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Nano-zinc Oxide and Zinc Sulfate in Broilers: Effect on Thyroid Hormones and Internal Intestinal Environments

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THE objective of this study was to evaluate of nano-zinc oxide and zinc sulfate on thyroid hormones, histomorphometry and microbial in the small intestine. A total of 108 Rose 308 chicks (1d old) were randomly divided into six groups with three replicates per group, each having 18 birds. Treatments include: 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 a significant increase in thyroid hormones and thyroid stimulating hormone (TSH), villi height, villi width, crypt height, crypt width, goblet cell, apparent surface area, Lactobacillus population with a reduction of E. coli in nano-zinc oxide and zinc sulfate compared with control and height ambient temperature. It was concluded that nano-zinc and zinc sulfate improved thyroid function, intestinal morphology and microbial populations in broiler.

Keywords: Broiler, Thyroids hormone, Nano-zinc, Zinc sulfate, Villi.

Introduction

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 [1,2]. 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 [3-5]. 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 [6]. Zinc is an essential nutrient for chickens [6] 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 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., ZnCl2, ZnSO4, 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.

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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 minimize the negative impact of such extreme temperatures. Zinc significantly improves broiler immunity, according to numerous studies. 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. Zinc 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 required dose is higher than the NRC recommendation [16], and zinc has the ability 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-ZnO is more bioavailable and bioactive than conventional ZnO, it can be used at lower doses and has greater 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 production, antioxidant defense, and intestinal health 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 was to evaluate of nano-zinc oxide and zinc sulfate in thyroid hormone, histomorphometry and microbial populations in the small intestine in broiler under heat stress.

Birds, housing, and management

A total of 108 Rose 308 chicks (1d old) were purchased from a commercial hatchery and utilized in the current study. The chicks were housed in the same experimental room. in an open-type 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 diet was developed to meet the American National Research Council (NRC) [10] recommendations and water was provided *ad libitum* over the study period (1-35 days), birds were randomly assigned to six groups of (18 birds per group) and each group had three replicates (6 birds / repeat). Chicks were assigned to one of the following treatments: - Group 1 control with basal diet, Group 2 ration provided with nano-zinc oxide at 40 mg/kg [27] , Group 3 ration containing zinc sulfate at 110 mg/kg (28) , Group 4 subjected to high ambient temperature 40±2 up to 4 h /day (8:00-12:00), Group 5 was exposed to high ambient temperature 40± 2 up to 4 h /day (8:00-12:00) and were dietary supplemented with

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

nano-zinc oxide 40 mg/kg Group 6 was exposed to high ambient temperature 40±2 up to 4 h / day (8:00-12:00) and were dietary supplemented 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) using gel tubes that were placed in a centrifuge for 15 min at a rate (3000 rps/mint) 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 thyroid hormone (T3, T4) and thyroid stimulating hormone (TSH) using an ELISA kit.

Microbial population

After slaughtering the birds, 1 g of the intestine components was taken and diluted with distilled water in four dilutions until reaching a concentration of 1 x 10^4, then 0.1 ml of the diluted solution was taken and planted in two types of dishes: - MacConkey agar for counting *Escherichia coli* and MRS agar for counting *Lactobacillus* [29] .

Intestinal histomorphology

Intestinal tissue samples were taken from Meckel's diverticulum after cleaning them with distilled water they were immersed in 10% formalin solution to make histological sections for the purpose of measuring the length, width of the villi, the depth of the crypts, the width of the crypts, thickness of the epithelium, apparent surface area and the percentage of goblet cells. These parameters were measured using a Chinese-origin Color digital camera HMDC-5 attached to an optical microscope this camera is equipped with image analysis software (Scope image-0.9) prepared to perform these measurements [29].

Data analysis

The statistical analysis among the groups was performed using one-way analysis of variance, and the significant within groups were calculated by using Duncan test were considered statistically significant at $P<0.05$. All statistical analyses were carried out using the SPSS 10.00 software package [30].

Results

Table (2) showed a decline in thyroid hormones and thyroid stimulating hormone at a significant level ($P<0.05$) for the heat stress group in compared with control. The two groups of zinc oxide nanoparticles and zinc sulfate alone were superior to the control in the level of the above criteria, while the nano-zinc oxide showed a significant raise in the level of hormones compared with zinc sulfate. The two heat stress groups treated with nano zinc oxide and zinc sulfate led to an elevate in the level of thyroid hormones and thyroid stimulating hormone compared to heat stress. Zinc oxide nanoscale reduced the negative effect of high ambient temperature on thyroid hormones and the stimulating hormone in returning them to their normal level close to control.

Table (3) revealed that heat stress reduced the number of Lactobacilli while increasing the number of *E. coli* at a significant level of ($P< 0.05$) in comparison with control. When compared to the control, treatment with nano-zinc oxide and zinc sulfate increased the number of Lactobacilli while reducing the numbers of *E. coli*. The nano zinc oxide group outperformed zinc sulfate in

TABLE 2. Effect of ZnO nanoparticles and Zinc sulfate on thyroid hormones during height ambient temperature

Parameters	T3 (µm/ml)	T4(µm/ml)	TSH (pg/ml)
Treatments			
Group 1	3.19 ± 2.82^{c}	22.11 ± 2.26^{c}	0.98 ± 1.11^{c}
Group 2	2.03 ± 1.09^{f}	19.53 ± 1.01^{f}	0.23 ± 0.31^{f}
Group 3	4.74 ± 3.11^{a}	24.11 ± 2.84^{a}	1.19 ± 1.24^{a}
Group 4	3.76 ± 2.98^{b}	23.62 ± 2.31^{b}	1.02 ± 1.19^{ab}
Group 5	3.10 ± 1.92^{cd}	21.81 ± 1.78^{d}	0.94 ± 1.02^{d}
Group 6	2.98 ± 1.21^{e}	20.47 ± 1.29^{e}	0.69 ± 0.76^{e}

-The different small letters within the same column indicate a significant diver among the groups at ($P< 0.05$).

terms of Lactobacilli count while decline *E. coli* count. When compared to heat stress, heat stress treatment with nano zinc oxide and zinc sulfate increased the number of Lactobacilli while decreasing the number of *E. coli*. The zinc oxide nano group with stress played an important role in mitigating the negative effects of stress by re-preparing the Lactobacilli and reduce the numbers of *E. coli* to normal, close to control levels.

It is noticed from the results indicated in Table (4) that there was a significant decrease at the probability level of ($P< 0.05$) in the height of the epithelium, the length of the villi, the width of the villi, the depth of the crypts, the width of the crypts, the percentage of goblet cells and the apparent surface area of the heat stressor group in comparison with the control. Treatment with nano-zinc oxide and zinc sulfate led to a significant raise in the above parameters in comparison with the control, and the nano-zinc oxide group outperformed zinc sulfate in these parameters. The heat stress treatment with nano-zinc oxide and zinc sulfate showed an increase in histological parameters compared with heat stress, and the heat stress group treated with nano zinc oxide outperformed the stress group with zinc sulfate in these above characteristics and the latter led to re-epithelial height, villi width and percentage of goblet cells The heat stress group treated with nano-zinc oxide improved the negative effect of heat stress by returning all values to their normal level similar to control.

TABLE 3. Effect of ZnO nanoparticles and Zinc sulfate on microbial population during height ambient temperature

Parameters / Treatments	Lactobacillus (cellx10^4/g of intestinal continent)	E. coli (cellx10^4/g of intestinal continent)
Group 1	876.89±13.25^{c}	459.84±6.75^{d}
Group 2	485.38±5.79^{f}	973.57±11.53^{a}
Group 3	1215.31±19.68^{a}	196.80±3.42^{f}
Group 4	985.79±17.39^{b}	219.17±4.31^{e}
Group 5	748.97±12.74^{d}	511.28±7.68^{c}
Group 6	673.94±9.82^{e}	568.74±9.39^{b}

-The different small letters within the same column indicate a significant diver among the groups at ($P< 0.05$).

TABLE 4. Effect of ZnO nanoparticles and Zinc sulfate on intestinal histomorphology during height ambient temperature

Parameters / Treatments	Epithelium Height (µm)	Villi length(µm)	Villi width(µm)	Crypts depth(µm)	Crypts width(µm)	Goblet cell%	Apparent surface area (µm x10^3)
Group 1	42.11 ±10.42^{e}	857.89 ±21.37^{d}	134.62 ±11.29^{e}	74.33 ±6.71^{d}	31.83 ±4.27^{d}	25.73 ±3.62^{e}	74.08 ±11.22^{e}
Group 2	28.14 ±8.57^{f}	516.78 ±17.26^{f}	103.74 ±9.82^{f}	57.14 ±5.28^{f}	20.71 ±1.64^{f}	14.33 ±1.25^{f}	49.32 ±8.08^{f}
Group 3	68.47 ±19.15^{a}	1076.81 ±30.62^{a}	197.42 ±25.74^{a}	167.59 ±15.62^{a}	41.85 ±8.72^{a}	43.69 ±9.83^{a}	195.86 ±11.59^{a}
Group 4	61.53 ±16.34^{c}	930.47 ±28.34^{b}	164.11 ±21.51^{b}	101.37 ±11.48^{b}	33.65 ±6.08b^{c}	39.21 ±6.11^{c}	98.76 ±9.62^{b}
Group 5	62.74 ±17.31^{b}	919.88 ±25.73^{c}	152.92 ±19.88^{c}	82.65 ±8.72^{c}	34.94 ±6.39^{b}	40.78 ±7.39^{b}	84.61 ±7.33^{c}
Group 6	58.39 ±14.19^{d}	832.57 ±19.81^{e}	144.82 ±15.63^{d}	73.59 ±6.34^{de}	28.56 ±2.17^{e}	32.63 ±5.09^{d}	79.52 ±5.19^{d}

-The different small letters within the same column indicate a significant diver among the groups at ($P< 0.05$).

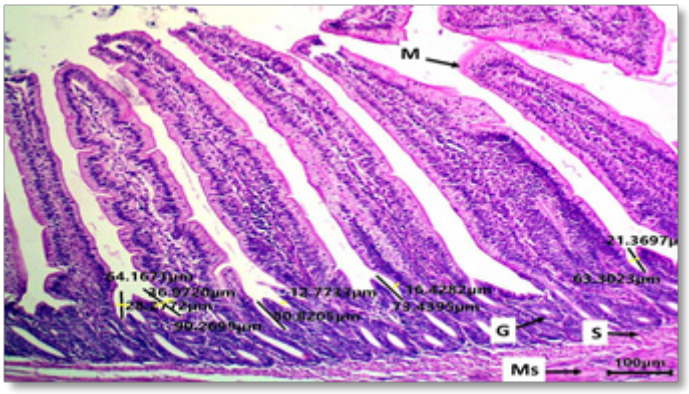

Fig. 1. A histological section of the small intestine of control showing normal histological features represented by the mucous layer lined with epithelial and goblet cells (M), the submucosa (S), the muscular layer (Ms), and the serous layer (Sr), as well as measurements of the length and width of the villi. H&E 100X.

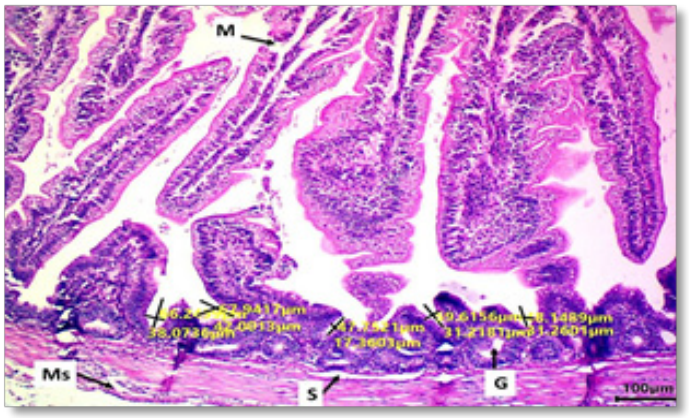

Fig. 2. Histological section of the small intestine from the group subjected to heat stress showing the lined mucosal layer with desquamation (M), the submucosal layer with edema (S), the muscular layer of light thickness with edema (Ms) and the intestinal glands (G), decrease in the length and width of the villi and crypts. H&E 100X.

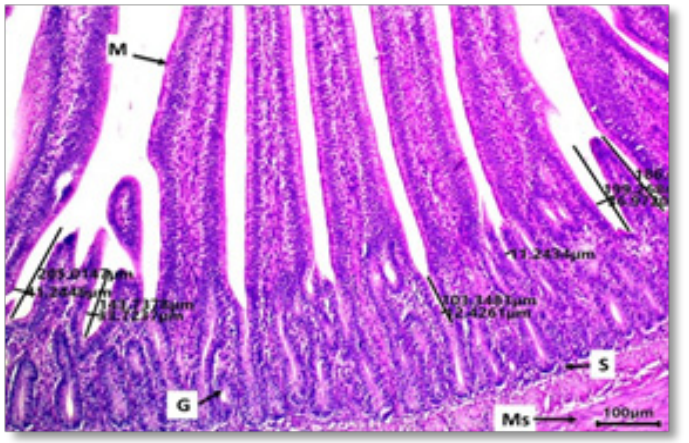

Fig. 3. Histological section of the small intestine of group with zinc nanoparticles (ZnO), showing normal histological features represented by the mucous layer lined with epithelial and goblet cells (M), the submucosal layer (S), the muscular layer (Ms), and the intestinal glands (G), with a raise in the length and width of the villi, crypts and goblet cell. H&E 100X

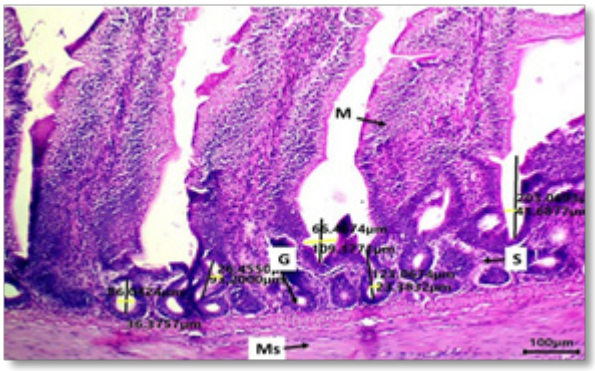

Fig. 4. Histological section of the small intestine of group with zinc sulfate (ZnSo4), showing normal histological features represented by the mucous layer lined with epithelial and goblet cells (M), the submucosal layer (S), the muscular layer (Ms), and the intestinal glands (G), with an elevate in the length and width of the villi, crypts and goblet cell. H&E 100X

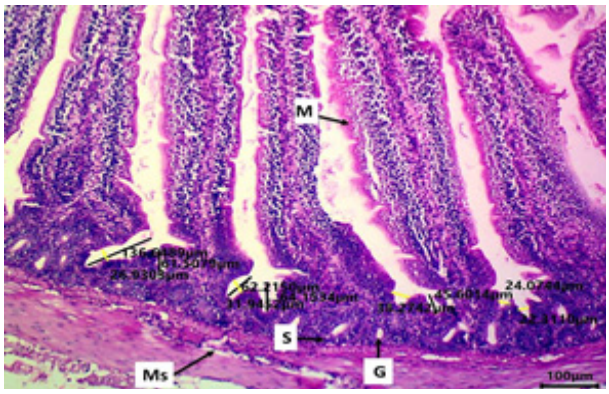

Fig. 5. Histological section of the small intestine of the group exposed to heat stress and treated with ZnO nanoparticles, showing an elevate in the thickness of the lining mucosal layer (M), submucosal layer (S), muscular layer and intestinal glands (G) with a raise in the length and width of the villi and crypts. H&E 100X.

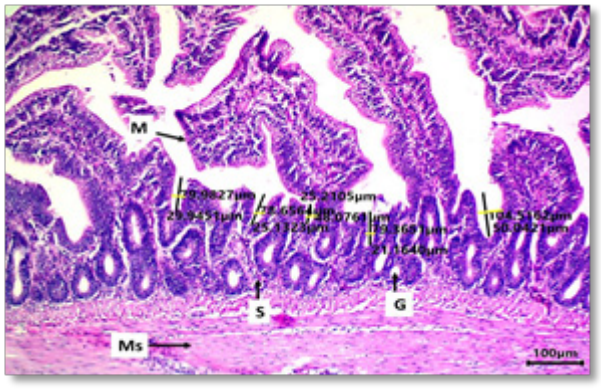

Fig. 6. Histological section of the small intestine of the group subject to heat stress and treated with ZnSo4 nanoparticles, showing an elevate in the thickness of the lining mucosal layer (M), submucosal layer (S), and muscular layer (Ms) and intestinal glands (G) with a raise in the length and width of the villi and crypts. H&E 100X

Discussion

High ambient temperature induces a variety of physiological changes, including oxidative stress and immune function inhibition, resulting in higher mortality along with reduced feed efficiency, body weight, and feed consumption [31,32]. In the currents study, heat stress decreased thyroid hormones, changes in intestinal morphology, increase *E. coli* with a reduction in Lactobacillus population in the intestine of broiler chickens. The addition of zinc as sulfate or nano-zinc lead to reverse this negative effect of high temperature. Thyroid hormones (T3 and T4) play an important role in controlling body metabolic rates of birds throughout their growth phases, so the thyroid gland plays an important role in making adjustments to high environmental temperatures [33]. Reduced T3 blood rate and corticosterone levels have been observed in broilers exposed to excess heat stress under various conditions [34]. Excessive rises in ambient temperature cause increases and decreases in plasma corticosterone and circulating thyroid hormones, respectively. This change has a negative impact on the neuroendocrine system's normal function, and as a result, the hypothalamic-pituitary-adrenal axes (HPA) become activated [35]. These various negative effects result in impaired metabolic activity (36). Extreme heat has a negative impact on the morphologies of the small intestine's duodenum, jejunum, and ileum, including changes in relative weight, villi height, villi surface area, crypt depth, and epithelial surface area [37]. Higher zinc intake (60 ~180 mg/kg) enhances animal digestion, including higher activity of digestive enzymes and improved intestinal morphology and function [38]. The crypt depth (which indicates the turnover rate of renewing villus) and intestinal villus (the sites of nutrient absorption) are important in digestive systems. In animals, the ratio of villi height to crypt depth is frequently used to assess nutrient absorption ability. Zinc may promote cell proliferation and protein synthesis in the crypt base; additionally, dietary zinc may improve intestinal morphology by raising villi height [39]. Previous research found that inorganic zinc (zinc sulfate) was more effective than organic zinc in enhancing jejunum villi height [40]. According to Hatab *et al.* [41] adding ZnO nanoparticles to the broiler diet enhanced villi high, width, epithelial surface area, and goblet cell. Broiler zinc oxide can elevate the concentration of sucrase in the small intestine, resulting in higher carbohydrate absorption [42]. Zinc in nanoparticles has antioxidant and anti-stress properties, and it impacts the intestinal gut bacteria of broiler chickens [43,44]. The small intestine, particularly its mucosal barrier, is critical for absorption and health maintenance, preventing tissue injury, and making sure sufficient nutritional nutrient provision to the entire body. The villus height and its ratio to crypt depth are widely used as key indicators of mucosal integrity and intestinal function, which are associated with better gut health and greater nutrient absorption [45]. The results of this study investigated the effect of ZONPs supplementation on the intestinal villi and crypt in broilers, the histological examination of intestine sections from broiler chicks fed a supplied diet with 40 mg/kg ZONPs revealed obvious desquamation of the intestinal villi, as well as a significant rise in cell producing of the intestinal villi and crypt. This was in line with the findings of Lei *et al*. [46], who discovered that replacing 60 mg inorganic zinc oxide with 45 and 30 mg of nano zinc/kg diet substantially elevate villi length and width, crypt depth, and villi length/crypt depth ratio compared to broiler fed the standard diet, indicating better nutrient absorption and feed utilization. El-Katcha *et al*. [47] confirmed previous findings in chicks fed diets containing supplemental ZONPs at 50 ppm. Bami *et al*. [48] reported that supplementing nano-ZnO at a rate of 40 mg is a significant feed additive for poultry with beneficial effects on intestinal changes in a more comprehensive experiment. In all parts of the small intestine of ZONPs supplemented birds, there was a significant increase in villus height, villus surface area, and total goblet cell count, as well as a high villus height: crypt depth ratio. Bahrampour *et al*. [49] explained that greater villus height could be due to enhanced bioavailability of zinc nanoparticles, thereby preserving epithelial barrier integrity and activity. Some other reason for a greater villus height in the gut segment could be that acidic mucin is resistant to bacterial degradation, resulting in less cellular damage [50]. Furthermore, crypt evolution is required to increase the rate of cell renewal and maturation in the gut. Because mucin-producing goblet cells are mostly found in the crypts, enhancing the crypt depth of chicken when supplemented with various rates of nano zinc/kg may provide more surface area for nutrient absorption by raising enterocyte

proliferation and enteric mucin secretion [51]. Because of its stem cell population, which divides continuously throughout life, the crypt plays an important role in the continuous renewal of villi (52). In this regard, Hu et al. [53] demonstrated that Zn addition enhanced villus dimensions by increasing absorptive surface and promoting villus crypt cell proliferation. Furthermore, zinc was shown to repair bowel injury by lowering the apoptotic index of ileal epithelial cells and rising villus height and crypt depth [54].It was concluded from this study that nan-zinc oxide improved the negative effect of heat stress by returning most values to their normal level similar to control.

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Conflict of Interests

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

Funding statements

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أوكسيد الزنك النانوي وكبريتات الزنك في فروج اللحم: تأثير على هرمونات الغدة الدرقية والبيئة المعوية الداخلية

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الهدف من هذه الدراسة هو تقييم تأثير أوكسيد الزنك النانوي وكبريتات الزنك على هرمونات الغدة الدرقية والمظهر النسجي والتجمع الميكروبي للأمعاء الدقيقة. 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. اظهرت النتائج زيادة معنوية في هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية ، وارتفاع الزغابات، عرض الزغابات، ارتفاع الخبايا، عرض الخبايا، الخلايا الكاسية، والمساحة السطحية الظاهرية، اعداد العصيات اللبنية مع انخفاض في اعداد الإيشيريشية القولونية لمجموعة أوكسيد الزنك النانوي وكبريتات الزنك مقارنة بالسيطرة مجموعة الحرارة العالية. نستنتج من هذه الدراسة ان أوكسيد الزنك النانوي وكبريتات الزنك أدى الى تحسن في وظيفة الغدة الدرقية، التركيب النسجي للأمعاء والتعداد الميكروبي في فروج اللحم.

الكلمات المفتاحية: فروج اللحم، هرمونات الدرقية، أوكسيد الزنك النانوي، كبريتات الزنك، الزغابات