (Ross 308) diets on the antibodies titer in serum directed against Newcastle parenchyma and infectious bronchitis

Treatments	Newcastle	Infectious bronchitis
T_1	4864.50±1013.61	^{B} 412.25±412.25
T_2	2971.75±379.80	^{B} 0.00±0.00
T_3	4166.00±654.91	^{A} 310.76±515.50
T_4	2778.00±544.44	^{A} 4385.50±2354.89
T_5	3761.00±1400.05	^{B} 364.00±221.84
T_6	4000.50±996.67	^{AB} 1013.50±586.60
T_7	4581.75±1111.68	^{B} 664.25±443.89
Significant level	N.S	*

* T_1 control treatment without addition, T_2, T_3, T_4 adding Nano zinc oxide at a concentration of 50,30,10 mg / kg feed for the treatments respectively, T_5, T_6, T_7 adding non-Nano zinc oxide at a concentration of 50,30,10 mg/kg feed for treatments respectively.
* The averages that have different letters indicate the presence of significant differences between the average treatments at ($P \leq 0.05$).
* N.S There were no significant differences between the average treatments.

As it plays an important role in increasing the number of T lymphocytes and antibodies and the effectiveness of killer cells and phagocytes cells. (Dardenne *et al*., 1985; Sun *et al*., 1993, Kidd *et al*., 1996, Khajarern *et al*., 2002) indicated that the high level of zinc supplementation of 75 versus 175 mg/kg has led to a higher antibody titer for Newcastle disease and infectious bronchitis. (Al-shemmari and Fahad 2019) reported that the addition of zinc and folic acid and their combination had a significant effect in increasing the antibodies against infectious bronchitis both.

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