Egypt. J. Vet. Sci. **Vol. 54,** No. 3, pp. 491-501 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Influence of Nano-zinc Oxide and Zinc Sulfate on Some Hematological Values and Liver Function in Broiler Under High Ambient Temperature

Falah S. Mahmood[1], Abd Al-Bar A. Al-Farha[1] and Hadeel M. Hameed[2]*

[1]*Animal Production Techniques, Technical Agricultural College, North Technical University, Mosul, Iraq.*

[2]*Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Iraq.*

Hadeel. M. Hameed, hadeel.mohammad@uomosul.edu.iq. ORCID no.: 0000-0002-4858-2391

Falah. S. Mahmood, falahalmawlaa@gmail.com ORCID no.: 0000-0003-3127-7806

Abd Al-Bar. A. Al-Farha , dr.abdalbar@nut.edu.iq. ORCID no.: 0000-1742-6350

THE present study was to estimate the influence of nano-zinc oxide and zinc sulfate on some hematological parameters, liver enzymes, glutathione and malondialdehyde. A total of 108 Rose 308 chicks (1d old) were randomly divided into six groups with three replicates per group (18 birds per group). Treatments included: group one (control with basal diet),group two diet supplemented with nano-zinc oxide 40 mg/kg , group three diet containing zinc sulfate 110 mg/kg , group four subjected to high ambient temperature 40± 2 up to 4 h /day , group five was exposed to high ambient temperature 40± 2 up to 4 h /day and were dietary supplemented with nano-zinc oxide 40 mg/kg , group six was exposed to high ambient temperature 40± 2 up to 4 h /day and were dietary supplemented with zinc sulfate 110 mg/kg. The results showed raise in RBCs and WBC count, Hb concentration, HCT, lymphocytes and heterophiles percentage and glutathione concentration accompanied with decrease in ALT, AST and malondialdehyde concentration in nano-zinc oxide and zinc sulfate compared with control and high ambient temperature. It concluded that nano-zinc oxide and zinc sulfate might improve negative effect of heat stress on some hematological values and liver function in boilers.

Keyword: Nano-zinc, RBC, ALT, AST, Glutathione.

Introduction

The poultry industry is well-known for making an important contribution to global nutrition and food safety by supplying low-cost protein, important nutrients, and energy to humans [1]. In many areas, elevated temperature is regarded to be among the most significant aspects causing stress in birds. As a result of global warming, high temperatures have appeared recently as one of the most substantial stressors influencing the poultry farming [1,3]. When chicks have been subject to elevated temperatures on a regular basis, it has a negative impact on their growth and immune system, and it can result in death, resulting in a significant economic loss in the livestock industry [4-6]. Zn is an essential nutrient for chickens [1] and serves three biological functions in the body: as a catalyst, regulator, and structural component [7]. Moreover, Zn is necessary because it functions as a cofactor in over 240 enzymes and aids in the metabolization of nutrients such as

*Corresponding author: Hadeel M. Hameed, E-mail: hadeel.mohammad@uomosul.edu.iq., Tel. 07724240820
(Received 05/02/2023, accepted 03/03/2023)
DOI: 10.21608/EJVS.2023.191733.1439

carbohydrates and proteins, thereby increasing growth and reproductive efficiency [8]. Zinc in broiler diets can be organic zinc (e.g., Zn protein, Zn amino acid, or Zn picolinate) or inorganic zinc (e.g., $ZnCl_2$, $ZnSO_4$, or ZnO) [9]. The National Research Council (NRC) [10] recommends a zinc level of 40 mg/kg in broiler diets, which can be supplemented with inorganic or organic forms. Poultry industry, on the other hand, is generally suboptimal in many countries, as evidenced by poor growth efficiency, suppression of the immune activity, respiratory disease severity, and a higher mortality rate [11], owing to the high ambient relative humidity and temperature in the areas. Zinc supplementation in the diet of heat-stressed broilers improved production performance while decreasing the feed conversion ratio (FCR) [12]. This suggests that zinc supplementation for broiler production may be more essential in order to diminish the unfavorable impact of such extreme temperatures. According to the previous study Zn significantly improves broiler immunity. It acts as a cofactor of thymulin, inducing proliferation and modulating cytokine release [13]. Sajadifar *et al*. [14] discovered that in broiler chicks, zinc acts as a nonpharmacologic immune booster. Zn works as an immunostimulant, boosting both the cellular and humoral immune systems [15]. Because many countries have imposed restrictions on the use of antibiotics in the diet, zinc's role as a stimulant of broiler immunity is required. However, the zinc doses required to improve broiler immune response vary between reports; some research suggests that the wanted rate is higher than the NRC recommendation [16], and zinc has the capability to boost antibody production. Trace minerals, such as Zn, are now commercially available in nanoparticle form, with the claim that they have higher bioavailability and potency than both inorganic and organic forms [17,18]. Because nano-zinc oxide (ZnO) is more bioavailable and bioactive than conventional ZnO, it can be used at lesser doses and give more benefits for both farm animals and the environment [19]. Several studies have found that nano-zinc outperforms conventional zinc sources in terms of broiler chicken product, antioxidant ability, and intestinal activity and function at the same or lesser doses [20]. Due to their tiny size and unique physical characteristics, trace minerals, such as Zn nanoparticles, can now be effectively used to meet the mineral requirements of birds [21, 22]. Nanomaterials have been shown to efficaciously supply minerals to birds while also increasing growth rate and feed efficiency [23-25]. Zinc oxide nanoparticles outperform conventional Zn sources and improve chicken performance and antioxidant defense [26]. The aim from this study was to investigate the effect of nano-zinc on broiler under heat stress and the ability of nano-zinc to overcome the undesirable effect of heat stress on some hematological value and liver enzymes.

Material and Methods

Birds, housing, and management

A total of 108 Rose 308 chicks (1d old) were obtain from a commercial hatchery and utilized in the current study. The chicks were all housed in the same experimental room, in an open-type

TABLE 1. Composition of starter and finisher ration.

Ingredients	Growth ration%	Production ration %
Maize	36	42
Wheat	22	22
Soy bean meal (24% protein)	35	30
Premix(40%protein)	5	4
Vegetable oil	1	1
Limestone	0.7	0.7
Salt	0.3	0.3
Total	100	100
Calculated Values		
Metabolizable energy (Kal/kg)	2821.8	2985.1
Crude protein%	24.270	21.998
Crude fiber%	3.975	3.650

hall and ground-based breeding was provided in which all the environmental conditions suitable for breeding were provided. As shown in table (1), a standard ration was developed to utilize the (NRC) [10] recommendations and water was provided *ad libitum* during the study period (1-35 days), birds were randomly assigned to six groups of (18 birds / group) with three replicates per group (6 birds / repeat). Chicks were assigned to one of the following treatments: (1) control with basal diet, (2) ration provided with nano-zinc oxide at 40 mg/kg [27] , (3) ration containing zinc sulfate at 110 mg/kg [28] , (4) subjected to high ambient temperature 40±2 up to 4 h /day (8:00-12:00), (5) was exposed to high ambient temperature 40± 2 up to 4 h /day (8:00-12:00) and were nutrient supplementation with nano-zinc oxide 40 mg/kg (6) was exposed to high ambient temperature 40± 2 up to 4 h /day (8:00-12:00) and were nutrient supplementation with zinc sulfate 110 mg/kg. ZnO nanoparticles (99%,10-30 nm: US3590, CAS#:1314-13-2).

Blood collection

On day 35, fresh blood samples were collected by cutting the jugular vein (6 birds/ group). The blood samples were placed in heparinized tube for the purpose of conducting blood tests including erythrocytes (RBCs), leukocytes (WBCs) leukocyte differential count (DLC), stress index (heterophils/lymphocytes), hemoglobin concentration (Hb) hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) according to Campbell [29]. To obtain serum using gel tubes that were placed in a centrifuge for 15 minutes at a rate (3000 rpm) and then the serum was isolated and preserved in Eppendorf tubes at temperature of (-20°C) for the purpose of conducting laboratory tests including estimation of Alanine transaminase (ALT), Aspartate transaminase (AST), glutathione and malondialdehyde.

Statistical analysis

The data analysis amongst groups was carried out using one-way variance analysis, and the substantial within groups were determined using the Duncan test. Results were regarded as statistically significant at $P<0.05$. All statistical analyses were carried out using the SPSS 10.00 software package [30].

Results

The results presented in Table (2) showed a significant decline ($P<0.05$) in red and white blood cells, hemoglobin concentration, and hematocrit for the heat stress group in comparison with the control. The treatment of zinc nanoparticles and zinc sulfate alone resulted in a significant raise in the red and white cells, hemoglobin concentration, and hematocrit compared with the control, with the superiority of the nano-zinc oxide group over the zinc sulfate group in blood parameters. The treatment of nano-zinc oxide and zinc sulfate with heat stress caused an elevate in most blood parameters compared to the heat stress group, and the treatment of nano-zinc oxide with heat stress showed a significant superiority in the numbers of white blood cells, the concentration of hemoglobin and hematocrit, compared with the treatment of zinc sulfate with heat stress with the possibility of this group in restoring the values of white blood cell numbers to their normal level close to the control.

The results shown in Table (3) led to a significant elevate ($P < 0.05$) in the MCV and MCH with a significant decline in the MCHC of the heat stress group in compression with the

TABLE 2. Impact of nano-zinc oxide and zinc sulfate on blood picture in broiler under high temperature

Groups / Parameters	RBCs $1x10^6/mm^3$	WBC $1 x10^3/mm^3$	Hb mg/100ml	HCT%
Control	$2.98±1.74^c$	$3.16±2.11^c$	$7.56±3.01^c$	$30.82±6.58^c$
Heat stress	$1.42±1.08^e$	$2.14±1.17^f$	$5.67±2.31^{ef}$	$26.54±4.62^f$
Nano-zinc oxide 40mg/kg	$3.24±2.09^a$	$3.98±2.52^a$	$8.74±3.85^a$	$33.72±7.38^a$
Zinc sulfate 110mg/kg	$3.11±1.95^b$	$3.52±2.39^b$	$8.39±3.67^b$	$31.67±7.11^b$
Nano-zinc oxide +heat stress	$2.90±1.86^d$	$3.07±1.47^{cd}$	$6.81±2.89^d$	$29.49±5.71^d$
Zinc sulfate + heat stress	$2.64±1.57^d$	$2.87±1.85^e$	$5.98±2.75^e$	$28.85±28.85^e$

Vertically varying letters represent significant differences among groups at the probability level ($P < 0.05$).
The above values presented as the mean (±) SE.

control group. Nano-zinc oxide and zinc sulfate alone showed a significant raise in the MCV and MCH in comparison with the control, in addition to a significant decrease for the treatment of nanoparticles of zinc oxide with a significant increase for the treatment of zinc sulphate in the MCHC compared with the control with the superiority of the zinc sulphate on the group of zinc oxide nanoparticles in the MCHC. Nanoparticles of zinc oxide and zinc sulfate with heat stress showed a significant decline in MCV and MCH compared with the heated stressor group. The group of zinc nanoparticles with heat stress showed a significant increase in MCHC compared with the heat stress, while the zinc sulphate group with heat stress indicates a significant decline in the MCH in compression with the heat stress group. The treatment of zinc oxide nanoparticles with heat stress resulted in the return of the MCV values to their normal level when compared with the control.

Our results indicate in Table (4) that there was a substantial decline ($P< 0.05$) in the lymphocytes with a significant raise in the heterophiles and the stress index of the heat stress group compared with the control. The treatment with zinc nanoparticles showed a significant elevate in the lymphocytes, heterophiles and stress index, with a significant decrease in the basophiles in compression with the control. While the zinc sulfate treatment caused a significant decline in the lymphocytes with a significant increase in the heterophiles and monocytes, basophiles and the stress index compared with the control group, the zinc sulfate group was superior to the treatment of zinc nanoparticles in the heterophiles, monocyte and the stress index, while nano-zinc oxide showed a significant superiority in the lymphocytes compared with zinc sulfate alone. Groups of zinc nanoparticles and zinc sulfate with heat stress indicate a significant raise in the lymphocytes and monocyte with a significant decrease in the heterophiles compared with the heat stress group, and the zinc sulfate group with heat stress showed a significant decline in the stress index when compared with the heat stress treatment. Treatment with zinc nanoparticles with heat stress caused a significant increase in the lymphocytes, heterophiles and stress index, accompanied by a significant decrease in the monocyte when compared with zinc sulfate treatment with heat stress. The two groups of zinc nanoparticles and zinc sulfate with stress returned the values of the heterophiles, monocyte and the stress index to their normal value and their superiority over the control.

Our results shown in Table (5) represent a significant raise ($P< 0.05$) in the levels of alanine aminotransferase and aspartate aminotransferase, accompanied by a significant decrease in the total protein of the heat stress group in compression with the control. The two groups of zinc nanoparticles and zinc sulfate alone showed a significant decline in the level of liver enzymes with a raise in the level of total protein in compression with the control group, and the group of nanoparticles of zinc oxide excelled in reducing the level of enzymes compared with the group of zinc sulfate. The two groups of zinc nanoparticles and zinc sulfate subjected to heat stress showed a significant decrease in the level of liver enzymes accompanied by an elevate in the level of total protein compared with the group of heat stress, while the group of nanoparticles of zinc oxide subjected to heat stress was superior in

TABLE 3. Impact of nano-zinc oxide and zinc sulfate on blood indices in broiler under high temperature

Groups / Parameters	MCV (fl)	MCH (pg)	MCHC(g/100ml)
Control	102.43± 2.10[e]	26.34± 3.11[c]	25.39± 3.08[b]
Heat stress	187.93± 3.91[a]	38.97± 4.21[a]	22.49± 1.89[e]
Nano-zinc oxide 40mg/kg	104.79± 2.32[c]	27.69± 3.87[b]	24.98 ±2.42[c]
Zinc sulfate 110mg/kg	103.82± 2.28[cd]	27.09± 3.76[b]	27.42± 3.73[a]
Nano-zinc oxide +heat stress	102.72± 2.14[e]	24.59±2.78[d]	23.95± 2.21[d]
Zinc sulfate + heat stress	110.31± 3.74[b]	23.67± 2.59[de]	21.77±1.65[f]

Vertically varying letters represent significant differences among groups at the probability level ($P< 0.05$).
The above values presented as the mean (±) SE.

TABLE 4. Impact of nano-zinc oxide and zinc sulfate on differential leukocyte count in broiler under high temperature

Parameters Groups	Lymphocytes%	Heterophils%	Monocytes%	Basophils%	Eosinophils%	H/L
Control	73.79±3.10^b	19.17±1.12^e	2.19±1.62^c	2.91 ±1.87^a	1.96 ±1.52^a	0.24 ±0.98^d
Heat stress	70.23±1.81^e	22.79±2.81^a	2.15 ±1.41^c	2.79±1.63^a	1.89±1.50^a	0.32±1.21^a
Nano-zinc oxide 40mg/kg	74.81±3.18^a	21.51±2.14^c	2.19±1.76^c	2.11±1.31^b	1.75±1.42^a	0.29 ±1.00^c
Zinc sulfate 110mg/kg	72.59 ±2.71^c	21.89±2.63^b	2.49±1.85^b	2.19±1.42^b	1.82±1.47^a	0.31±1.11^b
Nano-zinc oxide +heat stress	72.68±2.74^c	21.93±2.71^b	2.51±2.09^b	2.72±1.60^a	1.19±1.38^a	0.32±1.20^a
Zinc sulfate + heat stress	71.92±2.42^d	20.86±1.79^d	2.93±2.11^a	2.87±1.82^a	1.49±1.40^a	0.30±1.01^b

Vertically varying letters represent significant differences among groups at the probability level (P < 0.05).
The above values presented as the mean (±) SE.

reducing the level of liver enzymes and improving the level of total protein compared with sulfate Zinc subjected to heat stress. From the above results, it is noted the importance of zinc oxide nanoparticles, either alone or with heat stress, in improving the level of liver enzymes and total protein by restoring their values to the normal position close to the control.

It is noted through the results obtained in Table (6) that a significant decline (P < 0.05) in the level of glutathione, accompanied by a significant raise in the level of malondialdehyde for the heat stress group compared with the control group. Treatment with zinc nanoparticles and zinc sulfate alone led to a significant raise in glutathione accompanied by a decline in malondialdehyde compared with the control, and the zinc sulfate group showed a significant decrease in malondialdehyde in compression with the control, and the group of nano-zinc oxide excelled in the level of glutathione and reduced malondialdehyde compared with zinc sulfate. The two groups of zinc nanoparticles and zinc sulfate exposed to heat stress led to a significant elevate in the level of glutathione with a decrease in malondialdehyde compared to heat stress, and the group of zinc oxide nanoparticles with heat stress was superior in increasing the level of glutathione and a decrease in the level of malondialdehyde compared with zinc sulfate subjected to heat stress. It is noted that the nano-zinc oxide group showed a positive effect in improving the level of glutathione and reducing malondialdehyde when broiler exposure to heat stress by restoring its values to their normal position close to the control.

Discussion

Elevated environmental temperature induces a variety of physiological alteration, such as oxidative stress and immune activity depression, resulting in an elevate mortality as well as decline feed efficiency, body weight, and feed intake [31,32]. In the current study, heat stress decline most hematological value, total protein, glutathione with raise in the ALT, AST and malondialdehyde. Increased temperature affect poultry, and they have a difficult time balancing their body temperature due to a lack of sweat glands and the presence of feathers. If high temperatures have an impact on the performance and health of poultry, it can result in economic losses in poultry flocks [33]. Thermal stress has been linked to immune function abnormalities. Furthermore, a growing body of evidence suggests that humoral antibody response to a variety viral antigen does not enhance as expected following vaccine immunization in birds grown under hot conditions [34]. Furthermore, numerous studies have shown that poultry subjected to heat stress had lower levels of IgA, IgM, and IgG [35]. High ambient temperature causes superoxide anions to accumulate within mitochondria, causing oxidative stress and, as a result, rising MDA levels, a marker of lipid peroxidation [36]. Moreover, heat enhances the permeability of the intestinal wall, permitting for a substantial leak of endotoxins into the circulation even in healthy animals [37]. In the case of raise temperature, reduced portal blood flow combined with a slowdown in hepatic function significantly reduces the liver's detoxifying capacity, leading

TABLE 5. Impact of nano-zinc oxide and zinc sulfate on liver enzyme and total protein in broiler under high temperature

Parameters / Groups	ALT (IU/ml)	AST (IU/ml)	Total protein (mg/dl)
Control	118.53±2.11[d]	14.69±1.56[d]	2.25±1.17[b]
Heat stress	213.47±3.21[a]	26.87±2.29[a]	1.78±0.92[e]
Nano-zinc oxide 40mg/kg	103.62±1.33[f]	11.79±1.14[f]	3.23±1.89[a]
Zinc sulfate 110mg/kg	110.39±1.78[e]	12.83±1.28[e]	3.12±1.77[a]
Nano-zinc oxide +heat stress	121.47±2.89[c]	19.37±1.78[c]	2.01±1.01[c]
Zinc sulfate + heat stress	125.19±3.07[b]	21.14±1.88[b]	1.89±1.01[d]

Vertically varying letters represent significant differences among groups at the probability level ($P < 0.05$).
The above values presented as the mean (±) SE.

TABLE 6. Impact of nano-zinc oxide and zinc sulfate on glutathione and malondialdehyde in broiler under high temperature

Parameters / Groups	Glutathione (ng/ml)	Malondialdehyde (ng/ml)
Control	3.87±2.40[bc]	2.73±2.11[b]
Heat stress	1.54±1.18[f]	3.11±2.42[a]
Nano-zinc oxide 40mg/kg	4.29±2.92[a]	1.62±1.37[f]
Zinc sulfate 110mg/kg	3.94±2.52[b]	1.87±1.19[e]
Nano-zinc oxide +heat stress	3.18±2.01[d]	2.06±1.51[d]
Zinc sulfate + heat stress	2.85±1.79[e]	2.23±1.87[c]

Vertically varying letters represent significant differences among groups at the probability level ($P < 0.05$).
The above values presented as the mean (±) SE.

to reduced biotransformation of endotoxins and xenobiotics [38]. The high concentration of polyunsaturated fatty acids (PUFA) in the liver, especially in relation to the proportion of potential antioxidants, may also be linked to a higher hepatic sensitivity to heat stress [39]. These PUFA particles are especially prone to oxidation and thus susceptible to oxidative damage triggered by heat stress, exposing the organ to the harmful effects of raised environmental temperature [40]. Heat stress (HS) has also been linked to oxidative stress in broilers, with lower antioxidant property and blood concentrations of antioxidant vitamins and minerals (e.g., Zn) [41]. The adding of zinc oxide nanoscale and zinc sulfate to the ration of broiler result in reverse the negative effect of heat stress and this congruent with Sharideh *et al*. [42] who indicate that the administration of zinc oxide to broiler chickens enhanced the number of red and white blood cells while reducing the proportion of heterophile and the stress index. This result contradicts the findings of Salman et al. [43], who noticed that nano-ZnO (25-200 mg) substantially increased ALT and AST enzyme activities, one explanation for these differences could be the doses and length of time the animal was subjected to nano-ZnO. It has been revealed that nano-ZnO levels greater than 50 mg/kg induce oxidative stress and raise plasma ALT and AST levels [44]. In birds exposed to heat stress, nano zinc oxide reduces malondialdehyde and corticosterone while increasing glutathione [12]. Zinc is a trace element that is required for broiler production as well as the regulation of immune and antioxidant defenses [45]. The importance of zinc micronutrients in animal health is well established

[46]. This nutrient is not storable in the body. As a result, regular dietary zinc intake is required to meet function requirements [47]. Zn is a cofactor that is found in over 240 enzymes and has the power to influence oxidative processes. In general, chronic antioxidation creates greater sensitivity to certain oxidative stresses [48]. Zinc is an essential mineral for animals, as it aids in the stabilization of cell membranes and proteins by adjusting ROS production and scavenging due to its presence in antioxidant enzymes such as superoxide dismutase (SOD), furthermore, it has been demonstrated that Zn acts as an antioxidant to protect cell membranes from free radical damage [48]. In heat stressed broilers, Zn was linked to a rise in humoral immune responses [49]. A further crucial function proposed for Zn is its immunomodulatory function, which includes raising the number of peripheral T-cells and thymocytes as well as stimulating interferon production [50]. Zn enzymes are involved in the creation and/or breakdown of carbohydrates, lipids, proteins, and nucleic acids, and they comprise all known enzyme classes, they also make up hundreds of proteins that are involved in intermediary metabolism, hormone secretion pathways, and immune defense systems [51]. There is great deal of interest in using nanotechnology to enhance trace element utilization efficiency in farming practices, the use of ZnONPs in livestock feed as an alternative to conventional zinc sources could be beneficial [52]. The nanocomposite enhances the surface area obtainable for interaction with biological support, rises compound residence time in the GIT, and reduces the impact of intestinal clearance mechanisms, they reach deep into tissues via fine capillaries. These particles pass through the epithelial lining fenestration (e.g., liver), it allows for efficient cell uptake. Active compounds will be delivered to target sites in the body efficiently [53]. We were concluded from this research that zinc oxide nano-particles has positive effect to overcome the undesirable effect of heat stress in broiler by retain most of parameters to their normal value as in control.

Conclusion

It was concluded from this study that zin as nano-particles or sulfate have positive effect to overcome the undesirable effect of heat stress in broiler by retain most of parameters to their normal value as in control.

Acknowledgement

The authors thanks the College of Veterinary Medicine for supplying study facilities and financial assistance.

Conflict of Interest

The authors name appears above; they declare that they have no conflict of interest.

Funding statement

The authors indicate that on funding statement.

References

1. Hidayat, C., Jayanegara, A. and Wina, E. Effect of zinc on the immune response and production performance of broilers: a meta-analysis. *Asian-Aust J. Anim. Sci*., **33** (3), 465 (2020). doi: 10.5713/ajas.19.0146

2. Dosoky, W.M., Al-Banna, A.A., Zahran, S.M., Farag, S.A., Abdelsalam , R. and Khafaga, A.F. Zinc oxide nanoparticles induce dose-dependent toxicosis in broiler chickens reared in summer season. *Enviro. Sci. Pollu. Res. Int*., **29**(36), 54088-54107 (2022). https://doi.org/10.1007/s11356-022-19156-4

3. Lara, L.J. and Rostagno, M.H. Impact of heat stress on poultry production. *Anim*., **3**(2), 356-369(2013) https://doi.org/10.3390/ani3020356

4. Abdel-Latif, M.A., Abd El-Hack, M.E., Swelum, A.A., Saadeldin, I.M., Elbestawy, A.R., Shewita, R.S. and Abd El-Hamid, H.S. Single and combined effects of *Clostridium butyricum* and *Saccharomyces cerevisiae* on growth indices, intestinal health, and immunity of broilers. *Anim*., **8**(10), 184(2018). https://doi.org/10.3390/ani8100184

5. Rao, S.R., Prakash, B., Raju, M., Panda, A., Kumari, R. and Reddy, E.P.K. Effect of supplementing organic forms of zinc, selenium and chromium on performance, anti-oxidant and immune responses in broiler chicken reared in tropical summer. *Biol. Trace Elem. Res*., **172**,511–520(2016). https://doi.org/10.1007/s12011-015-587-x

6. Rossi, P., Rutz, F., Anciuti, M., Rech, J. and Zauk, N. Influence of graded levels of organic zinc on growth performance and carcass traits of broilers. *J. Appl. Poult. Res*., **16**, 219–225 (2007). https://doi.org/10.1093/japr/16.2.219

7. Stefanidou, M., Maravelias, C., Dona, A. and Spiliopoulou, C. Zinc: a multipurpose trace element. *Archi of Toxic*., **80**(1), 1-9 (2006). doi: 10.1007/s00204-005-0009-5.

8. Chand, N., Naz, S., Khan, A., Khan, S. and Khan, R.U. Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. *Inter. J. Biometeor*., **58**(10), 2153-2157 (2014). doi: 10.1007/s00484-014-0815-7.

9. Niknia, A.D., Vakili, R. and Tahmasbi, A.M. Zinc supplementation improves antioxidant status, and organic zinc is more efficient than inorganic zinc in improving the bone strength of aged laying hens. *Vet. Med. Sci*., **5**(8),2040-2049 (2022). https://doi.org/10.1002/vms3.896/v2/review1

10. National Research Council. (1994). Nutrient requirements of poultry: 1994. National Academies Press.

11. Jaiswal, S.K., Raza, M., Uniyal, S., Chaturvedani, A., Sahu, V. and Dilliwar, L. Heat stress and its relation with expression of heat shock proteins in poultry. *Int. J. Sci. Environ*., **6**,159-166(2017). https://doi.org/10.5455/ijlr.20170201093645

12. Ramiah, S.K., Awad, E.A., Mookiah, S. and Idrus, Z. Effects of zinc oxide nanoparticles on growth performance and concentrations of malondialdehyde, zinc in tissues, and corticosterone in broiler chickens under heat stress conditions. *Poult. Sci*., **98**(9),3828-3838(2019). https://doi.org/10.3382/ps/pez093

13. Maggini, S., Wintergerst, E.S. and Beveridge, S. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune response. *Br. J. Nutr*., **98**(1), S29–35(2007). doi:10.1017/S0007114507832971.

14. Sajadifar, S., Miranzadeh, H. and Moazeni, M. Effect of zinc on humoral and cell-mediated immunity of broilers vaccinated against coccidiosis. *Iran. J. Parasitol*., **8**(3),474(2013). https://doi.org/10.3923/ijps.2013.94.97

15. Akhavan-Salamat, H. and Ghasemi, H.A. Effect of different sources and contents of zinc on growth performance, carcass characteristics, humoral immunity and antioxidant status of broiler chickens exposed to high environmental temperatures. *Livestock Sci*., **223**, 76-83(2019). https://doi.org/10.1016/j.livsci.2019.03.008

16. Applegate, T.J. and Angel, R. Nutrient requirements of poultry publication: history and need for an update. *J. Appl. Poult. Res*., **23**,567-575(2014). https://doi.org/10.3382/japr.2014-00980

17. Swain, P.S., Rajendran, D., Rao, S.B.N. and Dominic, G .Preparation and effects of nano mineral particle feeding in livestock: a review. *Vet. World*, **8**,888–891 (2015). https://doi.org/10.14202/vetworld.2015.888-891

18. Gopi, M., Pearlin, B. and Dhinesh, R. Role of nanoparticles in animal and poultry nutrition: modes of action and applications in formulating feed additives and food processing. *Int. J. Pharmacol*., **13**,724–731(2017). https://doi.org/10.3923/ijp.2017.724.731

19. Hussain, Z., Khan, J.A., Anwar, H., Andleeb, N., Murtaza, S., Ashar, A. and Arif, I. Synthesis, characterization, and pharmacological evaluation of zinc oxide nanoparticles formulation. *Toxicol. Ind. Health*, **34**(11), 753–763(2018). https://doi.org/10.1177/0748233718793508

20. Mohammadi, V., Ghazanfari, S., Mohammadi-Sangcheshmeh, A. and Nazaran, M.H. Comparative effects of zinc-nano complexes, zincsulphate and zinc-methionine on performance in broiler chickens. *Br. Poult. Sci*., **56**,486–493 (2015). https://doi.org/10.1080/00071668.2015.1064093

21. Dosoky, W.M., Fouda, M.M., Alwan, A.B., Abdelsalam, N.R., Taha, A.E., Ghareeb, R.Y., El-Aassar, M. and Khafaga, A.F. Dietary supplementation of silver-silica nanoparticles promotes histological, immunological, ultrastructural, and performance parameters of broiler chickens. *Sci. Rep*., **11**,1–15(2021) https://doi.org/10.1038/s41598-021-83753-5

22. Mohammed, E.T. and Safwat, G.M. Grape seed proanthocyanidin extract mitigates titanium dioxide nanoparticle (TiO 2-NPs)–induced hepatotoxicity through TLR-4/NF-κB signaling pathway. *Biol. Trace Elem. Res*., **196**,579–589 (2020). https://doi.org/10.1007/s12011-019-01955-5

23. Bhattacharya, T., Maishu, S.P., Akter, R., Rahman, M., Akhtar, M.F., Saleem, A., Bin-Jumah, M., Kamel, M., Abdel-Latif, M.A. and Abdel-Daim, M.M. A review on natural sources derived protein nanoparticles as anticancer agents. *Curr. Top. Med. Chem*., **21**(13), 1014–1026 (2021). https://doi.org/10.2174/1568026621666210412151700

24. Fouda, M.M., Abdelsalam, N.R., El-Naggar, M.E., Zaitoun, A.F, Salim, B.M., Bin-Jumah, M., Allam, A.A., Abo-Marzoka, S.A. and Kandil, E.E. Impact of high throughput green synthesized silver nanoparticles on agronomic traits of onion. *Int. J. Biol. Macromol*., **149**, 1304–1317 (2020) . https://doi.org/10.1016/j.ijbiomac.2020.02.004

25. Ibrahim, K.A., Abdelgaid, H.A., El-Desouky, M.A., Fahmi, A.A. and Abdel-Daim, M.M .Linseed ameliorates renal apoptosis in rat fetuses induced by single or combined exposure to diesel nanoparticles or fenitrothion by inhibiting transcriptional activation of p21/p53 and caspase-3/9 through pro-oxidant stimulus. *Environ. Toxicol*., **36**,958–974(2021). https://doi.org/10.1002/tox.23097

26. El-Katcha, M., Soltan, M.A. and El-Badry, M. Effect of dietary replacement of inorganic zinc by organic or nanoparticles sources on growth performance, immune response and intestinal histopathology of broiler chicken. *Alex. J. Vet. Sci*., **55**,129-145(2017). https://doi.org/10.5455/ajvs.266925

27. Ramiah, S.K., Atta Awad, E., Hemly, N.I.M., Ebrahimi, M., Joshua, O., Jamshed, M. and Idrus, Z. Effects of zinc oxide nanoparticles on regulatory appetite and heat stress protein genes in broiler chickens subjected to heat stress. *J. Anim. Sci.,* **98**(10), 300(2020). doi: 10.1093/jas/skaa300

28. Kumar, A., Hosseindoust, A., Kim, M., Kim, K., Choi, Y., Lee, S. and Chae, B. Nano-sized zinc in broiler chickens: effects on growth performance, zinc concentration in organs, and intestinal morphology. *J. Poult. Sci*., **58**(1), 21-29 (2021). doi:10.2141/ jpsa.0190115

29. Campbell, T.W. *Avian Hematology and Cytology*, 2nd ed., Iowa: Iowa State University Press, pp.176-198 (1995).

30. Steel, R.D., Torrie, J.H. and Dickey, D.A. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. New York: McGraw-Hill Book Co; 1997. DOI: 10.4236/blr.2014.54024

31. Wastim S., Sah, N. and Mishra, B. Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Anim*., **10**(8),1266(2020)https://doi.org/10.3390/ani10081266

32. Attia, Y.A. and Hassan, S.S. Broiler tolerance to heat stress at various dietary protein/energy levels. *European Poult. Sci*., **81**, 10.1399(2017) https://doi.org/10.1399/eps.2017.171

33. Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. and Prasad, A. S. Role of dietary zinc in heat-stressed poultry: A review. *Poultry Science*, **88**(10), 2176-2183(2009)

34. Song, Z.H., Cheng, K., Zheng, X.C., Ahmad, H., Zhang, L.L. and Wang, T. Effects of dietary supplementation with enzymatically treated Artemisia annua on growth performance, intestinal morphology, digestive enzyme activities, immunity, and antioxidant capacity of heat-stressed broilers. *Poultry Science*, **97**(2), 430-437(2018).

35. Quinteiro-Filho, W.M., Calefi, A.S., Cruz, D.S. G., Aloia, T.P.A., Zager, A., Astolfi-Ferreira, C. S. and Palermo-Neto, J. Heat stress decreases expression of the cytokines, avian β-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. *Veterinary Immunology and Immunopathology*, **186**, 19-28(2017).

36. Elbaz, A.M., Ibrahim, N.S., Shehata, A.M., Mohamed, N.G. and Abdel-Moneim, A.M.E. Impact of multi-strain probiotic, citric acid, garlic powder or their combinations on performance, ileal histomorphometry, microbial enumeration and humoral immunity of broiler chickens. *Tropical Animal Health and Production*, **53**(1), 1-10(2021).

37. Rostagno, M. H. Effects of heat stress on the gut health of poultry. *Journal of Animal Science*, **98**(4), skaa 090(2020).

38. Epstein, Y. and Roberts, W. O. The pathopysiology of heat stroke: an integrative view of the final common pathway. *Scandinavian Journal of Medicine & Science in Sports*, **21**(6), 742-748(2011) https://doi.org/10.1111/j.1600-838.2011.01333.x

39. Lin, H., Decuypere, E. and Buyse, J. Acute heat stress induces oxidative stress in broiler chickens. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **144**(1), 11-17(2006). https://doi.org/10.1016/j.cbpa.2006.01.032

40. Di Nunzio, M., Valli, V. and Bordoni, A. PUFA and oxidative stress. Differential modulation of the cell response by DHA. *International Journal of Food Sciences and Nutrition*, **67**(7), 834-843(2016). https://doi.org/10.1080/09637486.2016.1201790

41. Arain, M.A., Mei, Z., Hassan, F.U., Saeed, M., Alagawany, M., Shar, A. H.and Rajput, I. R.Lycopene: a natural antioxidant for prevention of heat-induced oxidative stress in poultry. *World's Poultry Science Journal*, **74**(1), 89-100.(2018)

42. Sharideh, H., Zhandi, M., Zaghari, M., Akhlaghi, A., Hussaini, S.M.H. and Yousefi, A.R. Changes in broiler breeder hen's immunity by zinc oxide and phytase. *Iranian Journal of Veterinary Research*, **20**(2), 120(2019).

43. Salman, Z.A., Azeez, R.A., Yaseen, A. K. and Al-Rubaee, E. A. Effect of zinc oxide nanoparticles on serum alkaline phosphatase activity in male mice. *Biochem. Cell Arch*., **18**(2), 1715-1719(2018).

44. Sharma, V., Shukla, R.K., Saxena, N., Parmar, D., Das, M. and Dhawan, A. (2009). DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicology Letters*, **185**(3), 211-218(2009).

45. Naz, S., Idris, M., Khalique, M.A., Alhidary, I.A., Abdelrahman, M.M., Khan, R.U. and Ahmad, S. The activity and use of zinc in poultry diets. *World's Poultry Science Journal*, **72**(1), 159-167(2016).

46. El-Masry, K. and Nasr, A. Influences of season and dietary supplementation with selenium and vitamin E or zinc on some blood constituents and semen quality of New Zealand white rabbit males. *World. Rabbit. Sci.*, **2**, 79–86(2010).

47. Swain, P.S., Rao,S. B., Rajendran, D., Dominic, G. and Selvaraju, S. Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Anim., Nutr.,* **2**,134–141(2016).

48. Fathi, M., Haydari, M. and Tanha, T. Effects of zinc oxide nanoparticles on antioxidant status, serum enzymes activities, biochemical parameters and performance in broiler chickens. *Journal of Livestock Science and Technologies*, **4**(2), 7-13(2016).

49. Sharideh, H., Zhandi, M., Zaghari, M., Akhlaghi, A., Hussaini, S.M.H. and Yousefi, A.R. Changes in broiler breeder hen's immunity by zinc oxide and phytase. *Iranian Journal of Veterinary Research*, **20**(2), 120(2019).

50. Tsai, Y.H., Mao, S.Y., Li, M.Z., Huang, J.T. and Lien, T.F. Effects of nanosize zinc oxide on zinc retention, eggshell quality, immune response and serum parameters of aged laying hens. *Animal Feed Science and Technology*, **213**, 99-107 (2016). https://doi.org/10.1016/j.anifeedsci.2016.01.009

51. Jahanian, R., Moghaddam, H.N. and Rezaei, A. Improved broiler chick performance by dietary supplementation of organic zinc sources. *Asian-Australasian Journal of Animal Sciences*, **21**(9), 1348-1354(2008).

52. Al-Beitawi, N. A., Momani Shaker, M., El-Shuraydeh, K. N. and Bl´aha, J. Effect of nanoclay minerals on growth performance, internal organs and blood biochemistry of broiler chickens compared to vaccines and antibiotics. *J. Appl. Anim. Res.*, **45**,543-549(2017).

53. Asheer, M., Manwar, S.J., Gole, M.A., Sirsat, S., Wade, M.R., Khose, K.K. and Ali, S.S. Effect of dietary nano zinc oxide supplementation on performance and zinc bioavailability in broilers. *Indian Journals*, **53**(1),70-75(2018). DOI : 10.59588180.2018.00004.1-0974/.

تأثير أوكسيد الزنك النانوي وكبريتات الزنك في بعض القيم الدموية ووظائف الكبد في فروج اللحم تحت درجة حرارة محيطة عالية

[1]فلاح صلاح محمود و[1]عبد البر احمد نوري و[2]هديل محمد حميد
[1]تقنيات الإنتاج الحيواني - الكلية التقنية الزراعية - الجامعة التقنية الشمالية - الموصل - العراق.
[2]فرع الفسلجة والكيمياء الحياتية والأدوية - كلية الطب البيطري - جامعة الموصل- العراق.

هدفت الدراسة الحالية إلى تقدير تأثير أكسيد الزنك النانوي وكبريتات الزنك على بعض المقاييس الدموية وأنزيمات الكبد والكلوتاثيون والمالونديالديهايد تم تقسيم ١٠٨ فروج لحم من نوع روز ٣٠٨ (عمر يوم واحد) بشكل عشوائي إلى ست مجاميع بثلاث مكررات لكل مجموعة، كل منها بها ١٨ طائرًا. تشمل المجاميع: المجموعة الأولى (السيطرة علقة قياسية), المجموعة الثانية بنظام غذائي مكمل بأوكسيد الزنك النانوي ٤٠ ملغم / كغم , المجموعة الثالثة بنظام غذائي يحتوي على كبريتات الزنك ١١٠ ملغم / كغم , المجموعة الرابعة معرضة لدرجات حرارة عالية ٤٠ ± ٢ لمدة ٤ ساعات / يوم , المجموعة الخامسة المعرضة لدرجات حرارة عالية ٤٠ ± ٢ حتى ٤ ساعات / يوم مع عليقة حاوية على أوكسيد الزنك النانوي ٤٠ ملغم / كغم , المجموعة السادسة المعرضة لدرجات حرارة عالية ٤٠ ± ٢ حتى ٤ ساعات / يوم وتم تزويدها بكبريتات الزنك ١١٠ ملغم / كغم. أظهرت النتائج زيادة في اعداد خلايا الدم الحمر والبيض، تركيز الهيموكلوبين، حجم الخلايا المرصوصة، النسبة المئوية للخلايا اللمفاوية والمتغايرة، وتركيز الكلوتاثيون مصحوبًا بانخفاض في تركيز خميرة ناقلية الالنين، خميرة ناقلة الاسباتيت والمالوندالديهايد في مجموعة أوكسيد الزنك النانوي وكبريتات الزنك مقارنة بالسيطرة والمجموعة ذات الحرارة العالية. وخلصت الدراسة إلى أن اوكسيد الزنك النانوي وكبريتات الزنك قد يحسن التأثير السلبي للإجهاد الحراري على بعض القيم الدموية ووظائف الكبد في فروج اللحم.

الكلمات المفتاحية: الزنك النانوي، خلايا الدم الحمر، خميرة ناقلة الالنين، خميرة ناقلة الاسبارتيت، الكلوتاثايون