Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (2): 183-190, 2023
DOI: 10.9775/kvfd.2022.28922

RESEARCH ARTICLE

Promotives of Nano-Zinc Oxide as an Immune Stimulant in the Treatment of Lambs Suffering from Zinc Deficiency

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Article ID: KVFD-2023-28922 Received: 16.12.2022 Accepted: 04.03.2023 Published Online: 15.03.2023

Abstract: Zinc nanoparticles have a positive effect in enhancing growth and improving immunity, so this work aimed to investigate the role of Nano-zinc oxide as an immune stimulant and treatment of zinc deficient lambs in comparison with zinc in ordinary size. Thirty lambs were used and divided into three groups, the 1st group (N=10) control healthy group, the 2nd group (N=10) lambs suffered from zinc deficiency and treated with Nano-zinc oxide (nZnO), the 3rd group (N=10) lambs suffered from zinc deficiency treated with zinc oxide (ZnO) ordinary size. Serum samples were collected at zero-day (0-day), 7th, and 15th days of experiment. The serum analysis results of the zinc-deficient groups at 0-days revealed hypozincaemia, hypovitaminosis A and E, hypoproteinemia, hypoalbuminemia, hypoglobulinemia, decreased antioxidant (SOD, TAC, CAT, GSH) and immunoglobulin (γ2, γ1, β2, β1), while increased MDA, α2, and α1 compared to control healthy group. Most of parameters revealed rapid recovery from the 7th day of experiment in the nZnO group rather than the ZnO group. Some parameters of SOD, total protein, globulin, γ2, γ1, α2, and α1 appeared on the 7th day of experiment of the nZnO group significantly increased compared to the control healthy group, which revealed an improvement in the immune response. In conclusion; nZnO induced rapid recovery, and improved both immunity and antioxidants than zinc oxide ordinary sized.

Keywords: *Nano-zinc oxide, Zinc oxide, Vitamins, Antioxidants, Protein, Electrophoresis*

Bir Sinir Stimülatörü Kullanılarak Gerçekleştirilen Tavşan Brakiyal Pleksus Blokajında QX-314 ve Lidokainin Birlikte Uygulanması

Öz: Çinko nanopartikülleri büyümeyi arttırmada ve bağışıklığı geliştirmede olumlu bir etkiye sahiptir, bu nedenle bu çalışma Nano-çinko oksidin bağışıklık uyarıcı olarak rolünü ve normal seviyedeki çinko ile karşılaştırıldığında çinko eksikliği olan kuzuların tedavisini araştırmayı amaçlamıştır. Çalışmada 30 kuzu kullanılmış ve kuzular; 1. grup (N=10) sağlıklı kontrol grubu, 2. grup (N=10) çinko eksikliği olan ve Nano-çinko oksit ile tedavi edilen grup (nZnO) ve 3. grup (N=10) çinko eksikliği olan ve normal dozajlı çinko oksit ile tedavi edilen grup (ZnO) olmak üzere 3 gruba ayrılmıştır. Serum örnekleri, deneyin 0. gün, 7. gün ve 15. günlerinde alınmıştır. Çinko eksikliği olan grupların 0. gün analiz sonuçlarına bakıldığında, kontrol grubu ile kıyaslandığında, serum örneklerinde hipozinkemi, hipovitaminoz A ve E, hipoproteinemi, hipoalbuminemi, hipoglobulinemi, antioksidan (SOD, TAC, CAT, GSH) ve immünoglobulin (γ2, γ1, β2, β1) değerlerinde azalma, MDA, α2 ve α1 değerlerinde ise artış tespit edilmiştir. Parametrelerin çoğu, ZnO grubundan ziyade nZnO grubunda deneyin 7. gününden itibaren hızlı bir iyileşme göstermiştir. Deneyin 7. gününde nZnO grubunun SOD, toplam protein, globulin, γ2, γ1, α2 ve α1 gibi bazı değerleri kontrol grubuna kıyasla önemli ölçüde artmış ve bu da bağışıklık yanıtında bir iyileşme olduğunu göstermiştir. Sonuç olarak; nZnO hızlı bir iyileşmeye neden olmuş ve normal dozajlı çinko okside kıyasla hem bağışıklığı hem de antioksidanları iyileştirmiştir.

Anahtar Sözcükler: *Nano-çinko oksit, Çinko oksit, Vitamin, Antioksidan, Protein, Elektroforez*

How to cite this article?

Mansour MK, Yousif HM, Rezk RAESA, El Mahdy AM: Promotives of nano-zinc oxide as an immune stimulant in the treatment of lambs suffering from zinc deficiency. *Kafkas Univ Vet Fak Derg,* 29 (2): 183-190, 2023.
DOI: 10.9775/kvfd.2022.28922

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Introduction

Zinc is one of the foremost essential trace elements needed for the metabolic activity of more than 300 co-enzymes and is crucial for the synthesis of protein, DNA, and immune system function [1]. Zinc has a vital role in impacting animal growth, reproduction and the immune system by affecting enzyme activity and protein gene expression [2]. Also, it is a crucial part of the body's antioxidant system, which can prevent cell membrane oxidation. Once zinc deficiency occurs, lipid oxidation increases, which leads to oxidative damage [3]. Zinc has an interaction with vitamin A, while absorption, metabolism, hepatic release, conversion, and tissue utilization of vitamin A may depend on zinc status. Zinc deficiency may lead to impair synthesis of the protein, hepatic synthesis of cellular retinol-binding protein (cRBP) and retinol-binding protein (RBP), and mobilization of vitamin A within cells and liver [4]. Zinc deficiency may lead to elevated serum malondialdehyde (MDA) and a decrease in total antioxidant capacity (TAC), catalase (CAT), reduced glutathione (GSH), and SOD activity in sheep [5,6].

Nano minerals technologies are widely utilized in wide-range of sectors as well as agriculture, livestock animals, and food systems [7]. Nano zinc oxide particles have better bioavailability, bigger specific surface area, higher surface activity, high catalytic potency, and stronger adsorbing ability [8], also because it is often easily carried up by the gastrointestinal tract (GIT) and utilized within the animal system and reach deeper tissues more efficiently than the larger sized particles [9]. Nano-ZnO is often prepared by various methods, like the traditional high-temperature solid-state methodology, chemical precipitation, sol-gel synthesis, and hydrothermal methodology [10]. Nano-ZnO have enhanced responses once fed to livestock conventional diet improving growth, immunity, reproduction and feed efficiency [11].

The research aimed to investigate the efficacy of Nano-zinc oxide as an immune stimulant and anti-oxidative activator during the treatment of zinc deficient lambs.

Material and Methods

Ethical Statement

This protocol was approved by the Research Committee of the Animal Health Research Institute and authorized by The Institutional Animal Care and Use Committee (ARC-IACUC)/Agricultural Research Center (ARC/AH/22/22).

Experimental Design

This work carried out on a private sheep farm. Thirty fattening lambs aged 12-15 months were used and divided into three groups. Control apparently healthy group (N=10) 1st group. Twenty diseased lambs showing a clinical symptoms of zinc deficiency (wool eating and para-keratosis and confirmed by decreased serum zinc level), were divided into two groups according to treatment used; diseased lambs (N=10) were treated by zinc oxide nanoparticles prepared solution 10 mL/animal (contains 2 mg of Nano-zinc oxide) one dose orally/day for 7 days, (nZnO) 2nd group. Diseased lambs (N=10) were treated by zinc oxide powder 20 mg/kg DM mixed ration daily for 7 days, (ZnO) 3rd group.

Sampling

Blood samples were collected from the jugular vein of all animals at the time of disease detected and the beginning of treatment (0-day), at the end of treatment (7th day of the experiment) and after a week from the end of treatment (15th day of the experiment). Serum samples were obtained by centrifuging the blood samples at 5000 rpm for 5 minutes. Clear sera were transferred into clean dry Eppendorf tubes and stored at -20°C till biochemical analysis.

Nano-Zinc Oxide (nZnO) Preparation

Nano-zinc oxide solution was prepared according to Wang et al.[10]. Zinc oxide (ZnO) nanoparticles were characterized by TEM, XPS, XRD, and UV-visible spectrophotometry. The morphological characteristics of nZnO were investigated by a scanning electron microscope (SEM) and a transmission electron microscope (TEM). X-ray photoelectron spectroscopy (XPS) was used to identify the chemical bonding states of the Zn and O. The UV spectrum of the ZnO nanoparticles was recorded with a UV-visible spectrophotometer, and the max excitation wavelength was 325 nm. The solution contains Nano-zinc oxide (2 mg/10 mL).

Serum Biochemical Analysis

Zinc levels were detected by atomic absorption Spectrophotometer according to the method described by Maret and Henkin [12]. Special kits were used for calcium (Ca) and inorganic phosphorus (P) detection by the method described by Roberts et al.[13]. Special kits (Bio diagnostic Company) CAT. No. SD2521 was used for Superoxide dismutase (SOD) activity estimation according to Nishikimi et al.[14] and CAT. No. TA2513 was used for total antioxidant capacity (TAC) estimation according to Koracevic et al.[15], reduced glutathione (GSH) level was detected by a method of Pleban et al.[16], catalase (CAT) activity was detected by a method of Aebi [17] and malondialdehyde (MDA) was detected calorimetrically according to Lahouel et al.[18].

Total Protein and Electrophoretic Protein Estimation

The serum total protein and electrophoretic pattern were

estimated according to Sonnenwirth et al.[19] and Davis [20], respectively and calculated according to SynGene S. No. 17292*14518 sme*mpcs.

Serum Vitamins Examination

Determination of Retinol (vitamin A) and α-tocopherol (vitamin E) concentrations in serum samples were performed by High-Performance Liquid Chromatography (HPLC).

Chemicals and Reagents

Retinol, α-tocopherol, and 2, 6-di-tert-butyl-4-methyl phenol (BHT) were provided by Sigma-Aldrich. The grade Methanol and hexane of HPLC were obtained from Fisher Scientific. HPLC-grade Ethanol was obtained from Carlo Erba and the Purified deionized water was prepared using a Milli-Q system.

Chromatographic Conditions

HPLC system (Agilent 1200 series, Software - Agilent Chemistation Version B.040.01) SP1 (Agilent Technologies, Germany), with a pump, degasser, autosampler, DAD detector and Chromatographic column - Agilent C18, 100A (4.6 x 250 mm, 5 µm) as the stationary phase was used. The chromatographic condition was set according to Bystrowska et al.[21].

Stock, and intermediate standard solutions of retinol and α-tocopherol were prepared according to Bystrowska et al.[21]. The serum calibration curve was created by spiking blank lamb serum with varying intermediate standard solution volumes at concentrations ranging from 0.1 to 100 µg/mL. Three different levels of quality control (QC) samples were prepared in blank lamb's serum and were used for achieving the method validation requirements.

Extraction Procedures

Samples prepared for extraction were performed by a liquid-liquid extraction technique according to the procedure described by Siluk et al.[22] which was a modified version of an earlier reported procedure by Aebischer et al.[23].

This method was validated according to USP [24] via the determination of method precision, recovery, linearity, the limit of detection, and quantification.

The HPLC method was accurate with high recovery (95-99%) of good linearity ($r^2 \geq 0.999$) with a low LOD and LOQ; as LOD were 0.29 µg and 2.0 µg and LOQ were 0.87 µg and 6.1 µg for retinol (Vit. A) and α-tocopherol (Vit. E), respectively. Specificity and selectivity were illustrated with chromatogram of retinol (Vit. A), at 22.28 min retention time *(Fig. 1)* and chromatogram of α-tocopherol (Vit. E), at 12.29 min retention time *(Fig. 2)*.

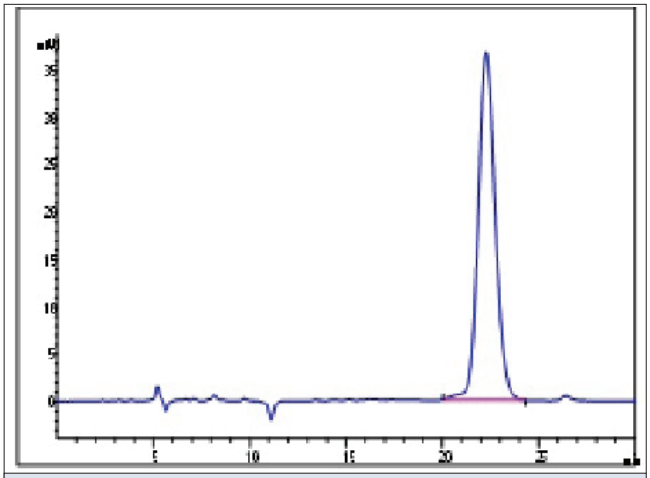

Fig 1. Chromatogram of Retinol (Vit. A), at 22.28 min retention time

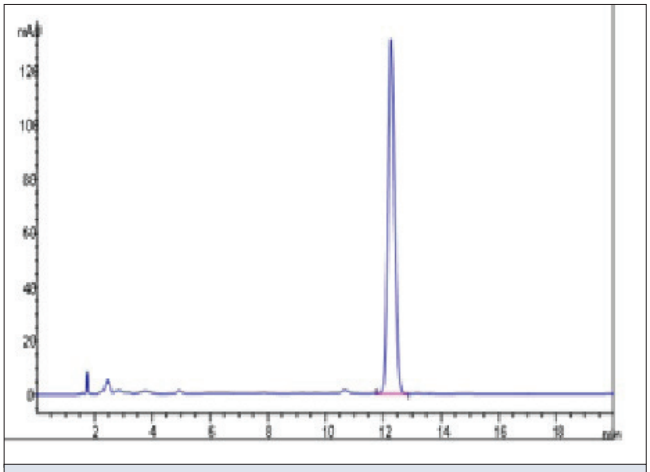

Fig 2. Chromatogram of α-tocopherol (vit. E), at 12.29 min retention time

Statistical Analysis

The data were statistically analyzed using two-way (ANOVA) by SPSS 22 software to test both the treatment and times. The results were demonstrated as means ± SE. The results were considered statistically significant at $P<0.05$.

Results

The results of serum minerals and vitamins analysis *(Table 1)* of the diseased groups on the day of detected zinc deficiency (0-day) revealed a significant ($P<0.05$) decrease in zinc levels and non-significant changes in calcium and phosphorus levels compared to the control healthy group. Treatment by Nano-zinc oxide (nZnO) revealed a significant ($P<0.05$) increase on the 7th day of experiment compared to the group treated by zinc oxide in ordinary size (ZnO) but non-significant with the control healthy group.

The serum vitamins analysis results *(Table 1)* revealed a significant decrease ($P<0.05$) in vitamins A and E at 0-day in diseased groups compared to the control healthy group. After treatment by (nZnO), there was a

Table 1. *The results of serum levels of minerals, vitamins, and anti-oxidants in the different experimental groups*

Parameters	Time	Groups		
		Control Healthy Group	Group Treated with nZnO	Group Treated with ZnO
Zinc (µg/dL)	0- day	105.98±9.33 ^{a,1}	75.83±9.62 ^{b,1}	76.49±9.33 ^{b,1}
	7th day	107.35±8.71 ^{a,b,1}	109.44±9.43 ^{a,2}	94.54±9.86 ^{b,2}
	15th day	106.57±10.16 ^{a,1}	108.47±10.24 ^{a,2}	103.75±8.46 ^{a,2}
Calcium (mg/dL)	0- day	8.774±0.466	9.294±0.612	9.292±0.403
	7th day	8.644±0.395	8.776±0.336	8.788±0.346
	15th day	8.74±0.34	8.704±0.278	8.732±0.33
Phosphorus (mg/dL)	0- day	4.868±0.325	4.69±0.3	4.67±0.34
	7th day	4.888±0.339	4.794±0.306	4.716±0.348
	15th day	4.854±0.303	4.824±0.261	4.794±0.276
Vitamin A (µg/mL)	0- day	57.6±4.04 ^{a,1}	3.61±1.08 ^{b,1}	3.82±1.04 ^{b,1}
	7th day	60±6.04 ^{a,1}	35.26±4.09 ^{b,2}	26.6±2.82 ^{c,2}
	15th day	59.6±5.55 ^{a,1}	60.8±4.55 ^{a,3}	51.52±3.04 ^{b,3}
Vitamin E (µg/mL)	0- day	52.6±4.67 ^{a,1}	2.016±0.849 ^{b,1}	2.064±0.748 ^{b,1}
	7th day	54±4.74 ^{a,1}	41.78±2.39 ^{b,2}	36.76±2.46 ^{c,2}
	15th day	53.4±4.72 ^{a,1}	54.2±3.27 ^{a,3}	47.8±3.35 ^{b,3}
SOD (U/mL)	0- day	35.314±4.359 ^{a,1}	21.918±4.319 ^{b,1}	22.894±3.785 ^{b,1}
	7th day	35.682±4.476 ^{b,1}	42.43±4.36 ^{a,2}	40.704±4.768 ^{a,b,2}
	15th day	36.704±4.556 ^{b,1}	45.204±4.222 ^{a,2}	41.908±4.312 ^{a,b,2}
TAC (mU/L)	0- day	1.778±0.08 ^{a,1}	1.058±0.171 ^{b,1}	1.104±0.123 ^{b,1}
	7th day	1.788±0.099 ^{a,1}	1.652±0.106 ^{a,2}	1.37±0.136 ^{b,2}
	15th day	1.784±0.116 ^{a,1}	1.802±0.084 ^{a,2}	1.618±0.121 ^{b,3}
CAT (U/mL)	0- day	16.97±0.98 ^{a,1}	12.664±1.193 ^{b,1}	12.762±1.048 ^{b,1}
	7th day	16.89±0.99 ^{a,1}	16.862±1.027 ^{a,2}	16.024±1.021 ^{a,2}
	15th day	17.044±0.944 ^{a,1}	17.036±1.121 ^{a,2}	16.884±1.069 ^{a,2}
GSH (U/mL)	0- day	5.302±0.473 ^{a,1}	3.746±0.569 ^{b,1}	3.764±0.565 ^{b,1}
	7th day	5.33±0.48 ^{a,1}	5.246±0.585 ^{a,2}	4.762±0.832 ^{a,2}
	15th day	5.352±0.465 ^{a,1}	5.362±0.589 ^{a,2}	5.23±0.7 ^{a,2}
MDA (nmol/mL)	0- day	1.764±0.159 ^{b,1}	2.228±0.185 ^{a,1}	2.208±0.193 ^{a,1}
	7th day	1.794±0.152 ^{a,1}	1.818±0.143 ^{a,2}	1.982±0.122 ^{a,2}
	15th day	1.784±0.162 ^{a,1}	1.766±0.158 ^{a,2}	1.796±0.132 ^{a,2}

Data are expressed as mean ± SE of 10 samples
$^{a, b, c}$ *Superscript letters: Mean significance difference between groups in the same time on P<0.05*
$^{1, 2, 3}$ *Superscript numbers: Mean significance difference among times of treatment in the same group on P<0.05*

gradually significant (P<0.05) increase in both vitamin A and E until become non-significant on the 15th day of experiment compared to the control healthy group and significantly (P<0.05) increased compared to (ZnO) group throughout treatment. The (ZnO) group revealed a gradually significant (P<0.05) increase in vitamin A and vitamin E levels but still significantly (P<0.05) decreased compared to the control healthy group.

The results of serum antioxidant *(Table 1)* of the diseased groups at 0-day revealed a significant (P<0.05) decrease in SOD, TAC, CAT, and GSH with a significant (P<0.05) increase in MDA in comparison with the control healthy group. After treatment with (nZnO), there was a significant (P<0.05) increase in SOD level on the 7th and 15th day compared to the control healthy group, at the same time non-significant in comparison with (ZnO) group, on the contrary, there was a non-significant change between (ZnO) group and control healthy at 7th and 15 days of experiment. Only the TAC level of (ZnO) group on the 15th day revealed a significant (P<0.05) decrease compared to either (nZnO) group or the control healthy

Table 2. *The results of serum levels of patterns of protein electrophoresis fractions and sub-fractions (g/dL) in the different experimental*

Parameters	Time	Groups		
		Control Healthy Group	Group Treated with nZnO	Group Treated with ZnO
Total Protein (g/dL)	0- day	5.18±0.31 ^{a,1}	3.458±0.255 ^{b,1}	3.44±0.22 ^{b,1}
	7th day	5.26±0.43 ^{b,1}	5.742±0.398 ^{a,2}	4.944±0.313 ^{b,2}
	15th day	5.39±0.31 ^{a,1}	5.428±0.324 ^{a,2}	5.136±0.216 ^{a,2}
Albumin (g/dL)	0- day	1.496±0.091 ^{a,1}	1.062±0.078 ^{b,1}	1.057±0.067 ^{b,1}
	7th day	1.52±0.12 ^{a,1}	1.576±0.109 ^{a,2}	1.224±0.078 ^{b,2}
	15th day	1.558±0.09 ^{a,1}	1.503±0.09 ^{a,2}	1.46±0.062 ^{a,3}
Globulin (g/dL)	0- day	3.68±0.22 ^{a,1}	2.396±0.177 ^{b,1}	2.383±0.151 ^{b,1}
	7th day	3.74±0.305 ^{b,1}	4.223±0.292 ^{a,2}	3.72±0.236 ^{b,2}
	15th day	3.83±0.22 ^{a,1}	3.925±0.234 ^{a,2}	3.676±0.155 ^{a,2}
Gamma 2 (γ2) (g/dL)	0- day	0.49±0.03 ^{a,1}	0.192±0.014 ^{b,1}	0.191±0.012 ^{b,1}
	7th day	0.495±0.04 ^{b,1}	0.54±0.04 ^{a,2}	0.46±0.03 ^{b,2}
	15th day	0.508±0.029 ^{a,1}	0.503±0.03 ^{a,2}	0.44±0.02 ^{b,2}
Gamma 1 (γ1) (g/dL)	0- day	1.362±0.082 ^{a,1}	0.541 ±0.04 ^{b,1}	0.538±0.034 ^{b,1}
	7th day	1.384±0113 ^{b,1}	1.639±0.113 ^{a,2}	1.326±0.084 ^{b,2}
	15th day	1.419±0.082 ^{b,1}	1.562±0.093 ^{a,2}	1.42±0.06 ^{b,2}
Beta 2 (β2) (g/dL)	0- day	0.682±0.041 ^{a,1}	0.358±0.026 ^{b,1}	0.356±0.023 ^{b,1}
	7th day	0.693±0.057 ^{a,1}	0.681±0.047 ^{a,2}	0.689±0.044 ^{a,2}
	15th day	0.71±0.04 ^{a,1}	0.674±0.04 ^{a,b,2}	0.648±0.027 ^{b,2}
Beta 1 (β1) (g/dL)	0- day	0.618±0.037 ^{a,1}	0.49±0.036 ^{b,1}	0.487±0.031 ^{b,1}
	7th day	0.628±0.051 ^{a,1}	0.616±0.043 ^{a,2}	0.498±0.032 ^{b,1}
	15th day	0.644±0.037 ^{a,1}	0.63±0.038 ^{a,2}	0.401±0.017 ^{b,2}
Alpha 2 (α2) (g/dL)	0- day	0.285±0.017 ^{b,1}	0.486±0.036 ^{a,1}	0.483±0.031 ^{a,1}
	7th day	0.29±0.024 ^{b,1}	0.394±0.027 ^{a,2}	0.409±0.026 ^{a,2}
	15th day	0.297±0.017 ^{b,1}	0.291±0.017 ^{b,3}	0.409±0.017 ^{a,2}
Alpha 1 (α1) (g/dL)	0- day	0.245±0.015 ^{b,1}	0.329±0.024 ^{a,1}	0.327±0.021 ^{a,1}
	7th day	0.249±0.02 ^{c,1}	0.296±0.021 ^{b,2}	0.334±0.021 ^{a,1}
	15th day	0.255±0.015 ^{b,1}	0.265±0.016 ^{b,3}	0.351±0.015 ^{a,1}

Data are expressed as mean ± SE of 10 samples
$^{a, b, c}$ *Superscript letters: Mean significance difference between groups at the same time on P<0.05*
$^{1, 2, 3}$ *Superscript numbers: Mean significance difference among times of treatment in the same group on P<0.05*

group. The other antioxidants (CAT, GSH, and MDA) had non-significant changes in both groups of treatment compared to the control healthy group from the 7th day of experiment.

Total protein, albumin, and globulin analysis *(Table 2)* of the diseased groups at 0-day revealed a significant (P<0.05) decrease in total protein, albumin, and globulin compared to the control healthy group. After treatment with nZnO on the 7th day of experiment, there was a significant (P<0.05) increase in total protein compared to either (ZnO) group or the control healthy group, while there were non-significant changes between groups in the 15th day of experiment. There was a significant (P<0.05) increase in albumin level in the (nZnO) group on the 7th day compared to the (ZnO) group, but non-significant in comparison with the control healthy group. The albumin level in the (ZnO) group gradually increased until become non-significant in comparison with either (nZnO) group or the control healthy group on the 15th day of experiment. The globulin level revealed a significant (P<0.05) increase in the (nZnO) group on the 7th day of experiment compared to either the (ZnO) group or the control healthy group, while non-significant changes between groups in globulin level on the 15th day of experiment.

Serum protein electrophoresis results *(Table 2)* of the diseased groups at 0-day revealed a significant (P<0.05)

decrease in (γ2, γ1, β2, β1) and significant (P<0.05) increase in (α2, α1) compared to control healthy group. After treatment with nZnO, there was a significant (P<0.05) increase in γ2 on the 7th day of experiment compared to either the (ZnO) group or the control healthy group. On the other hand, there was a significant (P<0.05) increase on the 15th day of experiment compared to the (ZnO) group but non-significant compared to the control healthy group. There was a significant (P<0.05) increase in γ1 of the (nZnO) group on the 7th and 15th day compared to either the (ZnO) group or the control healthy group, at the same time there were non-significant changes between the (ZnO) group and control healthy group. There were non-significant changes in the β2 levels between groups on the 7th day of experiment but only there was a significant (P<0.05) decrease in the (ZnO) group on the 15th day of experiment compared to the control healthy group and non-significant changes compared to (nZnO) group. There was a significant (P<0.05) decrease in the β1 level of the (ZnO) group on the 7th and 15th day of experiment compared to either the (nZnO) group or the control healthy group. There was a significant (P<0.05) gradually decreased in α2 and α1 levels of the (nZnO) group toward non-significant compared to the control healthy group on the 15th day and at the same time revealed significant (P<0.05) decrease compared to the (ZnO) group.

Discussion

Reduced zinc concentration in lamb serum are commonly used as a biomarker for zinc deficiency [6,25]. This result may be attributed to a decreased zinc level in the ration and/or an elevated calcium level in the ration which decreases zinc absorption and metabolism [5,6,25]. The rapid recovery of serum zinc level in the (nZnO) group on the 7th day of the experiment rather than the (ZnO) group that recovered on the 15th day of the experiment referred to the greater bioavailability, bigger specific surface area, higher surface activity, and rapid adsorption ability of Nano-zinc oxide particles [8].

The serum vitamins A and E levels showed decreased levels in 0-day of diseased groups with respect to the healthy. This observation is in accordance with Serdar and Funda [26] in calves suffering from dermatophytosis and decreased zinc levels and Nguta [27] in lactating cow. As absorption, metabolism, hepatic release, conversion, and tissue utilization of vitamin A may depend in zinc status, so zinc deficiency may lead to impairing synthesis of the protein, hepatic synthesis of cRBP and RBP, and mobilization of vitamin A within cells and from the liver [4]. Zinc deficiency may inhibit lipid absorption and/or vitamin E (lipid soluble vitamin) [28]. The recovery of vitamins A and E on the 15th day of treatment in the nZnO group before the ZnO group may be referred to nano-zinc can be rapidly absorbed by the GIT and reached deeper tissues and be utilized in the animal system, more efficiently than the larger sized particles [9].

The serum antioxidant results of the diseased groups at the 0-day revealed decreased SOD, TAC, CAT, and GSH while increased MDA were agree with Song and Shen [5] and Yousif et al.[6]. These results may be attributed to zinc deficiency which increasing lipid oxidation, and may provide oxidative damage [3]. After treatment with either (nZnO) or (ZnO) groups, there was an increase in SOD level compared to the healthy group, as zinc has an important role in the stability of cell membranes and protein as it helps in balancing reactive oxygen species (ROS) production because its presence in superoxide dismutase (SOD) [29]. The result of the recovery of antioxidants in the (nZnO) group is in accordance with Song et al.[30] who recorded similar results after zinc supplementation in buffalo calves. These results may be attributed to the role of zinc is a crucial component of the body's antioxidant system [3]. The rapid recovery of antioxidants (TAC, CAT, GSH, and MDA) in the (nZnO) group may be attributed to the rapid effectiveness role of nano-zinc in improving the antioxidant system in ewes and lambs [31].

In the present work, the results of decreased total protein, albumin, and globulin were in agreement with Fouda et al.[32]. Langenmayer et al.[33] recorded a significant decrease in serum albumin in zinc-deficient calves. These decreases may be attributed to suppression in feed consumption, decreased appetite, and disruptions in protein synthesis in the liver [34]. The decreased globulin level is agreed with El Maghraby and Mahmoud [35] who recorded similar results in zinc-deficient neonatal calves. This result may be related to the reduction of gamma globulins (γ2, γ1) and beta globulins (β2, β1) levels, which may be attributed to B-prolymphocyte mitogenesis dysfunction. Also attributed to a decrease in the serum immunoglobulin concentration and antibody response to T-dependent antigen [36]. Since the role of zinc in inducing the B cells to secrete globulin, and improving the immune function of animal B cell and enhancing the immunoglobulin synthesis ability, so zinc deficiency will lead to disorder of the immunoglobulin secretion [37]. The results of increased total protein and globulin in the (nZnO) group than either the (ZnO) group or the control group, similar to results were recorded in ewes and lambs [31] and goats [38]. These results may be referred to the higher elevation of gamma globulins (γ2, γ1) and beta globulins (β2, β1) levels in the nZnO group, which may be attributed to the role of Nano-zinc oxide in improving growth and immunity in livestock [11]. Also, Nano zinc oxide proved to be better than traditional zinc (ZnO) for improving growth performance [39], as well as zinc oxide nanoparticles can

be rapidly absorbed by GIT and reached to deeper tissues and utilized in the animal system with more efficacy than the larger sized particles [9], also attributed to the greater bioavailability, bigger specific surface area, and higher surface activity of zinc oxide nanoparticles [8].

In conclusion; Zinc has a crucial role in the body's physiology and important trace element required for most of the body's enzymes, metabolic activities, and immune system functions. Zinc deficiency leads to impairment of the antioxidant system and biochemical changes that affect immune response. Nano-zinc oxide supplementation has a rapid and effective recovery more than zinc oxide in ordinary size, not only but also, zinc oxide in nanoparticles has a higher and strong effect on immunity and antioxidants.

Availability of Data and Materials

The data sets analyzed during the current study are available from the corresponding author H. M. Yousif on reasonable request.

Acknowledgment

The authors are grateful to Prof. Dr. Mahmoud, H. H. Chairman of the Laboratory of Elemental and Isotopic Analysis, Nuclear Research Centre, Atomic Energy Authority, Egypt, for his kind assistance and funding in the identification and characterization of the prepared and used zinc oxide nanoparticles.

Funding Support

There was no funding support.

Conflict of Interest

No potential conflict of interest was reported by the authors. Authors only are responsible for the content and writing of the paper.

Author Contributions

H. M. Yousif designed the research and collect the samples. M. K. Mansour, H. M. Yousif, R. A. A. Rezk and A.M. El Mahdy performed the experimental duties of this study and analyzed the data. M. K. Mansour and H. M. Yousif did the statistical analyses. All authors participate in writing and approved the final version of the manuscript.

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