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Effect of Dietary Replacement of Inorganic Zinc by Organic or Nanoparticles Sources on Growth Performance, Immune Response and Intestinal Histopathology of Broiler Chicken

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ABSTRACT

Two hundred and twenty-five one day-old avian chicks were used to investigate the effect of different dietary supplemental zinc sources and levels on growth performance, blood picture and biochemical parameters, immune response, carcass characteristics and intestinal morphology of broiler chickens. Birds were randomly allocated into 9 groups which fed on the experimental basal diet supplemented with different zinc sources and levels; group 1 was fed basal diet supplemented with (60 ppm) inorganic zinc oxide, while groups 2, 3, 4 and 5 were supplemented with (60, 45, 30 or 15 ppm) organic zinc (polysaccharide-zinc complex) respectively and groups 6, 7, 8 and 9 were supplemented with (60, 45, 30 or 15ppm) zinc oxide nanoparticles respectively. Broiler chickens fed 60 or 45 ppm organic zinc improved growth performance while, lower supplementation levels (30 or 15ppm) had no significant effect. Moreover, zinc nanoparticles supplementation at 60, 45 or 30 ppm improved growth performance while lower level (15ppm) significantly reduced broiler performance and feed efficiency parameters. Using organic or nano zinc reduced total feed intake of broiler chicken. Organic or nano zinc supplementation non-significantly reduced blood serum lipids and increased GOT or GPT concentrations compared with those fed on diet supplemented with inorganic zinc. Moreover, organic or nano zinc had no significant effect on antioxidant enzyme activity while reduced abdominal fat weight. Organic zinc or nano zinc supplementation improved phagocytic activity, antibody production against Newcastle Disease vaccine, lymphoid organs weight and improve intestinal villi length, width and crypt depth of broiler chicken.

1. INTRODUCTION

Zinc (Zn) is an important mineral with diverse functions in mammals and birds such as in nutrient metabolism, as a component of numerous metalloenzymes, appetite control, regulation of the immune system, oxygen free radical scavenging, and in transcription factors. Furthermore, Zn enzymes are involved in the synthesis and/or breaking down of carbohydrates, lipids, proteins, and nucleic acids, and encompass all known classes of enzymes (Liu et al., 2011). The recommended Zn requirement that is based on performance criterion for broiler chickens is 40mg/kg of diet Nation Research Council (NRC, 1994). However, NRC recommended values for most trace minerals are based on older strains of broilers and may be out dated for the modern strains of broilers used in commercial production today (Leeson, 2005). Traditionally, inorganic trace minerals such as oxides and sulfates are supplemented in broiler diets above the NRC recommended level to maximize performance (Leeson, 2008). When inorganic trace minerals are fed, and reach the upper gastrointestinal tract they tend to dissociate due to the low pH environment. These dissociated minerals can interact with other minerals as well as other dietary components in the digesta, which make them unavailable for absorption across the small intestine (Yan and Waldroup, 2006). As a result, these unabsorbed and excess minerals not utilized by the birds are excreted in the feces, and may lead to environmental concerns when poultry manure is applied to crop land as fertilizer (Aksu

et al., 2010). Furthermore, high Zn supplementation may affect the balance of other trace elements in the body and reduce the stability of vitamins and other nutrients, and long-term application can cause Zn residue in the animal body (Zhao et al., 2014).

Dietary strategies should be implemented to avoid overfeeding dietary nutrients without jeopardizing animal health and performance (Ferket et al., 2002). One dietary strategy that may be utilized to reduce over supplementation of trace minerals is replacing inorganic trace minerals with organic sources or nano sources. Results from different studies have indicated that the use of organic trace minerals in broiler diets can enhance mineral uptake, improve body weight gain, and reduce mineral excretion (Bao et al., 2007). The high bioavailability of organic trace minerals can be explained in part by their stability in the upper gastrointestinal tract, which allows the minerals to reach the small intestine where they are absorbed (Ashmead, 1993). Recently, the development of nanotechnology and its related products has rapidly progressed in different scientific areas; in fact, this branch of science has fundamentally affected human, animal, environment, and industrial lives. In this regard, Zinc Oxide (ZnO) nanoparticles have attracted a great deal of attention because nano-formulation particulates exhibit novel distinguishing qualities such as size, shape, large surface area, high surface activity, high catalytic efficiency, and strong adsorbing ability (Wijnhoven et al., 2009).

Compared with ZnO, nano-ZnO has a stronger chemical activity and participates in oxidation reactions with a variety of organic compounds. In addition, the permeability of nano-ZnO can also help prevent adverse gastrointestinal reactions and improve the absorption of medicine (Zhao etal., 2014). However, it is unclear whether nano-ZnO can improve growth performance, mortality and antioxidant status of broilers.

Scare literature comparing inorganic, organic or nano zinc supplementation in broiler chicken ration. Therefore, the objective of this study was to determine the effect of inorganic zinc replacement with low levels of organic or nano sources on growth performance, immune response, some blood serum biochemical changes and intestinal histopathology of broiler chickens.

2. MATERIALS AND METHODS

This study was conducted at Department of Nutrition and Veterinary Clinical Nutrition. Faculty of Veterinary Medicine, Alexandria University, Egypt at the period from 15th November 2015 until end of December 2015.

2.1. Birds accommodation and management:

Two hundred and twenty-five, one-day old chicks were used in this experiment. The chicks were allotted into nine equal groups (25 chicks/group) randomly. The chicks were housed in a clean well ventilated room previously fumigated with formalin and a potassium permanganate. According to age of the birds the environmental temperature was adjusted using electric heaters. A clean wheat straw was used to form a deep litter of four centimeters' depth on the floor. Suitable feeders and waterers were provided. Different types of vaccine (Hitchner B1, Gumboro intermediate, Gumboro weak and cloned) were used to protect the broiler chicks against Newcastle and infectious bronchitis diseases.

2.2. Experimental design and feeding program:

Experimental diets were varied according to feeding stage and in between groups in zinc sources and levels; in starter stage was corn – soybean based and contain 24% (standard protein requirement according to Nation Research Council (NRC, 1994), recommendation), 21% crude protein in growing stage, 18% crude protein in finishing stage and nearly similar in other nutrients and metabolizable energy. Each diet formulated with different three zinc sources (inorganic zinc, organic zinc, nano zinc) and four concentrations (100%, 75%, 50%, 25%) of the NRC recommendation to formulate nine different experimental diets. The ingredient composition and chemical analysis of the experimental basal diets used for the starter, grower and finisher are presented in table (1). The applied experimental design is illustrated in table (2).

2.3. Growth performance:

Individual bird body weight at the beginning of the experiment was recorded. Body weight, weight gain and feed intake for each pen were recorded weekly; feed conversion ratio (FCR), protein efficiency ratio (PER), efficiency of energy utilization (EEU) and performance index (PI) were calculated.

2.4. Chemical composition:

Analytical DM contents of feed samples were determined by oven-drying at 105°C for 8 h (AOAC, 2000; method 930.15). Ash contents were determined by incineration at 550°C overnight. Crude protein was determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963).

Table 1: Ingredient composition of the used basal diet.

Ingredients %	Feed Type		
	Starter diet	Grower diet	Finisher diet
Yellow corn	53.65	58.15	61.65
Soybean meal (44%)	32.6	29.5	26.6
Corn gluten (60%)	8.0	6.5	5.5
Vegetable oil[1]	2.0	2.0	2.5
DCP[2]	1.7	1.5	1.7
Limestone[3]	1.3	1.6	1.3
Lysine[4]	0.05	0.05	0.05
DL-Methionine[5]	0.15	0.15	0.15
Salt	0.3	0.3	0.3
Premix (vitamin)[6]	0.15	0.15	0.15
Mineral premix [7]	0.1	0.1	0.1
Total	100	100	100
Chemical composition:			
Moisture %	12.14	12.98	13.16
Crude protein %	22.85	21.12	18.7
Ether extract %	4.68	5.25	5.55
Crude fibre%	2.79	2.65	2.67
Ash%	6.79	6.55	6.66
NFE%*	50.75	51.45	53.26
Calcium%	1.10	1.09	0.98
Total phosphorus%	0.73	0.68	0.69
Methionine%*	0.67	0.56	0.51
Lysine%*	1.23	1.19	1.18
ME Kcal/kg diet**	3039.8	3058.7	3096.46
Calorie/protein ratio***	133.03	144.82	165.59

[1]Vegetable oil (mixture of sunflower oil and cottonseed oil). [2]DCP= dicalcium phosphate (contain 18% P and 25% Ca). [3]Limestone (contain 34% calcium). [4]Lysine = lysine hydrochloride (contain 98.5% Lysine). [5]DL-Methionine (Produced by Evonic Co and contain 99.5% methionine). [6]The premix used was Heromix produced by Heropharm and composed of (per 1.5 kg) vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, thiamin 1000 mg, riboflavin 5000 mg, pyridoxine 1500 mg, cyanocobalamin 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid. [7] mineral premix: formulated and composed of (each 1 kg) 70000 mg Mn, 60000mg Zn (Using zinc oxide (ZnO) and replaced by zinc polysaccharide complex or nano zinc particles), 8000mg Cu, 1000mg I, 250mg Se and 150mg Co.

* NFE= Nitrogen free extract (calculated by difference "100- (moisture% + CP% + EE % + CF% + ash%)". **Calculated according to **Lodhi et al. (1976)** as follows: Metabolizable energy MJ/Kg = 1.549+ (CP%*0.102) + (EE %*0.275) + (NFE%*0.148) + (CF%*0.034). The results multiply by 0.239 X 1000 = Kcal/kg. ***Calorie/protein ratio = ME Kcal/CP%

Table (2): Experimental design outline.

Groups No.	Experimental diet	Inorganic zinc concentration	Organic zinc concentration	Nano-zinc concentration
1	Basal diet	60 ppm	--	--
2	"""	--	60 ppm	--
3	"""	--	45 ppm	--
4	"""	--	30 ppm	--
5	"""	--	15 ppm	--
6	"""	--	--	60 ppm
7	"""	--	--	45 ppm
8	"""	--	--	30 ppm
9	"""	--	--	15 ppm

***Inorganic zinc oxide**: Using zinc oxide (ZnO) as fed basis produced by El-Gomhoria Co., Egypt with guaranteed minimum of 80% Zn).
****Organic zinc**: Used zinc polysaccharide complex (Quali Tech, Chaska, MN) with guaranteed minimum of 30% Zn.
*****Nano zinc**: Used zinc oxide nano particles produced by Mknano Co. " M K Impex Corp, Canada" with 30nm.

2.5. Immune response measurements:

2.5.1. Hemagglutination Inhibition test for detection of Newcastle antibodies: Blood samples were collected at 21th, 28th, 35th and 42th day of age from five chickens of each group. Blood samples were left without anticoagulant to clot. The serum was separated by centrifugation at 3000 rpm for 10 minutes. Micro-technique of hemagglutination inhibition test was done according to Takatasy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

2.5.2. Phagocytosis and differential leukocytic counts: Four blood samples were collected from each group of the experimental birds at 42th days of age in a clean dry vials containing anticoagulant (0.1ml sodium citrate 3.8%) for determination of phagocytic activity, phagocytic index, some blood pictures (total leukocytic count (WBCs), red blood cells (RBCs) counts, hemoglobin, packed cell volume (PCV) and differential leukocytes count).

2.5.3. Determination of phagocytic activity and phagocytic index: Phagocytic activity was determined according to Kawahara et al. (1991). Fifty micrograms of *Candida Albicans* culture were

added to 1 ml of citrated blood, collected at the end of experiment slaughtering four birds from each group. Treated blood samples were put in shaker water bath at 23 – 25C for 3 – 5 hrs. Smears of blood were made and then stained with *Geimsa* stain. Phagocytosis was estimated by determining the proportion of macrophages which contain intracellular yeast cells in a random sample of 300 macrophages and expressed as percentage of phagocytic activity (PA). The number of phagocytized candida cells was counted in the phagocytic cells to calculate the phagocytic index.

2.5.4. Determination of total leukocytic count (WBCs) and other blood pictures (RBCs count, Hb % and PCV): They were determined after previous methods **according to Maxine and Benjamine (1985).**

2.5.5. Determination of differential leukocytic count: Blood film was prepared according to the method of Lucky (1977). Ten drops from *May-Grunwald* stain stock solution on a dry, unfixed smear were added to equal amount of blood, then mixed and left for 1 minute for staining. The dye was decanted without rinsing. Diluted Giemsa´s solution (10 drops of the dye were added to 10 ml of distilled water) was poured over the film as counter stain and left for 20 minutes, then rinsed in water current and examined under an oil immersion lens. The percentage and absolute value for each type of cells were calculated according to Schalm (1986).

2.5.6. Lymphoid organs weight and some carcass traits:

At the end of experimental period, four birds from each dietary treatment were randomly taken, fasted for 6 hours then weighed and slaughtered to complete bleeding and weighed to determine relative weight of immune organs (spleen, bursa and thymus gland) and some carcass traits (liver, gizzard, proventriclus and abdominal fat).

2.6. Assessment of some blood parameters:

At the 42^{th} day, of age blood samples were collected from four birds of each group, and the blood was left to drop on the side of the tube to prevent destruction of RBCs. Each blood sample was left to coagulate at room temperature. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 minutes. The clear serum was kept in a freezer (-20 C) until analysis for determination of serum total protein, globulin, albumin, GOT, GPT, ALP, uric acid, creatinine, calcium, phosphorus, serum lipids concentrations (cholesterol, triglyceride, HDL, LDL and VLDL), glucose and antioxidant enzymes (GSH-Px and MDA) were estimated using specific commercial kits (Roche Diagnostica, Basel, Switzerland).

2.7. Intestinal histopathology:

About 2.5 cm of the jejunum portion was sectioned. The tissues were collected and submersed in 10% neutral-buffered formalin for 3 days for tissue fixation. After that, the samples were dehydrated and rinsed in several times in absolute alcohol, and then embedded in paraffin. Serial 5-µm longitudinal sections were cut on Leica Rotary Microtome (RM 2145, Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. Then, slides routinely stained with hematoxylin and eosin (H&E). The histomorphometric analysis was performed using Image J analysis software (National Institutes of Health, MD, USA), whereas the villus height (measured from the tip of the villus to the villus-crypt junction), villus width from the mid of the villus and crypt depth (measured from the crypt-villus junction to the base of the crypt) (Law et al., 2007).

2.8. Statistical analysis:

Statistical analysis was made using Analysis of Variance (ANOVA) one-way analysis of variance for study the effect of different treatment groups on the different studied variables studied that includes (growth performance parameters, hematological, biochemical and gut morphology) variables using statistical analysis system (SAS, 2004) .

3. RESULTS

3.1.Growth performance and feed efficiency parameters:

Broiler fed 60 or 45mg zinc polysaccharide complex/kg instead of inorganic zinc oxide (table 3) improved final body weight, TBG, FCR, PER, EEU and performance index of broiler chicken while, lower supplementation levels (30 or 15mg/kg) had no significant effect. Moreover, zinc nanoparticles supplementation at 60, 45 or 30mg/kg improved the previous mentioned items while lower level (15mg/kg) significantly reduced broiler performance and feed efficiency parameters. Using organic or nano zinc instead of inorganic source reduced total feed intake of broiler chicken.

3.2.Blood picture:

Supplementation of polysaccharide zinc complex or zinc nanoparticles instead of inorganic source increased WBCs, RBCs counts, Hb % and PCV% (table, 4) except lowest level (15mg/kg) of nano zinc reduced total WBCs count.

3.3.Blood serum units

Broiler fed on the basal diet with 30mg of polysaccharide zinc complex or zinc nanoparticle/kg diet instead of inorganic zinc oxide significantly ($P \leq 0.05$) reduced blood serum glucose

level (table, 5) when compared with broiler chicks fed on the basal diet with inorganic zinc supplementation. However, other levels of organic or nano zinc supplementation non-significantly reduced blood serum glucose level. On the other hand, it was observed that dietary replacement of inorganic zinc oxide by lower levels of organic or nano zinc had no significant effect on blood serum total protein, albumin or globulin concentrations. However, supplementation of 60, 30 or 15mg of organic zinc or 30 and 15 mg of nano zinc/kg diet instead of inorganic zinc oxide non-significantly increased blood serum globulin level by about 21.7%, 29.3%, 2.2%, 18.5% and 5.4% respectively compared with broiler chicks fed on the basal diet with inorganic zinc supplementation.

3.4.Some blood serum minerals levels:

Regarding blood serum calcium and phosphorus levels (table, 6), it was observed that dietary replacement of inorganic zinc oxide with lower levels of organic or nano zinc increased blood serum calcium or phosphorus levels except higher levels of organic zinc (60mg/kg) reduced blood serum calcium level compared with broiler chicken fed on the basal diet with inorganic zinc oxide supplementation.

Liver and kidney blood serum related parameters:

Broiler fed on different levels of organic or nano zinc reduced blood serum creatinine concentration (table, 7), while had no significant (P≥0.05) effect on blood serum uric acid and GOT concentrations and non-significantly (P≥0.05) increased blood serum GPT and ALP activities when compared with broiler chicks group fed on the basal diet with inorganic zinc supplementation.

3.5.Blood serum lipid profile:

As shown in table (8), replacement of inorganic zinc with different levels of polysaccharide zinc complex or with zinc nano particles non-significantly (P≥0.05) reduced blood serum triglycerides while increased total cholesterol and HDL and had no significant effect on blood serum LDL and VLDL concentrations.

3.6.Antioxidant enzyme activity:

Replacement of dietary inorganic zinc with low levels of zinc polysaccharide complex had no significant effect on blood serum GPx or MDA activities (table, 9), while nano zinc supplementation non-significantly reduced blood serum GPx or MDA activities compared to broiler chicken fed on inorganic zinc supplemented diet.

3.7.Immune response:

3.7.1. Differential leukocyte:

Broiler fed on basal diet with 60mg/kg diet of organic or nano zinc significantly reduced heterophil% (table, 10), while other levels non-significantly reduced heterophil % compared with broiler chicks fed on the basal diet with inorganic zinc supplementation. Moreover, organic or nano zinc supplementation increased lymphocyte% and consequently reduced heterophil /lymphocyte ratio. On the other hand, replacement of inorganic zinc with lower levels of organic or nano zinc had variable effect of eosinophil, basophil and monocyte percentages.

Phagocytosis: Broiler fed on different levels of organic or nano zinc non-significantly (P≥0.05) improved phagocytic activity and index (table, 11) except 45mg of zinc nanoparticle/Kg diet significantly (P≤0.05) improved phagocytic index compared to broiler chicks group fed on the basal diet with inorganic zinc supplementation.

3.7.2. Antibody production: As presented in table (12), dietary replacement of inorganic zinc oxide with lower levels of organic zinc or zinc nano particles improved antibody titer against New castle disease vaccine at 21th, 28th, 35th and 42th day of broiler age.

Immune organs: As shown in table (13), dietary replacement of inorganic zinc oxide with lower levels of organic zinc or zinc nano particles improved (P≥0.05) thymus and spleen weight and relative weights while reduced (P≥0.05) bursa weight and relative weight except higher level of zinc polysaccharide complex increased bursa weight when compared with broiler chicks group fed on the basal diet with inorganic zinc supplementation.

2.8. Some carcass traits:

Dressing percentage (table, 14) improved (P≥0.05) with dietary replacement of inorganic zinc by lower levels of organic or nano zinc while decreased liver and abdominal weight and their relative weight.

2.9. Intestinal morphology:

It was observed that dietary replacement of 60mg inorganic zinc oxide with 30 mg of organic zinc or by 45 and 30 mg of nano zinc/kg diet significantly (P≤0.05) increased jejunum villi length while other levels of organic and nano zinc non-significantly improved villi length (table, 15 and figures 1 -3). Moreover, different levels organic supplementation instead of inorganic zinc oxide had no significant effect on villi width and crypt depth while, nano zinc supplementation increased villi width and 45mg of nan zinc increased crypt depth compared with broiler chicks group fed on the basal diet with inorganic zinc oxide supplementation. On the other hand, birds fed on the experimental diet with 30 or 15mg of organic zinc and 30 mg of nano source increased (≤0.05) villi length/crypt depth ration compared with group fed on diet supplemented with inorganic zinc source.

Table (3): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on body weight (g/chick) development of broiler chickens.

Parameters	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	**Organic zinc**				**Nano zinc**			
	60	**60**	**45**	**30**	**15**	**60**	**45**	**30**	**15**
Initial Wt (g/chick)	41.28 ±0.66^{a}	41.35 ±0.77^{a}	40.09 ±0.90^{a}	41.94 ±0.55^{a}	40.90 ±0.65^{a}	41.64 ±0.73^{a}	40.30 ±0.63^{a}	40.81 ±0.60^{a}	40.15 ±0.61^{a}
Final body Wt (g/chick)	1660.23 ±72.27^{ab}	1700.42 ±42.89^{a}	1675.00 ±66.16 ^{ab}	1655.20 ±54.04 ^{ab}	1661.00 ±53.90 ^{ab}	1623.33 ±51.96 ^{ab}	1588.96 ±48.07 ^{ab}	1658.26 ±59.32 ^{ab}	1500.60 ±51.70 ^{b}
Total gain (g/chick)	1619.19 ±71.72^{ab}	1659.07 ±42.20^{a}	1635.91 ±65.34^{ab}	1613.25 ±53.51^{ab}	1620.10 ±53.27^{ab}	1581.99 ±51.35^{ab}	1548.65 ±47.52^{ab}	1617.44 ±58.91^{ab}	1460.15 ±51.11^{b}
Total Feed intake (g/chick)	3708.74	3686.77	3678.99	3591.40	3635.20	3540.60	3446.60	3571.00	3345.80
Average FCR value	2.41 ±0.13^{a}	2.26 ±0.06^{c}	2.33 ±0.10^{b}	2.29 ±0.08^{c}	2.31 ±0.08^{b}	2.29 ±0.07^{c}	2.29 ±0.07^{c}	2.31 ±0.12^{b}	2.36 ±0.09^{ab}
Average PER value	2.14 ±0.09^{c}	2.21 ±0.06^{a}	2.18 ±0.09^{b}	2.20 ±0.07^{a}	2.19 ±0.07^{ab}	2.18 ±0.07^{b}	2.19 ±0.07^{ab}	2.21 ±0.08^{a}	2.14 ±0.07^{c}
Average EEU value	7.54 ±0.39^{a}	7.07 ±0.19^{c}	7.30 ±0.31^{b}	7.17 ±0.25^{c}	7.23 ±0.26^{b}	7.18 ±0.23^{c}	7.16 ±0.23^{c}	7.22 ±0.38^{b}	7.40 ±0.27^{ab}
Average performance index	75.33 ±6.19 ^{bc}	77.67 ±3.81^{a}	77.09 ±6.04^{a}	76.28 ±5.07^{ab}	75.92 ±4.83^{bc}	74.16 ±5.05^{c}	72.62 ±4.37^{d}	77.07 ±5.02^{a}	67.40 ±4.60^{e}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (4): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on some blood pictures of broiler chickens.

Items	**Zinc source and supplementation levels (mg/Kg diet)**								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
WBCs count (10^6)	29.50±2.90^{ab}	29.50±2.32^{ab}	33.00±3.55^{ab}	28.75±2.09^{ab}	34.00±2.27^{ab}	35.50±2.72^{a}	32.75±1.93^{ab}	29.50±2.63^{ab}	26.75±1.43^{b}
RBCs count (10^3)	1.57±0.11^{a}	1.67±0.2^{a}	1.72±0.2^{a}	1.72±0.2^{a}	1.75±0.2^{a}	1.65±0.11^{a}	1.70±0.2^{a}	1.75±0.2^{a}	1.65±0.2^{a}
HB (%)	10.00±0.70^{b}	11.50±0.28^{ab}	12.50±0.64^{a}	12.25±0.47^{a}	11.50±0.86^{ab}	9.75±0.25^{b}	12.00±0.40^{ab}	12.00±0.40^{a}	11.25±0.62^{ab}
PCV (%)	30.25±1.10^{ab}	31.50±0.64^{ab}	32.50±0.64^{a}	31.00±0.70^{ab}	30.25±1.31^{ab}	29.75±0.47^{b}	32.0±0.40^{ab}	32.25±0.62^{ab}	31.0±0.40^{ab}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P < 0.05

Table (5): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on some blood serum units of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Glucose (mg/dl)	210.6 ±2.19ab	212.93 ±.88a	207.86 ±1.33abc	202.36 ±1.95c	207.26 ±3.26abc	209.40 ±2.63abc	211.10 ±3.31ab	202.66 ±2.11c	206.13 ±3.10abc
T. Protein (g/dl)	5.66 ±0.30a	5.61 ±0.34a	5.72 ±0.24a	5.58 ±0.27a	5.41 ±0.43a	5.59 ±0.39a	5.53 ±0.26a	5.60 ±0.37a	5.85 ±0.18a
Albumin (g/dl)	4.73 ±1.01a	4.49 ±0.22a	5.00 ±0.12a	4.39 ±0.34a	4.4 ±0.32a	4.02 ±0.24a	4.80 ±0.16a	4.51 ±0.10a	4.88 ±0.14a
Globulin (g/dl)	0.92 ±0.35a	1.12 ±0.48a	0.72 ±0.30a	1.19 ±0.42a	0.94 ±0.25a	0.67 ±0.17a	0.61 ±0.26a	1.09 ±0.38a	0.97 ±0.20a

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (6): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on some blood serum minerals (calcium and phosphorus) levels of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Ca (mg/dl)	8.60 ±0.28ab	6.95 ±0.05b	10.00 ±0.24a	9.67 ±0.48a	10.50 ±0.38a	9.20 ±0.26ab	10.15 ±0.32a	9.67 ±0.60a	10.22 ±0.28a
P (mg/dl)	4.00 ±0.15b	4.67 ±0.22ab	4.85 ±0.20ab	5.10 ±0.17a	5.10 ±0.26a	4.52 ±0.33ab	5.07 ±0.25a	4.82 ±0.51ab	4.97 ±0.51ab

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (7): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on some blood serum parameters related to kidney function of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Creatinine (mg/dl)	1.12 ±0.18a	0.97 ±0.58ab	0.72 ±0.21ab	0.32 ±0.13bc	0.21 ±0.2c	0.82 ±0.2ab	0.82 ±030ab	0.29± 0.10bc0	0.67 ±0.15ab
Uric acid (mg/dl)	5.86 ±0.2a	5.94 ±0.2a	4.23 ±0.38a	5.89 ±0.2a	5.83 ±0.2a	5.83 ±0.2a	5.88 ±0.2a	5.76 ±0.2a	5.96 ±0.2a
GOT (µ/L)	55.50 ±4.09a	56.25 ±4.23a	57.75 ±1.75a	57.75 ±2.95a	53.50 ±2.10a	55.00 ±4.08a	55.00 ±3.48a	51.50 ±2.32a	60.50 ±1.89a
GPT (µ/L)	77.25 ±7.98a	80.25 ±4.19a	80.00 ±6.17a	94.25 ±3.47a	94.75 ±5.66a	88.50 ±5.42a	89.00 ±3.93a	93.50 ±7.04a	85.50 ±4.19a
ALP (µ/L)	1256.98 ±50.88a	1411.90 ±66.12a	1399.19 ±59.20a	1389.08 ±65.99a	1309.16 ±70.21a	1406.20 ±51.88a	1448.76 ±77.99a	1369.69 ±90.22a	1289.96 ±59.99a

Values = means ±standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (8): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on blood serum lipids of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Triglycerides (mg/dl)	209.76 ±1.38a	201.10 ±1.24a	201.40 ±1.40a	199.03 ±1.16a	201.00 ±1.45a	199.36 ±1.00a	200.23 ±1.59a	198.36 ±1.79a	199.32±1.14 a
T. cholesterol (mg/dl)	200.66 ±4.12b	208.30 ±2.58ab	206.03 ±2.90ab	205.46 ±2.16ab	206.70 ±1.59ab	206.36 ±1.90ab	209.96 ±1.58a	210.13 ±2.74a	207.32±02.5 7ab
HDL (mg/dl)	38.33 ±2.33b	42.33 ±1.76ab	46.33 ±1.20ab	41.66 ±1.85ab	40.00 ±1.15ab	44.00 ±4.00ab	47.33 ±1.85a	44.33 ±3.17ab	39.33±3.84a b
LDL (mg/dl)	38.33 ±2.33a	42.33 ±1.76a	46.33 ±1.20a	41.66 ±1.85a	40.00 ±1.15a	44.00 ±4.00a	47.33 ±1.85a	44.33 ±3.17a	39.33 ±3.84a
VLDL (mg/dl)	40.15 ±0.27a	40.22 ±0.24a	40.28 ±0.28a	39.80 ±0.23a	40.20 ±0.29a	39.87 ±0.20a	40.04 ±0.31a	39.67 ±0.35a	39.80 ±0.31a

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (9): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on activity of some antioxidant activity of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Glutathione peroxide (GPx) (U/ml)	2282.40 ±19.84^{a}	2284.63 ±19.04^{a}	2303.93 ±14.18^{a}	2262.73 ±36.20^{a}	2280.53 ±55.82^{a}	2212.23 ±2887^{a}	2126.40 ±19.03^{a}	2134.60 ±86.49^{a}	2251.50±2 6.8^{a}
Malondialdehyde "MDA" (nmol/ml)	9.59± 0.2^{ab}	9.79 ±0.24^{ab}	9.81 ±0.2^{ab}	9.93 ±0.2^{ab}	10.08 ±0.2^{a}	9.35 ±0.26^{ab}	9.37 ±0.2^{ab}	9.25 ±0.24^{ab}	9.32 ±0.38^{b}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (10): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc on activity of some antioxidant activity of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Heterophil%	65.33 ±1.22^{a}	59.60 ±1.45^{b}	65.83 ±2.09^{a}	63.10 ±2.11^{ab}	63.30 ±1.99^{ab}	60.43 ±2.19^{b}	64.83 ±2.31^{a}	64.38 ±2.54^{a}	63.63 ±1.87^{ab}
Eosinophil%	7.30 ±0.34^{b}	5.95 ±0.28^{bc}	9.20 ±0.45^{a}	6.35 ±0.43^{b}	3.93 ±0.11^{c}	9.85 ±0.29^{a}	5.00 ±0.36^{bc}	6.08 ±0.28^{b}	5.90 ±0..29^{bc}
Basophil%	3.43 ±0.09^{b}	1.78 ±0.17^{c}	1.68 ±0.11^{c}	3.50 ±0.23^{b}	3.39 ±0.22^{b}	9.85 ±0.68^{a}	1.78 ±0.11^{c}	2.75 ±0..16^{bc}	1.38 ±0.12^{c}
Lymphocyte%	19.55 ±1.02^{b}	25.58 ±0.79^{a}	21.20 ±1.22^{b}	24.13 ±1.61^{a}	25..50 ±1.29$_{a}$	23.63 ±1.29^{ab}	24.28 ±1.61^{a}	23.40 ±1.22^{b}	24.03 ±1.11^{a}
Monocyte%	4.40 ±0.11^{a}	5.10 ±0.14^{a}	2.10 ±0.27^{b}	2.93 ±0.19^{b}	3.33 ±0.08^{ab}	3.45 ±0.27^{ab}	4.12 ±0.22^{a}	3.40 ±0.36^{ab}	5.08 ±0.31^{a}
H/L ratio	3.34	2.33	3.11	2.62	2.48	2.56	2.67	2.75	2.65

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (11): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc replacement on phagocytosis of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Phagocytic activity%	34.30 ±3.17[a]	42.50 ±0.66[a]	43.75 ±1.25[a]	41.02 ±.0.72[a]	42.57 ±1.63[a]	43.20 ±0.92[a]	44.07 ±0.75[a]	39.10 ±0.71[a]	40.00 ±1.76[a]
Phagocytic index%	1.54 ±0.27[bc]	2.14 ±0.12[ab]	1.96 ±0.24[ab]	1.91 ±0.20[ab]	2.00 ±0.23[ab]	2.23 ±0.36[b]	2.43 ±0.13[a]	1.63 ±0.25[c]	1.89 ±0.34[ab]

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (12): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc replacement on HI titer against New castle disease vaccine of broiler chickens.

Age/day	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
21	5.85 ±0.20[a]	6.57 ±0.24[a]	7.35 ±0.27[a]	7.85 ±0.37[a]	6.72 ±0.44[a]	7.35 ±1.88[a]	6.72 ±1.76[a]	7.92 ±.48[a]	6.95 ±0.37[a]
28	4.70 ±0.24[c]	5.27 ±0.14[bc]	5.62 ±0.39[abc]	6.25 ±0.17[abc]	5.47 ±0.33[bc]	6.00 ±0.22[abc]	6.52 ±0.25[a]	6.25 ±0.13[ab]	5.87 ±0.44[abc]
35	5.50 ±0.28[b]	7.25 ±0.25[a]	7.75 ±0.47[a]	6.50 ±0.28[ab]	6.50 ±0.64[ab]	7.00 ±0.40[ab]	6.75 ±0.25[ab]	6.50 ±0.64[ab]	7.00 ±0.40[a]
42	5.15 ±0.57[a]	6.35 ±0.40[a]	6.20 ±0.51[a]	6.20 ±0.45[a]	6.30 ±0.45[a]	6.50 ±0.32[a]	6.50 ±0.64[a]	6.50 ±0.57[a]	6.00 ±0.40[a]

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

4. Discussion:

Zinc (Zn) is an essential trace element, which plays important roles in various biological activities of animals. The comparable body weight of lower supplementation levels with normal level of inorganic zinc might be due to higher bioavailability of organic zinc and that could have reduced the amount of zinc supplementation required for body weight development. Our results were inconsistent with the findings of Feng et al. (2010) who observed that comparable average daily gains in broilers fed on dietary Zn supplementation of 90 mg Zn/kg diet from Zn-glycine and 120 mg Zn/kg diet from $ZnSO_4$. Similar, Jahanian and Rasouli (2015) stated that dietary substitution of inorganic Zn sources by zinc methionine (ZnMet) caused improvements ($p < 0.01$) in final body weight.

This higher body weight in zinc-polysaccharide supplemented group may be related to organic minerals are good vehicle to supply broiler with more mineral without increasing dietary mineral levels (Abdallah et al., 2009) also, nano zinc supplemented groups might be due to higher uptake of zinc nano particles in the gastro-intestinal tract. Because of particle size of nano zinc a faster diffusion through GIT membrane has taken place to reach the cells of the intestinal lining. Our data are in agreement with those obtained by Zhao et al. (2014) who stated that after 14 days, birds in the 20 and 60 nano-ZnO groups had significantly greater weight gains and better feed conversion ratios. However, the body weight of birds in the 100-nano-ZnO group was dramatically reduced after 28 days. In contrast, Siddhartha et al. (2016) found that body weight was significantly ($P \leq 0.05$) higher in broiler chick group fed on the basal diet with 60mg of nano zinc/kg diet when compared with chicks group fed on diet supplemented by organic or inorganic zinc sources.

Comparing body weight development of broiler chicken fed on basal diet with ZnO-NP or organic zinc, it was observed that nano zinc supplementation at 60, 45 or 15mg/kg diet non-significantly ($P \geq 0.05$) reduced final body weight by about 4.5%, 5.1% and 9.7% respectively when compared with broiler chicks group fed on the basal diet with 60, 45 or 15mg of organic zinc/kg diet. While inclusion of 30mg/kg of both nano or organic zinc sources showed nearly the same body weight.

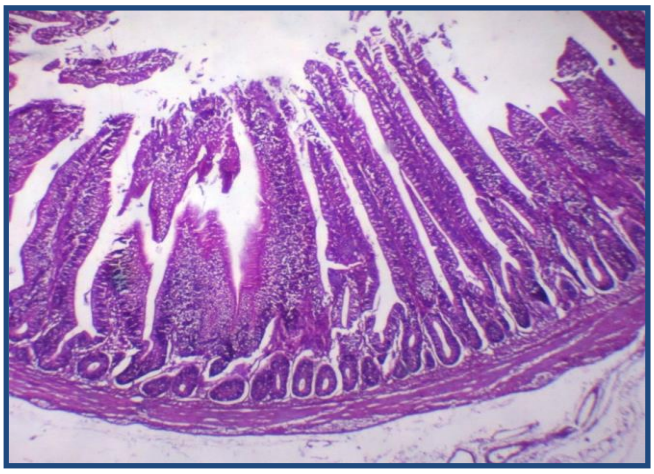

Fig. (1): Jejunum of chicken supplemented with inorganic Zn showing normal intestinal villi

Table (13): Effect of dietary inorganic zinc replacement by organic or nano zinc sources on weight and relative weight of some immune organs of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Thymus wt (g)	1.78 ±0.17^{b}	2.63 ±0.76^{a}	2.30 ±0.21^{ab}	2.50 ±0.81^{ab}	2.73 ±0.99^{a}	2.35 ±0.41^{ab}	2.45 ±0.55^{ab}	2.45 ±1.12^{ab}	2.08 ±0.47^{ab}
Thymus %	0.12 ±0.02^{a}	0.17 ±0.04^{a}	0.17 ±0.01^{a}	0.18 ±0.06^{a}	0.18 ±0.06^{a}	0.15 ±0.03^{a}	0.17 ±0.04^{a}	0.17 ±0.06^{a}	0.17 ±0.04^{a}
Bursa wt (g)	1.88 ±0.09^{ab}	2.00 ±0.15^{ab}	1.03 ±0.13^{cd}	1.40 ±0.35^{a}	1.18 ±0.18^{cd}	1.53 ±0.26^{bc}	1.15 ±0.16^{cd}	0.98 ±0.22^{cd}	0.75 ±0.14^{d}
Bursa %	0.12 ±0.01^{bc}	0.14 ±0.02^{ab}	0.07 ±0.01^{cd}	0.10 ±0.02^{c}	0.08 ±0.01^{cd}	0.10 ±0.02^{cd}	0.08 ±0.01^{cd}	0.07 ±0.01^{cd}	0.06 ±0.01^{d}
Spleen wt (g)	1.98 ±0.34^{b}	2.33 ±0.45	2.38 ±0.42	2.47 ±0.33	2.08 ±0.19	1.90 ±0.45	2.28 ±0.33	2.04 ±0.31	2.25 ±0.34
Spleen %	0.13 ±0.03^{a}	0.15 ±0.02^{a}	0.17 ±0.02^{a}	0.17 ±0.01^{a}	0.14 ±0.01^{a}	0.13 ±0.03^{a}	0.15 ±0.02^{a}	0.15 ±0.02^{a}	0.19 ±0.03^{a}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (14): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc replacement on some carcass traits of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Dressed carcass %	67.46 ±1.95^{b}	69.04 ±1.43^{a}	71.13 ±1.36^{a}	69.62 ±1.38^{a}	63.79 ±1.93^{c}	69.03 ±1.05 ^{a}	69.67 ±1.06^{a}	68.00 ±2.94^{b}	69.58 ±2.35^{a}
Liver wt (g)	36.28 ±4.41^{a}	37.65 ±2.39^{a}	33.63 ±4.64^{ab}	29.27 ±4.09^{b}	34.53 ±4.34^{a}	36.85 ±5.26^{a}	32.25 ±3.66^{ab}	33.35 ±4.10^{ab}	30.98 ±3.33^{ab}
Liver %	2.27 ±0.08^{ab}	2.50 ±0.14^{a}	2.39 ±0.17^{a}	1.99 ±0.04^{b}	2.35 ±0.20^{a}	2.24 ±0.10^{a}	2.17 ±0.13^{a}	2.58 ±0.25^{a}	2.50 ±0.29^{a}
Abdominal fat wt (g)	11.48 ±4.02^{cd}	7.00 ±2.95^{f}	13.45 ±2.39^{b}	15.43 ±4.21^{a}	12.68 ±4.37^{bc}	9.35 ±2.10^{ef}	10.90 ±5.28^{de}	5.65 ±0.90^{g}	2.60 ±0.38^{h}
Abdominal fat %	0.65 ±0.18^{ab}	0.43 ±0.16^{ab}	1.04 ±0.26^{a}	1.00 ±0.17^{a}	0.91 ±0.33^{a}	0.59 ±0.15^{ab}	0.70 ±0.29^{ab}	0.42 ±0.03^{ab}	0.21 ±0.04^{b}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P \leq 0.05$).

Table (15): Effect of dietary replacement of inorganic zinc with lower levels of organic or nano zinc replacement on morphological histology of jejunum of broiler chickens.

Items	Zinc source and supplementation levels (mg/Kg diet)								
	Inorganic zinc	Organic zinc				Nano zinc			
	60	60	45	30	15	60	45	30	15
Villi length (µm)	680.63 ±49.55^{d}	792.53 ±184.75^{cd}	846.55 ±56.99^{bcd}	1101.42 ±47.96^{b}	839.41 ±116.42^{bcd}	757.64 ±180.29^{cd}	981.40 ±148.21^{bc}	1434.86 ±103.66^{a}	760.63 ±124.38^{cd}
Villi width (µm)	114.46 ±40.24^{b}	106.11 ±24.49^{b}	136.04 ±30.07^{b}	122.26 ±49.61^{b}	107.01 ±16.37^{b}	231.03 ±55.61^{a}	119.24 ±31.24^{b}	122.45 ±15.48^{b}	139.72 ±43.03^{b}
Crypt depth (µm)	128.44 ±20.50^{bc}	128.85 ±15.56^{bc}	155.08 ±45.91^{abc}	150.29 ±20.70^{abc}	87.86 ±10.17^{c}	169.14 ±18.22^{ab}	215.85 ±41.43^{a}	175.28 ±40.37^{ab}	165.09 ±51.37^{abc}
Villi length/crypt depth ratio (µm : µm)	5.30 ±0.64^{c}	6.15 ±0.86^{c}	5.45 ±0.46^{c}	7.32 ± 0.72^{b}	9.89 ± 0.62^{a}	4.48 ± 1.36^{c}	4.54 ± 0.94^{c}	8.18 ± 0.42^{ab}	4.60 ±0.83^{c}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

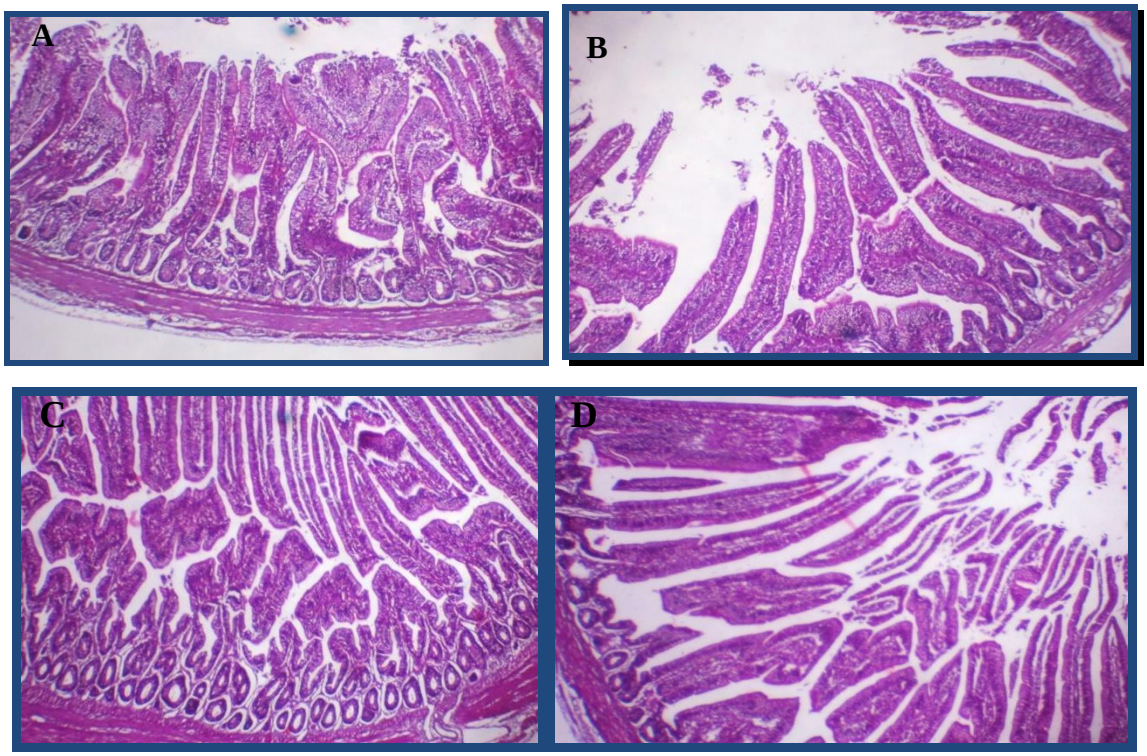

Fig. (2): Left Jejunum of birds supplemented with 60, 45, 30 or 15mg organic Zn/kg (A, B, C and D respectively) showing slight increase of villi length and thickness (A, B & D) and marked increase in villi length and thickness (C).

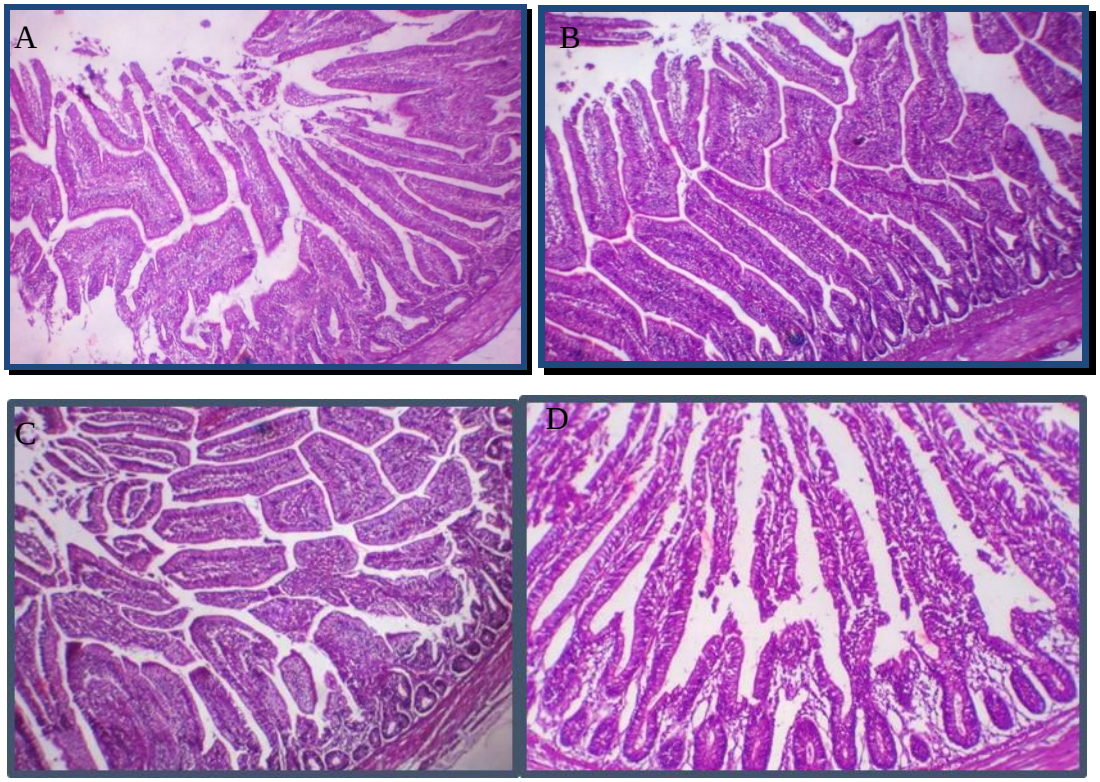

Fig. (3): Left Jejunum of birds supplemented with 60, 45, 30 or 15mg nano Zn/kg (A, B, C and D respectively) showing slight increase of villi length and thickness (A and D), and marked increase in villi length and thickness (B and C).

The present data are in harmony with those obtained by Sahoo et al. (2016) who indicated that organic zinc at 15ppm induced better growth than inorganic and nano zinc sources at the same supplementation level but, in contrast with those obtained by Zhao et al. (2014), wherein chickens fed 20 or 60 mg/kg nano-ZnO resulted increased in body weight at all the weeks until the end of the experiments of 42 days. Also, Siddhartha et al. (2016) showed that nano zinc supplementation improved broiler chicks weight.

Blood serum globulin level improvement in accordance with Fawzy et al. (2015) who observed that protein profile in broiler chicks supplemented with selenium and zinc showed an elevation in total proteins, albumin, and globulin levels with a decline in albumin/globulin ratio. Similarly, plasma total protein was increased with Zn dietary supplementation in broiler (Feng et al., 2010). Higher calcium and phosphorus levels in broiler blood serum fed on organic or nano zinc are in agreement with those obtained by Yenice et al. (2015) indicated that using the organic form of the trace mineral mixture significantly increased the serum Ca concentration compared with the inorganic form. However, Güçlü and İşcan (2004) reported that organic Mn and Zn supplementation of laying hen diets did not affect serum Ca, P, and Mg concentrations. In another study, dietary Zn supplementation at different levels increased plasma Ca concentration, but plasma Ca was not affected by different Zn sources (Bahakaim et al., 2014). Under the conditions of the present study, addition of the trace mineral mixture in the organic form positively affected calcium and phosphorus bioavailability of broiler chicken. It is estimated that the organic form (polysaccharide zinc complex) or zinc nano particles decreased the amount of free trace mineral ions in the small intestine and calcium and phosphorus were prevented from forming insoluble compounds with these minerals, thereby increasing the absorption of Ca. Idowu et al. (2011) similarly reported that organic Zn resulted in higher Ca bioavailability relative to a control group and to inorganic sources.

Organic or nano zinc had no adverse effect on kidney function and slight increase in liver enzyme. These data are in contrast with Fathi (2016) who reported that nano zinc increase blood serum creatinine kinase activity which reflect on higher creatinine concentration. The finding of the current study is adverse with Fazilati (2013) who observed that zinc oxide nanoparticles (25-200 mg) had significantly increased ($P<0.05$) activity of ALT and AST enzymes in serum male rats. Possible reason for these differences is probably related to using doses and time of animal exposed, as, it has been reported that, level above 50 mg/kg of nano-ZnO induce the oxidative stress and increase the plasma level of ALT and AST (Sharma et al., 2009). The present data are in harmony with Ahmadi et al. (2014) who reported nano-ZnO had no significant effects on ALT and AST activities in serum of broilers. Also, [35] indicated that the supplementation diet with nano ZnO had no significantly ($P>0.05$) affects the ALT, AST and LDH activates. Some researchers have also concluded Zn supplementation increased ALP activity in plasma (Levengood et al., 2000; Peretz et al., 2001). The significant increase in serum ALP activity in birds by 20 mg/kg of nano-ZnO as compared to other group may be attributed to the action of vitamin D_3, which has several effects on the intestine, kidneys and bones, increasing absorption of calcium into the extra cellular fluid and possibly promoting the formation ALP in the epithelial cells (Guyton and Hall, 2006). In addition, increased ALP activity may be attributed to numerically increased concentrations of cholesterol by nano ZnO (Table 7). Zaghari et al. (2009) reported that progesterone injection of broiler breeder pullets (20 week of age) affects serum glucose, triglycerides and cholesterol concentrations of hens. Therefore, the increase in the corticosteroids hormones secretion, epinephrine and norepinephrine leads to elevated ALP activity, but the mechanism is not totally clear (Al-Darraji, 2008).

The highest level was observed by broiler chicks group fed on the basal diet with 45 or 30mg nano zinc/kg diet. Our data are supported by those obtained by Fathi (2016) who stated that 20 mg/kg nano-ZnO tended to increase the serum cholesterol. Moreover, these results are consistent with those of Roberson and Edwards (1994) who reported that there is a significant elevation in the serum HDL and cholesterol for the subjects in the zinc supplemented group. These researchers suggested that increased level of HDL and cholesterol is probably due to improvement in calories and fat intake after zinc supplementation. Additionally, it has been reported that zinc-deficient diets are accompanied by decreased plasma total cholesterol, LDL, HDL, and triglyceride concentrations. This can be due to diminished absorption of dietary lipids in addition to decreased intake of fat and calories intake (Wu and Sun, 2004). It is also congruent with

the findings of Hazim et al. (2011) that showed that supplementation zinc of diet in broilers increased plasma total cholesterol. These researchers suggested that an increase in the amount of cholesterol ingested slightly increases the plasma cholesterol concentration. Moreover, a change of cholesterol levels in blood plasma may be due to the zinc's role in enzyme action in that zinc forms an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption (Hazim et al., 2011).

Zinc nanoparticle supplementation instead of inorganic source improve antioxidant ability of broiler chicken better than organic form as increased MDA concentration is an important index for lipid peroxidation and oxidative damage caused by ROS in cell (Nielsen et al., 1997). Zinc is considered a cofactor and component of more than 240 enzymes that play a central role in oxidative processes and protected cells from oxidative damage by reduction in the formation of OH from H2O2 and O2- through the antagonism of redox-active transition metals. The obtained data are in harmony with those reported by Ahmadi et al. (2014) who indicated that nano zinc supplementation decreased MDA concentration compared to control. Also, Liu et al. (2015) stated that supplemental zinc decreased malondialdehyde (MDA) level in the liver. We could speculate that dietary Zn might strengthen the oxidative defenses and decrease the MDA content of muscles, which would contribute to higher quality and longer shelf life of meat.

The ratio between heterophils and lymphocytes, an index of stress in birds, was wider when the diets were supplemented with inorganic ZnO. However, a perceptible decline in the ratios was observed with dietary replacement of inorganic zinc with different levels of zinc-polysaccharides complex or zinc nanoparticles. Higher Zn availability from organic or nano sources more than inorganic one was useful in reducing stress and stimulate immune response in young broilers. Zinc is required for the normal development of lymphocytes (Fawzy et al., 2016) and a deficiency of Zn leads to thymocyte depletion in the thymus and reduction in peripheral T-cell numbers and T-cell helper functions (Kid Ferket and Qureshi, 1996). This finding disagreed with Donmez et al. (2002) who reported that Zn supplementation did not affect peripheral blood leukocyte counts. Probably Zn supplemented at 30 ppm from organic or nano source was adequate to support optimum development of lymphocytes, which alleviated stress, as observed from the present study.

Higher immune response of broiler chicken fed on the basal diet with zinc-polysaccharide complex or zinc nanoparticles instead of inorganic source was observed through improvement of cellular and humoral immun. Broiler chicken provided diets supplemented with organic or nano zinc might have increased thymulin activity; therefore, enhancing immune response through increased maturation of T-lymphocyte and activation of B lymphocytes by T-helper cells (Hudson et al., 2004). Also, the immune system is dependent on the functions of cellular metabolism. Zinc is ubiquitous in cellular metabolism and functions both structurally and catalytically in metalloenzymes (O'dell, 1992). These data are in harmony with those obtained by Soni et al. (2013) who concluded that cellular immunity and antibody production significantly improved with organic zinc supplementation of broiler breeder. Weights of lymphoid organs are influenced by dietary zinc sources and zinc-polysaccharide complex or zinc nanoparticles supplementation supported better humoral and cell-mediated responses. These findings were similar to those observed by Bartlett and Smith (2003). On the other hand, low levels of supplemental Zn showed a relative reduction in the size of lymphoid organs with the possible decrease in T-cell function (Kid Ferket and Qureshi, 1996).

The highest dressing percentage was obtained by broiler chicks group fed on diet supplemented by 45mg organic zinc (71.13%) instead of inorganic zinc and followed by broiler chicks fed on diet supplemented by 45mg nano zinc (69.67%). The present data are supported by those obtained by Wojciech et al. (2007) who reported that different source of zinc (organic form of zinc) resulted in a 4.15% increase of the dressing percentage of the birds compared to the dressing percentage of the birds from the treatment that fed diet inclusion of zinc-oxide. Also, Lina et al. (2009) reported that the slaughter percentage of the broiler with nano zinc oxide at the level of 40mg/kg was extremely higher than the control group ($P \leq 0.01$) at 42 days.

The intestinal mucosal barrier is essential in maintaining health and preventing tissue injury and several diseases, and in ensuring adequate provision of dietary nutrients to the whole body. Dietary replacement with lower levels of organic (polysaccharide zinc complex) or nano particles

increase jejunum villi length which indicate improvement of absorptive capacity of different nutrients and improved feed efficiency. The present data are in harmony with those obtained by Geyra et al. (2001) indicated that zinc repaired intestinal injury by reducing the apoptotic index of ileal epithelial cells, enhancing villus height and the villus-height/crypt- crypt development is essential to increase cell renewal rate and maturation in the gut. The increase in crypt depth of chicken supplemented with different levels of nano zinc/kg might provide more surface area for nutrient absorption by increasing enterocyte proliferation and intestinal mucin secretion because mucin-producing goblet cells are present mainly in the crypts (Tsirtsikos et al., 2012). The villous height/crypt ratio most widely used as a marker of mucosal integrity and intestinal function (Clarke et al., 2006; Lamb-Rosteski et al., 2008). An improvement of villous length/crypt depth ratio was observed after dietary replacement of inorganic zinc oxide by different levels of organic or 30mg/kg of nano zinc in the present study, and this may indicate an improvement of mucosal barrier functional capacity.

Conclusion:

The obtained data indicated that dietary replacement of inorganic zinc oxide by 45mg of polysaccharide zinc complex or 30mg of zinc nano particles/kg diet improve broiler chicken growth performance and feed efficiency parameters. Moreover, organic or nano zinc using instead of inorganic zinc in broiler chicks diet improve blood serum minerals levels, immune response and intestinal health. It would be of further interest to investigate what is the absorption mechanism and metabolic pathways of Nano-Zn.

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