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The effect of dietary zinc oxide nanoparticles on liver enzymes activity and some of blood parameters from hatch to 14 days of broiler age (1 to 14 days of age)

BY:

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This research was carried out to investigate the effect of different levels of **zinc oxide nanoparticles on:**

1) **liver enzymes activity (TAC, SOD, Gpx) as** Antioxidants indicators
2) **Some of blood parameters**

Introduction

Growth rate, feed efficiency, and carcass traits are factors that determine the performance of a broiler flock
(Lesson and Summers, 2005)

Several nutritional factors influence those parameters, including Zn and Se trace mineral status. These minerals participate in many metabolic pathways influencing nutrient metabolism, growth, immune response, and skin quality
(McDowell, L. R. 2003)

Zinc participates as a cofactor or component of more than 250 enzymes, being important for protein and carbohydrate metabolism, growth, and reproduction.
(Burrell et al, 2004).

Zinc function in the body of live organism

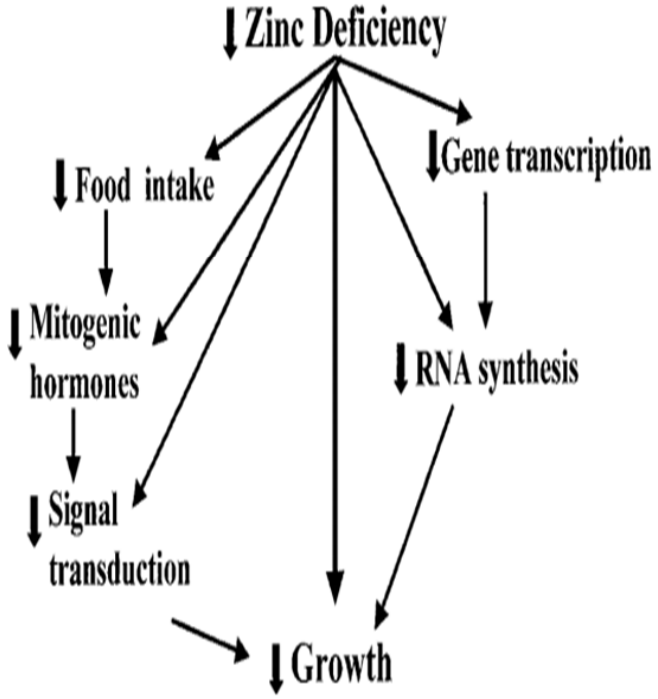
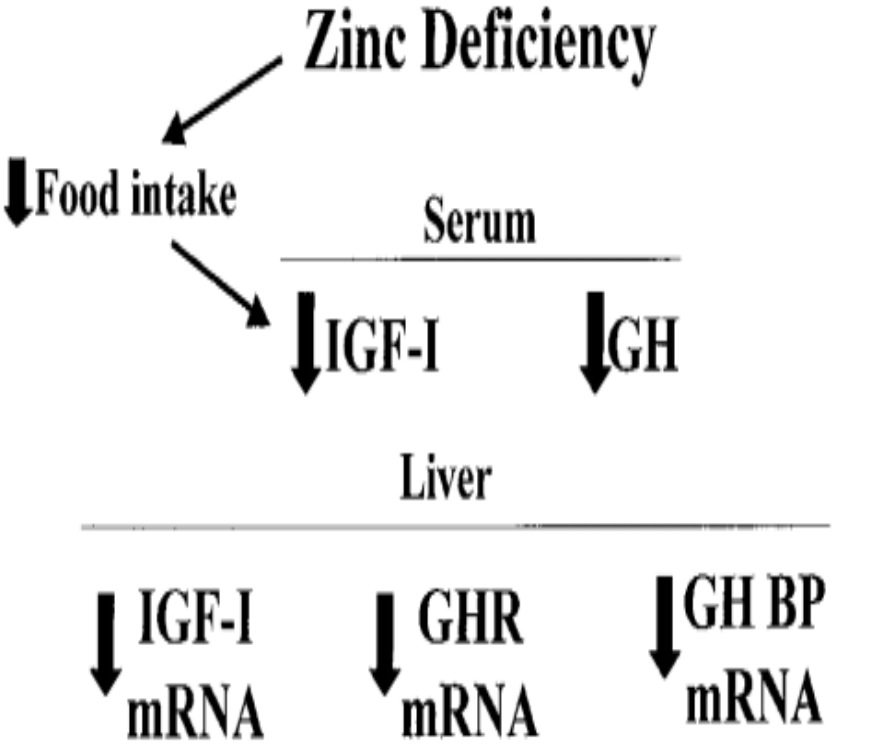


Figure 1

(Prasad and Kucuk, 2002).



insulin-like growth factor-I (IGF-I)

GHR, growth hormone receptor.

GHBP, growth hormone binding protein

Figure 2

Table 1. *Concentration of Zn in body fluids and tissues of fast growing pigs*[a]

Item	Concentration
Blood	
Plasma (μg/l)	740
Serum (μg/l)	600
Erythrocytes (μg/g packed cells)	7·7
Leucocytes[b]	21·5
Tissues[b]	
Bone	113
Brain	70
Heart	96
Kidney	141
Liver	151
Red muscle	137
Mixed muscle	89
White muscle	67
Pancreas	161
Spleen	107
Integuments[b]	
Hair	201
Skin	28

[a] Data compiled from Hoekstra *et al.* (1956, 1967), Cassens *et al.* (1967), Miller *et al.* (1968), Crofton *et al.* (1983) and Zhou *et al.* (1994).

[b] Data of leucocytes, tissues and integuments are expressed as p.p.m. on a DM basis.

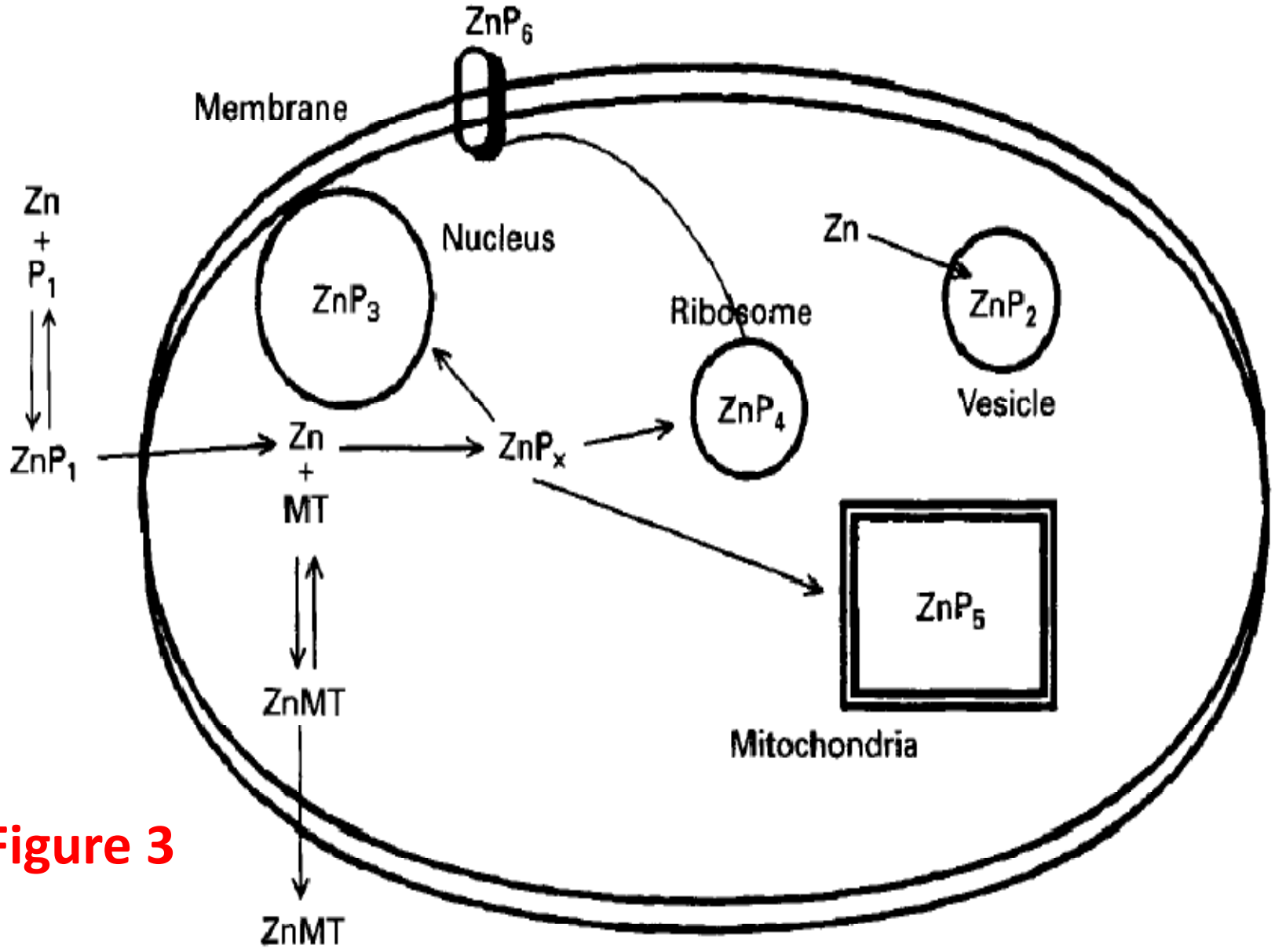


Figure 3

MacDowell, 2006; Park et al, 2004

Nanotechnology and Nanoscales

Recently, nanotechnology and related products had rapid progress in different scientific areas; in fact this branch of science had fundamental affected on the all parts of human life, animal, environment, and industrial life.

(Wijnhoven et al, 2009).

A) Nanotechnology has the potential to provide the tools and the research to change the future of food and feed technology.

(Mongillo, 2007)

B) By Applying the principles of nanotechnology, researchers can produce more nutritious food/feed; improve food and feed packaging.

(Mongillo, 2007)

C) Increasing monitor food or feed safety and the health of food and livestock products (due to produce and developing special biosensors.

(Mongillo, 2007)

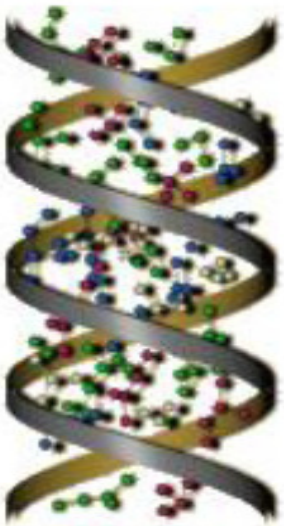
To now, material nanoscales that used in animal production are:

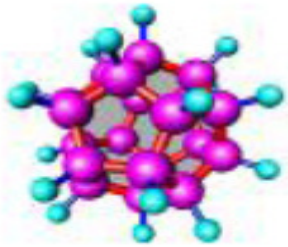
Silver nanoparticles (as antimicrobial),
nano-selenium , cupper oxide and **zinc oxide nanoparticles** **(as nano nutrients)**

The mention nanomaterials **(especially Se, CuO and ZnO)** interested extend attention because nano-formulation particulates exhibit novel distinguishing quality such as:

size, **shape**, **large surface area**, **high surface activity**, **high catalytic efficiency**, **S/V ratio** and **strong adsorbing ability.** **(Li *et al*, 2010)**

Cell





Dimensions
100 μm
Plant, Animal Cell
10 μm
Bacteria
1 μm
100 nm
Virus
Protein
10 nm
Simple Molecules
DNA "turn"
1 nm
DNA "base"
Atoms
0.1 nm

Materials and Methods

❑ Location research:

❖ This research was carried out in the poultry farm of, Kurdistan Azad University, Sanandaj Branch, Iran.

❑ Design and Statistical Population:

❖ A total of 300 one-day male broiler chickens (Ross-308) provided from (Warock Hatchery Co., Kurdistan state, Sanandaj city, Iran), Initial weight mean (44±0.2g).

❖ Broilers were distributed in a CRD including:
fives treatments, four replicates and 15 birds in each pens.

❑ **Condition production,** *Ad libitum*, ventilation, lighting system and…

Experimental diets (or Experimental treatments) were:

1) **Control group; fed on basal diet only that inclusion of : 36.12 mg zinc (ZnO, purity %72). (basal diet formulated as NRC)**

2) **Basal diet plus 30 mg zinc oxide nanoparticles**

3) **Basal diet plus 60 mg zinc oxide nanoparticules**

4)**Basal diet plus 90 mg zinc oxide nanoparticules**

5)**Basal diet plus 120 mg zinc oxide nanoparticules**

Requirent of boiler to the zinc during 24 hour is 40 mg per kg of diet

(NRC, 1994)

Fig 3. Zinc oxide nanoparticles powder (provided from nanomaterial Co. Huston, TX, USA. Purity> 99.99%,

Table 2. Ingredients and composition of basal diet

Ingredient (%)	Pre starter stage (1-14 d)
Corn grain	57.25
Soybean meal (%44)	35.30
Soybean oil	2.20
Dicalcium phosphate (DCP)	1.73
DL- methionine	1.63
Limestone	1.09
Common salt	0.25
Premix mineral[2]	0.25
Premix vitamin[2]	0.25
L-Lysine Hcl	0.05
Total	100
Nutrient composition	
ME (kcal/kg)	3127
Crude Protein (%)	22.16
Methionine (%)	0.49
TSAA (%)	0.91
Lys (%)	1.17
Arginine	1.29
Calcium (%)	0.92
Nonphytate phosphorus	0.45

[1]Supplied the following per kilogram of diet: 11,025 IU of vitamin A; 3,528 IU of vitamin D3; 33 IU of vitamin E; 0.91 mg of vitamin K; 2 mg of thiamin; 8 mg of riboflavin; 55 mg of niacin; 18 mg of Ca pantothenate; 5 mg of vitamin B6; 0.221 mg of biotin; 1 mg of folic acid; 478 mg of choline; 28 µg of vitamin B12; 40 mg of iron; 64 mg of manganese; 10 mg of copper; 2 mg of iodine; and 0.3 mg of selenium.

Zinc= 36.12 by analysis (Absorption Atomic spectrophotometer method)

Data collection

- At the end of trial, 4 birds per treatment (one bird per pen) as randomly selected. The blood samples collected from the brachial vein and divided two portions with and without EDTA.

- Serum removed by using centrifuge (3000×*g*,15min, 4°C) and then stored in -20 °C until analysis time.

- The blood parameters were measured using standard kits (Sigma Chemical Co., St. Louis, MO 63178-9916).

The sampling liver and measuring different enzymes activity

Total antioxidant capacity was measured by the ferric reducing ability of plasma method. This method is based on the ability of plasma to reduce Fe^{+3} to Fe^{+2} in the presence of TPTZ. The reaction of Fe^{+2} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (Benzie and Strain, 1996).

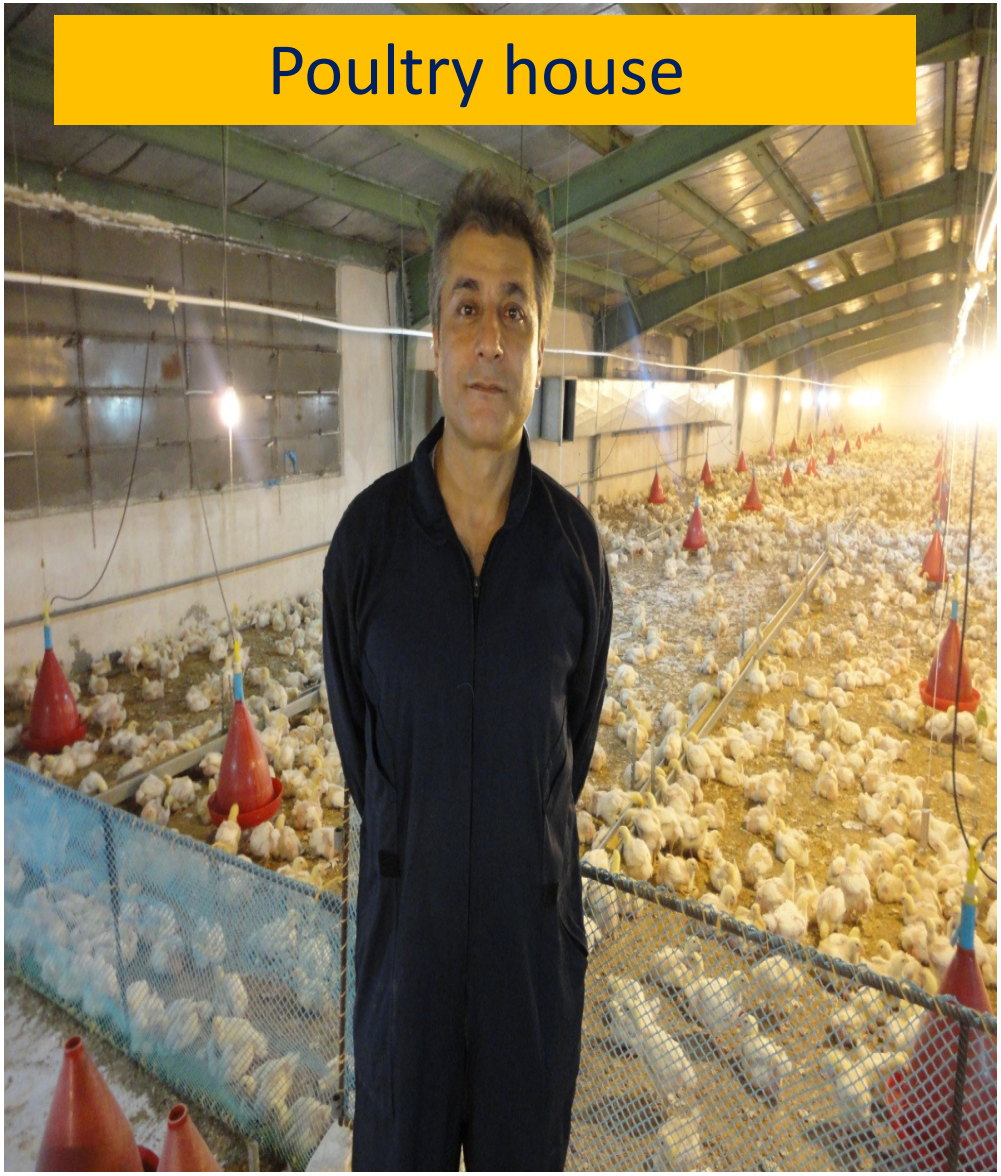
To measure of SOD activity used a commercial kit (**Ransod kit, Pars Azmon, Tehran, IRAN**). Measurement of the enzyme was expressed on the production of superoxide radicals resulted by xanthine and xanthine oxidase and then reacted with 2-(4-iodophenyl)-3-(4-nitrofenol) 5-phenyltetrazolium chloride (INT) to form a red color, and then was read at 505 nm.

Based on definition, one unit of SOD activity clarifies equal with the amount of enzyme necessary to produce 50 percent inhibition in the INT reduction rate.

The amount of GPx activity was determined by using a commercially kit provided by the mention company by measuring the rate of oxidation of nicotinamide adenine dinuclotide phos phate (NADPH) at 340 nm.

A unit of enzyme was determined as the amount of enzyme that needs to oxidize 1 nmol of NADPH oxidase per minute.

Poultry house



Experimental pens in the section of poultry house

Processing of Samples and data Collection

1) Birds selected

On day 14 study (Research was lasted 21 days), from any treatment 4 birds selected as randomly.

2) Sampling blood

3) Centrifuging and Serum Collection

Statistical analysis

Experimental data analyzed by one-way ANOVA using the SAS [Copyright (c) 1999–2001 by SAS Institute, Cary, NC, USA] program. The results were expressed as the mean values and standard errors. Also, during the differences were significant, Duncan's multiple range tests were performed.

Mean values we reconsidered significantly different at $P<0.05$. The model of experimental was used:

$$Y_{ij}=\mu+T_i+\varepsilon_{ij}$$ **Where**

Y = amount of single observation for any parameters

μ = the general mean;

T = The effect of different levels of ZnO-NPs and

ε = Experimental Error.

Results And Discussion

A) Liver enzymes activity

Table 2. Effect of different levels of dietary Zno-NPs on the liver enzymes activity during prestarter (1-14 d)

Enzymes activity	Experimental diets (Different levels of ZnO-NPs) [1]						
	T1. control (Basal diet)	T2. 30 mg	**T3. 60 mg**	**T4. 90 mg**	**T5. 120 mg**	SEM	P-value
TAC[1] (nmol/mg of protein)	0.132 b	0.158 a	**0.157 a**	**0.155 a**	**0.118 c**	0.02	0.0329
SOD (IU/mg of protein)	8.51b	8.67 b	**9.13 a**	**9.18 a**	**7.09 c**	1.07	0.0351
Gpx (IU/mg of protein))	287.6 **b**	284.1 **b**	**320.11 a**	**314.5 a**	**228.7 c**	1.02	0.0485

a-c Means in a row with different superscripts are significant (P <0.05).

[1] Each value represents the mean of 4 pens with 15 chicks per pen.

[2] Total Antioxidants Capacity;

B) Serum enzymes activity

Table 2. Effect of different levels of dietary Zno-NPs on serum enzymes activity of at 14 d.

Serum Enzymes Activity	Experimental diets (Different levels of ZnO-NPs) [1]						
	T1. Control	T2. 30 mg	T3. 60 mg	T4. 90 mg	T5. 120 mg	SEM	P-value
LDH (IU/L)	**1527.3 a**	**1526.5 a**	**1512.2 b**	**1509.6 b**	**1518.1 a**	**89.28**	**0.0192**
MDA[2] (nmol/L)	**4.25 c**	**4.12 b**	**4.17 b**	**4.51 b**	**4.97 a**	**1.02**	**0.0485**
ALP (IU/L)	44.5	43.6	40.25	39.8	46.3	6.041	0.1451
AST (IU/L)	89.3	89.2	80.1	81.6	91.3	2.084	0.3053
ALT (IU/L)	121.2	123.2	128.3	134.2	144.2	17.050	0.2458

Decreasing trend →

a-c Means in a row with different superscripts are significant (P <0.05).

[1] Each value represents the mean of 4 pens with 15 chicks per pen.

[2] Malondialdehyde

Table 4. Effects of silver nano-particle on blood parameters at 42 d of broiler age.

Blood parameters	Treatment						
	T1. Control	T2. 30 ppm	T3. 60 ppm	T4. 90 ppm	T5. 120 ppm	SEM	p-value
Total Protein (mg/dl)	3.69	3.76	3.91	2.97	3.52	0.013	0.2936
Albumin (mg/dl)	1.62^{b}	1.66^{b}	1.79^{a}	1.78^{a}	1.64^{b}	0.046	0.0371
Globulin (mg/dl)	2.07^{b}	2.09^{a}	2.17^{a}	2.19^{a}	1.94^{b}	0.070	0.0451

$^{a\text{-}c}$ Values in the same row not a common superscript differ significantly ($P < 0.05$).

D) Relative weight of immune organs

Table 3. Effect of different levels of dietary Zno-NPs on on relative immune organ weight in broiler chickens during prestarter (1-14d)

	Experimental treatments (Diets)						P-value	
Item, (%LBW)	Control (BD)	30 ppm	60 ppm	90 ppm	120 ppm	S.E.M	Linear	Quadratic
Spleen	0.06	0.09	0.09	0.10	0.02	0.02	0.182	0.712
Bursa of Fabricius	0.05**b**	0.06**b**	0.09**a**	0.09a	0.05**b**	0.02b	0.0318	0.0462

[a,b] Means within the same row with no common superscripts differ significantly.

Conclusion

1) Results present research indicated that inclusion of zinc oxide nanoparticles could be improvement antioxidant state and this condition may be increasing growth performance.

2) The activity enzymes in liver and serum of birds that fed diet supplementation with 60 and /or 90 mg ZnO-NPs were higher than control and other treatment.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