



Supplementation of broiler drinking water with zinc sulfate and its impact on physiological performance

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Abstract

This study was conducted with three hundred one-day-aged broiler chicks (Ross 308) to determine the impact of adding two different doses of zinc sulfate to drinking water on the physiological traits of broilers. At first, 300 broiler chicks were randomly distributed into three groups. They were reared in floor cages until they were 42 days old, as follows: The 1st group (control group) was given drinking water without any additives. The 2nd and 3rd groups were given drinking water fortified with 20 and 40 mg of zinc sulfate/L of water, respectively. The results showed that the zinc sulfate significantly improved the hematological traits and the level of high-density lipoproteins, significantly reducing triglycerides. In contrast, total serum protein and albumin levels were significantly increased. Globulin was significantly enhanced when the dose was given at 40 mg/L of water. Also, zinc sulfate improved the antioxidant status, as it significantly raised the level of glutathione. In contrast, the corticosterone hormone level and aspartate aminotransferase activity were significantly decreased. The addition of zinc sulfate did not affect the levels of leptin and insulin-like growth factor. In conclusion, zinc sulfate enhanced most physiological parameters and maintained the other biochemical parameters within normal healthy values.

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Introduction

Zinc is a trace mineral and an important element that the body needs, and it serves multiple vital roles in poultry. It promotes broiler growth, bone development, feathering, nutrient digestibility, feed consumption, and feed conversion ratio (1). It is also necessary for enzyme structure and function (2) and raises the laying hens' egg production rate and eggshell thickness (3). Zinc increases the secretion of endocrine glands such as: growth hormone, insulin, and thyroid hormone, which need zinc for their secretion and functions (4-6). Furthermore, zinc has a role in protein synthesis as well as carbohydrates and lipid metabolism (7). Zinc plays a major role in redox processes (8), and it boosts antioxidant capacity by acting as a catalytic for over 300 immune-boosting metalloenzymes (9-11). The supplement

of zinc is often not sufficient for the poultry feeds, or that most of their feeds contain a minimal zinc concentration. Therefore, recently, breeders have been interested in adding zinc to the diets at double the recommended concentration (6) because of its importance in the poultry industry as a daily requirement to carry out the body's physiological functions (12). Inorganic zinc is the most common type of zinc used in chicken feed, in addition to zinc oxide because of its market availability and low cost (8), which is used as a zinc dietary supplement according to the NRC recommended for poultry diets of 40 to 70 mg/kg (13). It is also used to reduce zinc deficiency and enhance the production performance of broilers (6,8).

As a result, we used zinc sulfate in our research to learn about its effects on hematological and physiological traits as well as the antioxidant status of the broiler.

Materials and methods

Three hundred one-day-old broiler chicks were distributed into three separate groups of 100 birds each, with four replicates per group. Chicks were reared in floor cages until they were 42 days old, as described in: 1stgroup: as a control group, it was given a standard ration and tap water without any additives. 2ndgroup: it was given a standard ration and the tap water contained 20 mg of zinc sulfate/L of water. 3rd group: it was given a standard ration and the tap water contained 40 mg of zinc sulfate/L of water.

Chick husbandry requirements were considered depending on the company's recommendations in the Ross broiler guide. *Ad libitum* feed and water were given in line with the nutritional requirements of chickens (13), it contained: a starter and grower ration (21% and 19% crude protein and 2900 and 3100 kcal/kg respectively).

On the forty-second day of age, 6 broiler chickens were slaughtered from each group, and samples of blood were obtained immediately in two different kinds of tubes: those with EDTA for hematological parameters: red blood cells (RBCs) and packed cell volume (PCV) were carried out utilizing the method described in (14), as well as blood hemoglobin (Hb), which was performed with Drabkin's method using Biosystems kit (Spain-made) (15). After that, the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) have been estimated as mentioned in (14,16). Other tubes were plain tubes (containing gel without anticoagulant) for serum isolation, which was done according to (17), then kept at -20°C for estimating the glucose, cholesterol, triglycerides, high-density-lipoprotein-cholesterol (HDL-C), protein, albumin, globulin, ALT and AST activities by using Biosystems (Spain) (18). A

competitive ELISA kit by Elabscience Biotechnology company (USA) was utilized to estimate the levels of serum chicken Insulin-like Growth Factor (IGF), corticosterone hormone, and glutathione, while a sandwich ELISA kit by Elabscience Biotechnology company (USA) was utilized for measure the chicken leptin hormone.

Ethical approve

This work is approved by scientific board of Department of Animal Production, College of Agriculture and Forestry, University of Mosul in its third meeting dated October 23, 2021.

Statistical analysis

SAS program version 19.0 was used to analyze data with a one-way analysis of variance (19). The Duncan's Multiple Range Test at $P \leq 0.05$ was used to determine the significance differences between the means as reported in Steel and Torrie (20).

Results

It was noted from the results of the current study in table 1, that the addition of zinc sulfate at both doses to broiler drinking water led to significant increases in RBCs, Hb, and PCV values in comparison to control group, while it did not affect the values of each of MCV, MCH, and MCHC.

As seen in table 2, zinc sulfate had no significant impact on blood glucose and cholesterol levels, while serum triglycerides were significantly decreased when zinc sulfate was added at 40 mg/L, whereas the level of HDL-C increased significantly when adding at 20 and 40 mg zinc sulfate/L water compared with the control group.

Table 1: Impact of zinc sulfate on a broiler's blood picture

Groups	RBCs ($10^6/\text{mm}^3$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg/cell)	MCHC (g/dl)
G1	2.75±0.09 b	10.72±0.29 b	30.00±0.84 b	109.56±4.31 a	39.21±1.96 a	35.81±1.13 a
G2	3.18±0.07 a	12.16±0.59 a	32.80±0.86 a	103.34±3.60 a	38.33±2.08 a	37.13±1.80 a
G3	3.31±0.07 a	13.20±0.42 a	34.20±0.37 a	103.63±2.55 a	39.95±1.16 a	38.61±1.25 a

Means (\pm SE) with vertically different letters indicate statistically significant differences at $P \leq 0.05$. G1= control, G2= 20 mg of zinc sulfate/L water, G3= 40 mg of zinc sulfate/L water.

Table 2: Impact of zinc sulfate on a broiler's glucose, cholesterol, triglycerides, and high-density lipoprotein levels

Groups	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
G1	134.75±6.07 a	127.52±4.89 a	46.14±5.07 a	74.58±3.52 b
G2	148.40±8.44 a	126.90±6.02 a	45.80±3.86 a	88.67±5.79 a
G3	147.17±4.14 a	117.58±2.87 a	32.68±3.07 b	96.50±3.32 a

Means (\pm SE) with vertically different letters indicate statistically significant differences at $P \leq 0.05$. G1= control, G2= 20 mg of zinc sulfate/L water, G3= 40 mg of zinc sulfate/L water.

It was observed from Table 3 that zinc sulfate supplementation increased blood protein and albumin levels significantly in broilers at 20 and 40 mg/L addition, while the level of globulin increased significantly when Zn-sulfate was added at 40 mg/L in comparison to the control treatment. There was no significant difference in leptin and IGF levels between all groups.

The results in table 4 showed a decrease in the activity of the AST enzyme when adding zinc to the drinking water of

broilers, and it reached a significant level when adding 40 mg/L of zinc sulfate compared to the control group. As we can notice from the same table, an increase in broiler serum GSH levels which reached a significant level when adding 40 mg/L of zinc sulfate in comparison to the control group. The corticosterone hormone was significantly reduced by the addition of 40 mg/L zinc sulfate in comparison to the other groups.

Table 3: Impact of zinc sulfate on a broiler's blood proteins, leptin, and insulin-like growth factor

Groups	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Leptin (pg/ml)	ILGF (pg/ml)
G1	3.38±0.07 b	2.00±0.05 b	1.38±0.06 b	536.60±33.23 a	36.57 ±1.11 a
G2	3.86±0.05 a	2.32±0.09 a	1.54±0.05 ab	632.27 ±30.75 a	37.94 ±1.21 a
G3	3.98±0.12 a	2.38±0.11 a	1.60±0.07 a	705.83±31.09 a	39.00 ±1.57 a

Means (±SE) with vertically different letters indicate statistically significant differences at P≤0.05. G1= control, G2= 20 mg of zinc sulfate/L water, G3= 40 mg of zinc sulfate/L water.

Table 4: Impact of zinc sulfate on a broiler's AST, ALT, glutathione, and corticosterone levels.

Groups	AST (U/L)	ALT (U/L)	GSH (µmol/L)	Corticosterone (ng/ml)
G1	145.62 ±9.02 a	38.20 ±2.96 a	0.474 ±0.04 b	92.03 ±4.95 a
G2	139.24 ±9.65 ab	33.84 ±2.49 a	0.574 ±0.02 ab	88.42 ±5.88 a
G3	114.12 ±5.80 b	33.70 ±2.70 a	0.620 ±0.04 a	71.58 ±5.37 b

Means (±SE) with vertically different letters indicate statistically significant differences at P≤0.05. G1= control, G2= 20 mg of zinc sulfate/L water, G3= 40 mg of zinc sulfate/L water.

Discussion

In general, hematological characteristics and serum biochemical traits are essential indicators of the body's physiological status (21). The findings in Table 1 show that zinc sulfate added to broiler drinking water led to a significant increase in RBCs, Hb, and PCV values. This result agrees with Jwad *et al.* (22) who mentioned that the number of RBCs, Hb, and PCV were significantly increased when zinc sulfate was added to the ration by 30 and 45 mg/kg feed. It is known that zinc supplementation is an essential factor in erythropoiesis, as well as iron and vitamin B12, and adding it improves the blood components (23,24). Also, zinc affects many vital activities of the organism, including the activity of the thyroid gland and its secretion of T3 and T4 hormones (25), which have a vital and important function in red blood cell formation by stimulating erythropoietin (26) and activating the process of erythropoiesis (27), which leads to an increase in the RBC count and hemoglobin, and this was observed by Bucci *et al.* (28), who reported that serum T4 levels increased after zinc sulfate supplementation.

Zinc sulfate supplementation also improves the blood picture by another mechanism; it protects the erythrocyte membrane from lipid peroxidation (29), as well as prevents its damage and regulates its function (30,31).

It is clear that the Zn-sulfate did not have an effect on glucose, and cholesterol levels, which agrees with

Yalcinkaya *et al.* (32) who stated there was no impact of Zn on the level of these parameters when added to the broiler ration. The current outcomes agree with the finding of Ibrahim *et al.* (33) who mentioned that the organic and inorganic zinc additions had no significant effect on glucose levels of laying hens. Also, results agreed with Lu and Combs (34), who stated that adding zinc to a diet had no impact on the serum cholesterol of chickens. The supplementation of zinc maintains normal glucose and cholesterol levels, which could be because of zinc's role in protecting pancreatic tissues from oxidative damage and the effects of free radicals, as it helps them function properly, including their hormonal and digestive enzyme secretions, which maintain the level of blood glucose in a stable and balanced manner (35,36).

The current results did not agree with Kucuk *et al.* (37), who mentioned that zinc reduces the level of serum glucose and cholesterol, and with Aksu *et al.* (38), who stated that inorganic zinc reduced the level of cholesterol in the blood of broiler chickens, as well as did not agree with Al-Daraji and Amen (39), who stated that inorganic zinc addition led to a significant increase in plasma glucose, and cholesterol of broiler breeders. But the findings of current research agree with those of Fawzy *et al.* (31), who found that inorganic zinc decreases triglycerides and increases high-density lipoprotein (HDL). Also, it agreed with El-Gogary and Abo EL-Maaty (40), who mentioned that zinc administration

elevated the amount of HDL in broiler blood serum. Wu *et al.* (41) stated that zinc reduces dietary lipid absorption, whereas, Jahanian and Rasouli (42) explained that organic zinc utilization inhibits disagreeable fat metabolism and boosts the bioavailability of HDL.

From Table 3, we noticed a significant increase in protein and albumin levels at 20 and 40 mg/L Zn-sulfate and globulin (at 40 mg/L Zn-sulfate), these findings agree with those of Fawzy *et al.* (31), who found a significant increase in blood protein and globulin levels in comparison to the control. Kucuk *et al.* (37) observed a similar outcome, reporting that adding zinc caused broilers' serum total protein to increase significantly. As well as agreed with Al-Daraji and Amen (39) who noted that adding inorganic zinc to the diets of broiler breeder chickens caused a significant elevation in blood total protein.

The present study's findings contradict Hidayat *et al.* (43) and Barman *et al.* (44) in which they found that dietary zinc had no significant impact on blood protein level. Perhaps zinc improved the digestive coefficient of the birds that took it (45), and this was confirmed by Sahin and Kucuk (46) in their research, as they observed that the digestibility of white quail increased when given zinc sulfate as a supplement in the diet.

Besides, we note that the significant increase in serum protein concentration in G2 and G3 in comparison to G1 (control group) may be due to the reduction in corticosterone levels, which may inhibit or reduce gluconeogenesis, so it prevents serum protein exhaustion.

Furthermore, we noticed that the levels of leptin and insulin-like growth factor improved by adding zinc to the water, and their levels remained at the level of the control group and did not increase significantly over them. Muszynski *et al.* (47) found that zinc affects serum leptin levels and activates insulin-like growth factor. In addition, zinc is required for protein synthesis as well as the metabolism of carbohydrates, proteins, and lipids (48), and when zinc deficiency occurs, the insulin-like growth factor and growth hormone will be reduced in the blood, which leads to a decrease in growth (49). Yu *et al.* (50) observed that zinc availability affects IGF-I gene transcription. Also, according to Tomaszewska *et al.* (51), zinc is vital in the proliferation and differentiation of the cells and has an influence on cellular activity via hormones and growth factors. Leptin is synthesized and released by adipocytes of the body, and its circulating levels reflect the changes in the energy stores in the adipose cells (52).

The concentration of leptin in the blood is affected significantly by zinc status, and both leptin and zinc participate in the physiological regulation of the body's energy homeostasis (53,54). That means the leptin levels decrease in reaction to zinc deficiency and increase in response to zinc excess. That is, leptin levels are related to changes in cellular zinc levels (55), due to zinc's acting as a cofactor for many metalloenzymes as well as being

important for the physiological activities of many hormones, including leptin and insulin-like growth factor.

The results of this research demonstrated that broiler blood levels of AST, GSH, and corticosterone significantly affected in G3 after Zn-sulfate was added to the water. The levels of AST and corticosterone were significantly decreased, while the serum glutathione level of broilers increased significantly when 40 mg/L Zn-sulfate was added to the water. There was a significant reduction in the AST value of the third group at 40 mg Zn/L water, in contrast to what was reported by Yalcinkaya *et al.* (32) and Ibrahim *et al.* (33) who reported that there was no effect of the dietary zinc on the activity of AST, possibly due to the type of zinc.

As shown in Table 4, adding 40 mg of zinc sulfate/liter of water leads to significant increase in the level of glutathione, and significant decrease in the concentration of corticosterone hormone in the broiler blood. This is maybe because zinc has a vital role in suppressing free radicals, it serves as a cofactor for the antioxidant enzyme Cu-Zn-SOD (56), as well as because it prevents glutathione depletion, which reduces lipid peroxidation (8), also it competes with other metals in binding to the cell membrane and thus exerts a direct antioxidant effect by reducing the production of free radicals (57). As Naz *et al.* (5) mentioned, the most significant function of zinc is involved in the Fenton reaction via competing for transition metal binding sites and functioning as electron donors in these interactions. It also acts by preventing hydroxyl radicals' formation from H₂O₂.

Although zinc is a trace mineral and very rare in the diet, it's an essential component for the function and formation of the Cu-Zn-SOD. Therefore, from Table 4, it is seen that serum corticosterone decreased in G3 at 40 mg zinc sulfate/L water. The current result agrees with Sahin *et al.* (58) when they added zinc to a 30 mg/kg diet. Sahin *et al.* (8) suggest that zinc may boost the production of metallothionein, a metal-binding protein with a high cysteine content that scavenges free radicals and thus decreases serum corticosterone levels.

Conclusions

The findings of this research indicate that the addition of zinc sulfate to broiler drinking water has a significant impact on the physiological status of the bird, as it has significantly increased the red blood cell counts, hemoglobin, and PCV. Moreover, zinc enhanced the serum lipid profile, also, total protein. On the other hand, zinc sulfate contributed to improving antioxidant status as evidenced by an increase in the glutathione value, and lowering the stress index. Finally, it is still necessary to study the effectiveness of zinc in other aspects of both the physiological and productive performance of broilers, and more research is needed to determine how the zinc will affect broilers' physiological and productive performance.

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Conflict to interest

There is no conflict of interest in publishing this work.

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تعزیز ماء شرب فروج اللحم بكبريتات الزنك وأثره في الأداء الفسلجي

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الخلاصة

أجريت هذه الدراسة على ثلاثمائة فرخاً بعمر يوم واحد (روز ٣٠٨) لمعرفة تأثير إضافة جرعتين مختلفتين من كبريتات الزنك إلى مياه الشرب في الصفات الفسلجية لفروج اللحم. في البداية، وزع عشوائياً ٣٠٠ فرخاً من فروج اللحم في ثلاث مجموعات وربييت في أقفاص أرضية إلى عمر ٤٢ يوماً على النحو الآتي: المجموعة الأولى (مجموعة السيطرة) أعطيت ماء شرب بدون أية إضافات. أما المجموعتان الثانية والثالثة فقد أعطيت ماء شرب مدعم بـ ٢٠ و ٤٠ ملغم كبريتات الزنك/لتر من الماء على التوالي. تبين من النتائج أن كبريتات الزنك حسنت معنوياً من الصفات الدمية ومستوى البروتينات الدهنية عالية الكثافة مع انخفاض معنوي في مستوى الدهون الثلاثية، في حين ارتفع معنوياً مستوى البروتين الكلي والألبومين في مصل الدم، وتحسن معنوياً الكوليوليون عند إعطاء الجرعة ٤٠ ملغم/لتر ماء. كما حسنت كبريتات الزنك حالة مضادات الأكسدة، إذ رفعت معنوياً مستوى الكلوتاثيون بينما خفضت معنوياً من مستوى هرمون الكورتيكوستيرون ونشاط الإنزيم الناقل لمجموعة الأمين الاسبارتيت. لم يكن لإضافة كبريتات الزنك أي تأثير في مستوى اللبتين وعامل النمو الشبيه بالأنسولين. نستنتج من ذلك أن كبريتات الزنك قد عززت معظم المؤشرات الفسلجية وحافظت على المعايير الكيموحيوية الأخرى ضمن القيم الصحية الطبيعية.