Research Article

Effect of Different Sources and Levels of Zinc Used in Poultry Birds: A Review

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Abstract | Proper growth and health require proper trace mineral supplementation in poultry. Zinc (Zn) is the second most important micronutrient in animal metabolism. It is involved as a cofactor of more than 300 metallothionein, a constituent of several enzymes, and almost every biochemical pathway in the body requires it in some of the reactions, which is why it is critical for the normal physiology of living things. As Zn cannot be stored in monogastric animals, such as poultry birds, it must be added to the poultry diet as a supplement to fulfill the birds' requirements. Inorganic Zn sources like zinc-sulfate or zinc-oxide are preferably used by feed manufacturers due to their low cost, while organic Zn (amino acid chelates) has more bioavailability. Owing to their different physiochemical properties, such as higher surface area, Zn nanoparticles (NPs) reduce the quantity used in the feed and antagonize other divalent ions. Zinc plays a key role, and its different Zn sources (inorganic, organic, and NPs) have a positive impact on feed intake and conversion ratio, carcass characteristics, weight gain, weight of immune organs, blood antibodies, hot weather stress, and gene expression. The interaction of Zn with other minerals was also safe and improved bird performance. Inconsistent results have been reported by different researchers regarding the effectiveness of different Zn sources and levels on the performance and health of birds. Different studies have compared its recommended concentration by the National Research Council of the USA with varying supplementation doses from different sources. This review highlights the outcomes of these studies and the potential role of zinc in poultry nutrition.

Keywords | Carcass characteristics, Gene expression, Growth performance, Heat stress, Immunity, Poultry, Trace element, Zinc nanoparticles

Received | May 20, 2025; **Accepted** | September 10, 2025; **Published** | October 20, 2025
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Citation | Asnan M, Sharif M, Hamid MMA, Abbas W, Tarteel H, Sarwar MK, Hassan S (2025). Effect of different sources and levels of zinc used in poultry birds: A review. J. Anim. Health Prod. 13(4): 1016-1034.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.4.1016.1034
ISSN (Online) | 2308-2801

INTRODUCTION

Zinc (Zn) is one of the most extensively studied trace minerals in poultry nutrition owing to its vital function in maintaining health and productivity in production systems (Hu *et al.*, 2024). The enhanced genetic capabilities of modern poultry breeds, along with challenges such as oxidative stress, high-density housing, and climate variability, have highlighted the importance of effective trace mineral management (Ao and Pierce, 2013). As nutritionists aim to refine feed formulations for optimal performance, Zn remains a key component because of its involvement in various metabolic activities and its ability to influence immune function, growth, and resilience in birds (Sahin *et al.*, 2009).

Traditionally, poultry diets have relied on inorganic Zn sources, such as Zn sulfate and Zn oxide (ZnO), owing to their affordability and availability (Ezzati *et al.*, 2013). However, these forms often demonstrate limited bioavailability and can interact negatively with other dietary components, leading to excessive supplementation and increased environmental Zn excretion. In contrast, organic Zn sources, such as Zn methionine, Zn proteinate, and amino acid chelates, have shown improved gastrointestinal stability and absorption (Abd El-Hack *et al*., 2017; Sahin *et al*., 2005; Suttle, 2022). More recently, Zn nanoparticles (NPs) have emerged, offering enhanced bio-efficacy at lower inclusion levels owing to their high surface area and better cellular uptake (Sukhanova *et al*., 2018; Wijnhoven *et al*., 2009). The National Research Council (NRC, 1994) recommends dietary Zn inclusion of 40–60 mg/kg for poultry, depending on age and physiological status. However, the efficacy of Zn supplementation depends largely on the chemical form of Zn used and its interactions with other nutrients in the diet (Hambidge, 2010; Lonnerdal, 2000).

The functions of Zn are closely related to those of other micronutrients. It enhances vitamin A metabolism by promoting retinol-binding protein synthesis (Gilbert *et al*., 2019; Rahman *et al*., 2002) and acts synergistically with vitamin E and selenium to regulate oxidative stress through the activation of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase (Aly and Mantawy, 2012; Marreiro *et al*., 2017). This synergism becomes especially critical under heat stress conditions, where oxidative damage, immune suppression, and reduced performance are common challenges. The Zn supplementation has been shown to alleviate the adverse effects of heat stress by enhancing antioxidant capacity, maintaining intestinal integrity, and supporting immune functions (Hu *et al*., 2025; Ren *et al*., 2025). These effects are further amplified when Zn is combined with other antioxidants, such as selenium and vitamin E (Bahrampour *et al*., 2021; De Grande *et al*., 2021). However, mineral interactions must be carefully managed, as high dietary levels of calcium, phosphorus, iron, and copper can inhibit Zn absorption through competitive transport mechanisms in the gut (Lonnerdal, 2000; Pang and Applegate, 2007; Spencer *et al*., 1984).

This review aims to critically evaluate the impact of different Zn sources and supplementation strategies on poultry nutrition. This highlights their comparative bioavailability, physiological roles, and gene-level effects, with a particular focus on the functional implications of Zn in immunity and birds under stress conditions.

Role of zinc in poultry

The Zn is a type-2 nutrient that is important for metabolism and is found throughout the body (King, 2011). The structure and catalytic activity of over 300 metalloenzymes are associated with Zn (Sanna et al., 2018). Zn plays a number of roles in poultry, which include (i) preserving normal growth, including the development of the skeleton and feathers as well as the health of the skin and foot; (ii) strengthening the immune system and preventing infectious diseases; (iii) changing the metabolism of proteins, lipids, and carbohydrates by increasing the activities of glutamic dehydrogenase, alcohol dehydrogenase, alkaline phosphatase, and RNA polymerase; (iv) boosting antioxidant capacities by increasing copper-Zn superoxide dismutase and zinc metalloenzyme activities; and (v) affecting gene expression by changing DNA and chromatin structure. By controlling the release of reproductive hormones during sexual maturation and protein synthesis in the epithelium during egg formation, dietary Zn supplementation improves egg production in layer and breeder birds (Figure 1) (Tabatabaie *et al*., 2007). Almost every signaling and metabolic pathway includes at least one Zn-dependent protein (Suttle, 2022). Additionally, adding Zn to the diet improves the immunological state of hens (Alirezaei *et al*., 2024) and the survival of their offspring when exposed to an *Escherichia coli* challenge (Virden *et al*., 2003). The Zn has been suggested to stimulate mineralization, bone production, and bone mass preservation (Mohd Yusof *et al*., 2022). It has been claimed that changes in the Zn status influence the expression of an array of genes via activation (Adam et al., 2024; Shi *et al*., 2020). Because of its role in gene regulation, it is necessary for the synthesis of several proteins. Two important structural proteins, keratin and collagen, require Zn (Suttle, 2022).

The Zn interacts with many nutrients, making it a potential key component of micronutrient malnutrition (Welch and Graham, 2012), including the intake, production, and metabolism of fatty acids, which influences the circulating lipid profile and may increase the risk of cardiovascular disease (Banaszak *et al*., 2021; Olechnowicz *et al*., 2018). Excessive Zn intake can limit the absorption of copper (Cu) and Fe, causing Cu deficiency and anemia, respectively (Stiles *et al*., 2024). The Zn is used as a supplement to alter the bird reproductive system, in addition to its role as a nutrient (Pereira *et al*., 2020; Prabakar *et al*., 2021). Zn lowers the negative impacts on the environment by reducing uricase activity in poultry litter to lower atmospheric ammonia emissions (Hunde *et al*., 2012). At physiological or appropriate levels, Zn acts as an antioxidant and protects against oxidative stress (Maret and Krężel, 2007; Özcelik *et al*., 2012). Metallothionein (MT) is a cysteine-rich, low-molecular-weight protein that regulates metal homeostasis and detoxifies the body. However, both Zn deficiency and excess impair MT function; low Zn lowers antioxidant defense, whereas high Zn increases MT expression but may have pro-oxidant effects by displacing other metals or affecting cellular redox signaling, resulting in oxidative stress under certain circumstances (Lee, 2018; Maret, 2019). Zinc has a synergistic antioxidant effect with both water-soluble (vitamin C) and fat-soluble (vitamin E) antioxidants (Maggini *et al*., 2017; Song *et al*., 2017).

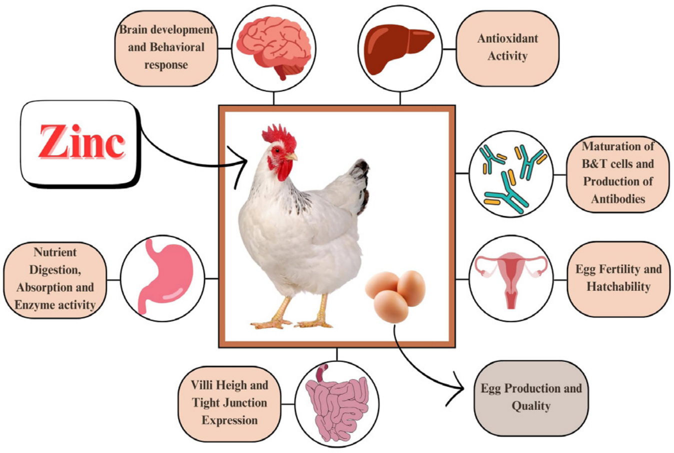

Figure 1: Different roles of dietary Zn in poultry (created in canva.com).

Sources of dietary zinc

Inorganic zinc sources

The Zn can be derived from both organic and inorganic sources, with ongoing debate regarding the relative effectiveness of the two sources (Salama *et al*., 2003) (Figure 2). Zinc sulfate ($ZnSO_4$) is favored as a source of Zn in poultry diets because of its cost-effectiveness and easy accessibility (Ezzati *et al*., 2013). Other inorganic sources include ZnO, Zn tetrabasic chloride, and Zn carbonate, which are used in the animal feed industry. Some researchers have suggested that the total amount of Zn in the diet should not exceed the NRC guidelines, which include Zn from natural components and mineral premixes. Although limiting dietary Zn according to NRC standards is favorable for the environment, it may not be optimum for certain physiological functions, and it is more critical when less bioavailable Zn sources are added to poultry diets (Burrell *et al*., 2004).

Organic zinc sources

Recent research has indicated that Zn from organic sources, such as Zn-methionine and Zn-propionate, is more bioavailable for production than Zn from $ZnSO_4$ or Zn oxide (Abd El-Hack *et al*., 2017; Sahin *et al*., 2005). Based on the tibia mineral content, Zn from Zn-methionine or Zn-propionate is more bioavailable than Zn from inorganic sources (Rahman *et al*., 2002; Wedekind *et al*., 1992). The efficiency of Zn is determined by its gastrointestinal absorption and bioavailability in the blood (Naz *et al*., 2016). Metal-specific amino acid complexes, metal-proteinates, metal-amino acid chelates, and metal-amino acid complexes are examples of organic mineral sources. Amino acids are coupled with soluble metal salts in a 1:1 molar ratio, which may encourage birds to use minerals more frequently (Burrell *et al*., 2004). The Zn picolinate is a good and highly absorbable supply; additional forms include citrate, gluconate, and acetate, whereas sulfate is poorly absorbed (Barrie *et al*., 1987). Jahanian and Yaghoubi (2010) reported similar results; they found that adding Zn-methionine or Zn-lysine to broiler diets increased Zn bioavailability. Its high bioavailability improves nutrient absorption and utilization in animals, resulting in lower amounts in feed and less excretion without impairing performance (Mohammadi *et al*., 2015).

Nano zinc sources

In recent years, nanotechnology and its related products have rapidly advanced in various scientific fields (Kandeel *et al*., 2022). The Zn-NPs have received considerable interest in this regard because nano-formulation particulates have distinct differentiating properties, such as size, shape, wide surface area, high surface activity, high catalytic efficiency, and strong adsorption ability (Wijnhoven *et al*., 2009). They are more likely to change the biological consequences caused by them than their bulk materials (Patra and Lalhriatpuii, 2020), and the total amount required also decreases (Sukhanova *et al*., 2018). The Zn-NPs have recently received considerable attention owing to their small particle size and improved surface activity (El-Bahr *et al*., 2020a; Mohd Yusof *et al*., 2019). Numerous studies attempting to learn more about the effects of Zn nanoparticle addition on poultry development rate, intestinal architecture, and immune response have used Zn-NPs because of their good biocompatibility, low cost, and minimum toxicity (Jiang *et al*., 2018).

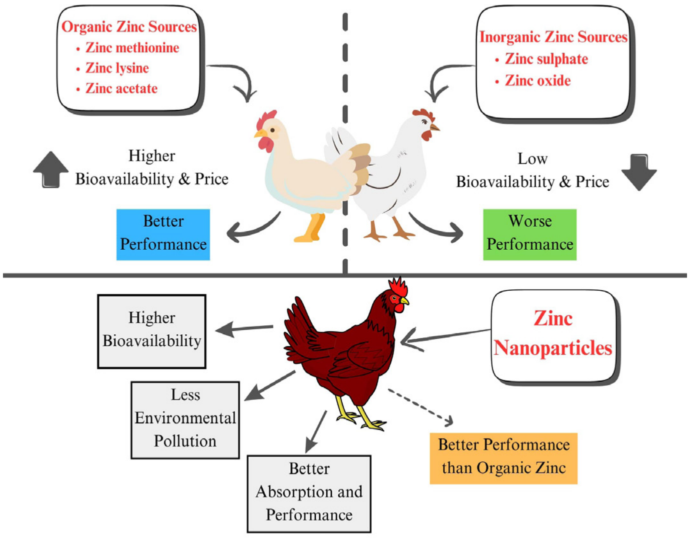

Figure 2: Advantages and disadvantages of different sources of Zn in poultry (created in canva.com).

Effect of zinc levels and sources on growth performance

Several studies have been conducted to determine the effect of Zn on broiler chicken BWG, FI, and FCR, with different Zn sources optimized at various supplementation amounts (Table 1). Zn deficiency in broiler chickens reduces feed consumption (Quarterman, 1968; Quarterman *et al*., 1969). Furthermore, high dietary Zn reduces FI and growth rates in livestock and poultry (Naz *et al*., 2016). There is a need to determine the ideal dosage for supplementation in poultry. According to previous studies, adding Zn to birds boosts their growth performance. Sarvari *et al*. (2015) reported that the addition of 0.01% ZnO to the diet significantly increased (P<0.05) BWG (2120 vs. 1973 g), FCR (1.9 vs. 1.96), and FI (4043 vs. 3870 g) in broilers at 42 days of age. Similarly, Sahraei *et al*. (2013) found that supplementing 100-200 ppm of Zn from Bioplex® Zn or $ZnSO_4$ was more effective than control and ZnO in terms of FCR (1.3 vs 1.4) during the broiler grower phase. In Ross-308 broilers, the ZnO-NP (40 ppm) group had a higher (P < 0.001) FCR than the control, ZnO, and Zn-Lys groups. FI decreased linearly (P = 0.003) in Ross-308 broiler chickens when supplemented with Zn lysine (Zn-Lys), ZnO, and ZnO-NPs at a level of 40 ppm in four different groups, and Zn-NPs (40 ppm) improved (P<0.001) BWG compared to ZnO and Zn-Lys supplementation (Alian *et al*., 2023).

Table 1: Effect of Zn on growth performance.

Dose (ppm)	Effect	Reference
40 (Zinc oxide, zinc lysine, and nano zinc oxide)	FI decreased linearly, and BWG and FCR increased	(Alian *et al*., 2023)
40 (Nano zinc oxide and conventional zinc oxide)	No difference in FI, BWG was higher and positive effect on FCR in the nano-zinc group.	(Qu *et al*., 2023)
20, 40 and 60 (Nano-zinc) and 60 (Organic zinc)	No influence on FI, higher BWG was observed in 40 ppm during the finisher phase and significant change in FCR observed in nano-zinc (60 ppm).	(Mozhiarasi *et al*., 2023)
40 and 60 (Nano zinc oxide)	Increased FI, increased BWG at 40 ppm zinc, and better FCR than control	(Hatab *et al*., 2022)
2.5, 5, 10, 20 and 40 (Nano-zinc) and 40 (Inorganic zinc)	2.5 ppm improved FI, BWG and FCR	(Hussan *et al*., 2022)
24, 54, 84 and 114	No influence on FCR	(Zaghari *et al*., 2018)
0.01%	FI increased, and better FCR and BWG	(Sarvari *et al*., 2015)
30, 60, 90 and 120 (Nanoparticles)	Positive effect on FI, BWG and FCR	(Ahmadi *et al*., 2013)
3, 6, and pre-biotic (With or without)	No effect on FI, BWG and FCR in quail	(Abd El-Samee *et al*., 2013)
50, 75, 100 and 125	Positive effect on FI and BWG but on effect on FCR	(Ezzati *et al*., 2013)
20-140 (Inorganic)	No effect on FI, BWG and FCR	(Liao *et al*., 2013)
10, 20, 40, or 80 (Inorganic and chelate)	Better daily FI	(Liu *et al*., 2013)
100-200 (Inorganic and chelate)	Better FCR	(Sahraei *et al*., 2013)
10, 20 or 40 (Organic) and 10, 20, 40 or 100 (Inorganic)	No effect on FI, BWG and FCR	(Vieira *et al*., 2013)
40, 60, 80, or 100 (Inorganic and organic)	No effect on FI, BWG but better FCR in organic zinc treatment	(Yogesh *et al*., 2013)
20, 40, 60 and 80 (Organic and inorganic)	No effect on FI, BWG and FCR	(Anil *et al*., 2012)
40 (Organic)	No effect on FI, BWG and FCR	(Salim *et al*., 2012)
5, 10, 15, 20 and 40 (Organic or inorganic)	No difference in FI, BWG but better FCR	(Star *et al*., 2012)
100 and 150 (Organic or inorganic)	Higher FI, BWG and better FCR	(Sahraei *et al*., 2012)
140 (Inorganic and chelate)	Better FI, BWG in layer birds	(Idowu *et al*., 2011)
60, 120 or 180	Increased daily FI but no effect on BWG and FCR	(Liu *et al*., 2011)
20, 40 and 80	No effect on FI, BWG and FCR	(Salim *et al*., 2011)
60 and 90 (Inorganic and organic)	Better daily FI, BWG and improved FCR	(Feng *et al*., 2010)
40 (complexed zinc) and 80 (Inorganic)	Increase in weight gain and improved FCR	(Saenmahayak *et al*., 2010)

FI: Feed intake; BWG: Body weight gain; FCR: Feed conversion ratio.

Conversely, Liao *et al.* (2013) observed no significant influence (P>0.54) on BWG, FI, and FCR in broilers fed a corn-soya diet containing Zn (20-140 ppm) from $ZnSO_4$. They suggested that the Zn content in the base diet was sufficient for proper broiler growth during the finishing phase of production. Similarly, Vieira *et al.* (2013) discovered that supplementing Zn at levels of 10, 20, or 40 ppm, whether from $ZnSO_4$ or Mintrex® Zn, did not lead to any noticeable change in FI, FCR and BWG at any stage or over the entire duration of the experiment in broiler chickens. This could be attributed to potential Zn recycling through litter consumption or the initial Zn concentrations present in the basal diet, which contained 58, 46, 41, and 38 ppm of Zn in each respective growth phase. Zhang *et al.* (2018) conducted research on Ross 308 broiler chickens supplemented with $ZnSO_4$ at 0, 20, 40, 60, 80, 100, or 120 ppm in the starter diet and 0, 16, 32, 48, 64, 80, or 96 ppm in the grower diet. The results showed that Zn supplementation had no effect (P>0.05) on BWG, FI, and FCR at 21 and 42 d of age. This could be linked to Zn availability and supplementation levels (Leeson and Summers, 2009). This could also be due to the presence of phytate and calcium, which have been shown to form an insoluble combination with Zn, limiting its absorption in the small intestine (Salim *et al.*, 2008).

Mozhiarasi *et al.* (2023) reported that dietary supplementation with organic Zn (60 ppm) or ZnO-NPs (20, 40, 60 ppm) in Cobb-400 broilers had no significant effect (P>0.05) on FI throughout the trial. The BWG was unaffected during the pre-starter and starter phases, but supplementation with 40 ppm ZnO-NPs significantly (P<0.05) improved the BWG during the finisher and overall periods. For FCR, 20 ppm ZnO-NPs differed significantly (P<0.05) from organic Zn and higher ZnO-NP levels during the pre-starter phase, whereas no significant differences were observed among the groups during the starter, finisher, and overall phases. In contrast, Hussan *et al.* (2022) observed that supplementation of 2.5 ppm ZnO-NPs in Cobb-400 broilers significantly (P<0.05) improved FI, BWG, and FCR compared to higher levels of ZnO-NPs (5–40 ppm) and inorganic Zn (40 ppm). The authors attributed these effects to enhanced nutrient digestion, absorption, and higher Zn bioavailability in NPs form, highlighting 2.5 ppm ZnO-NPs as the optimal inclusion level. The BWG was considerably increased by adding Moringa oleifera leaf powder extract stabilized with Zn-NPs (2.0 and 3.0 cm^3/L). The additive had no detrimental effects on feed efficiency, as evidenced by the lack of significant variations in FI and FCR between the treated and control groups (El-Abbasy *et al.*, 2025).

An experiment was conducted by Lail *et al.* (2023) on broilers infected with *Eimeria tenella* and treated with 60 mg/kg ZnO-NPs. In addition to reducing oocyst shedding and the anti-coccidial index, ZnO-NPs considerably (P<0.05) enhanced the growth performance of the infected birds. One possible explanation for this could be that Zn improves the antioxidant capability of chickens. Similarly, Hatab *et al.* (2022) reported that supplementation with ZnO-NPs (40 and 60 ppm) significantly (P<0.05) increased FI and BWG and improved FCR in broiler chickens compared to the basal diet, with 40 ppm showing better effects on body weight gain than 60 ppm. Qu *et al.* (2023) demonstrated that Arbor Acres broilers supplemented with 40 ppm ZnO-NPs (21 and 82 nm) had significantly (P<0.05) higher final BWG and improved FCR compared to conventional ZnO, although no differences were observed in FI. Collectively, these findings suggest that the beneficial effects of Zn supplementation in broilers are mainly attributed to the source (ZnO-NPs vs. conventional/organic Zn), whereas particle size differences exert a negligible influence on performance parameters.

The use of 40 ppm Zn in broiler feed is recommended by the NRC (1994). Some researchers, including those cited in previous studies (Rossi *et al.*, 2007; Salim *et al.*, 2011, 2012), argue that this 40 ppm level is sufficient for optimal broiler growth, with no discernible benefits from increasing Zn levels in poultry diets. However, in broilers fed a corn-soya diet supplemented with Zn propionate (Zn-P), $ZnSO_4$, or ZnO at varying levels (40, 60, 80, or 100 ppm), Yogesh *et al.* (2013) observed no significant (P>0.05) impact on FI and BWG up to 42 days of age. However, a lower FCR (P<0.05; 2.3 vs. 2.4) was observed in the Zn-P group than in the $ZnSO_4$ and ZnO groups. Dibaiee-Nia *et al.* (2017) investigated the effects of a wheat-based diet supplemented with different levels of Zn (20, 40, 60, 80, and 100 mg/kg). There was a significant improvement (P<0.05) in FCR with Zn supplementation, and 60 mg/kg had the best FCR among other treatments.

Effect of zinc levels and sources on carcass characteristics

Responses to Zn supplementation in poultry can lead to considerable variations in the weight and yield of various organs (Table 2). These discrepancies may stem from the diverse carcass characteristics examined in different studies. Mohammadi *et al.* (2015) observed significant differences (P<0.05) in quantitative carcass traits among dietary treatments, which included both dry and wet basal diets supplemented with Zn (100 or 200 ppm) from ZnO-NPs. The dry control diet containing 200 ppm ZnO-NPs resulted in increased carcass weight percentages (60.7 vs. 55.5) compared to other dietary treatments. However, Zaghari *et al.* (2018) found that as Zn supplementation (ranging from 24 to 114 ppm) increased in corn-SBM diets already containing dietary Zn (24 ppm), carcass weights (2053 vs. 1917 g) and percentages (78 vs. 75) decreased

Table 2: Effect of Zn on carcass characteristics.

Dose (ppm)	Effect	Reference
20, 40 and 60 (Nano-zinc) and 60 (Organic zinc)	No effect on water holding capacity but significantly increased the dressing percentage and reduced the meat pH	(Mozhiarasi *et al.*, 2023)
40 and 60 (Nano zinc oxide)	Carcass yield increased significantly and lower gizzard weight observed at 40 ppm	(Hatab *et al.*, 2022)
2.5, 5, 10, 20 and 40 (Nano-zinc) and 40 (Inorganic zinc)	No difference in cook yield, WHC, meat pH, giblet organs, abdominal fat, and breast yield was observed	(Hussan *et al.*, 2022)
100 or 200 (Nanoparticles)	Carcass weights percentages increased by dry control diet with 200 ppm zinc	(F. Mohammadi *et al.*, 2015)
0.01%	No effect on the carcass, abdominal fat, heart, and liver	(Sarvari *et al.*, 2015)
24, 54, 84 or 114	Carcass weights were decreased as zinc supplementation increased	(Zaghari *et al.*, 2018)
100 and 200 (Organic, inorganic, and nano)	ZnO had a lower carcass percentage	(Selim *et al.*, 2014)
30, 60, 90, or 120 (Nanoparticles)	No effect on supplementation	(Ahmadi *et al.*, 2013)
40, 60, 80 or 100	No effect on dressed, eviscerated, and giblet organ weights	(Yogesh *et al.*, 2013)
20, 40, 60, or 80 (Inorganic or organic)	No significant difference in the ready-to-cook yields and the liver weights	(Anil *et al.*, 2012)
40 and 80	No effect on WHC	(Saenmahayak *et al.*, 2012)
60, 120, or 180 (Organic, inorganic, or chelate)	No effect of zinc levels and sources	(Liu *et al.*, 2011)
20, 40 and 80 (Organic)	WHC of the breast was increased	(Salim *et al.*, 2011)

WHC: Water holding capacity.

($P<0.01$). This lack of response could be attributed to the utilization of fiber and phytate sources in the corn-SBM diet. Eskandani *et al.* (2021) reported significant improvements in Zn content of breast muscle, breast meat lightness and redness indexes, as well as breast meat shear force and pH, when supplemented with 70 mg Zn amino acid complex and 30, 50, 70, and 90 mg Zn-NPs. However, 30 mg of Zn-NPs is recommended for supplementation in broilers to enhance quality. In contrast, Sevim *et al.* (2021) reported non-significant results of carcass and meat quality in broilers when supplemented the diet with inorganic Zn and Zn-NPs.

Mozhiarasi *et al.* (2023) found that broilers (Cobb-400) had no effect ($P>0.05$) on water holding capacity (WHC), but significantly increased ($P<0.05$) the dressing percentage and reduced the meat pH when supplemented with ZnO-NPs compared to inorganic and organic Zn. In contrast, Hussan *et al.* (2022) found no differences ($P>0.05$) in the cook, giblet organ, abdominal fat, or breast yield of Cobb-400 broilers at 42 d of age. No significant effect ($P>0.05$) was observed on the WHC and pH of meat when supplemented with ZnO-NPs (2.5, 5, 10, 20, and 40 ppm). Hatab *et al.* (2022) found that the carcass yield increased significantly ($P<0.05$) in the groups with supplemented ZnO-NPs than the control group. The highest carcass yield was observed in the 40 ppm ZnO-NP-treated group. A significantly lower ($P<0.05$) relative gizzard weight was observed in the 40 ppm ZnO-NP group than in the control group, but the 60 ppm ZnO-NP group showed no difference from the control group. No significant intergroup differences were observed in the heart weight of the broilers. Dong *et al.* (2023) supplemented methionine hydroxyl analog chelated Zn in broiler diets at a dose of 90 mg/kg in low-protein diets and found non-significant results regarding carcass and meat quality. In an experiment, Abd El-Hack *et al.* (2024) supplemented the basal diets with 0.2 and 0.4 mg Zn-NPs/kg diet and found significant improvement in carcass and meat quality. Treatment with 0.4 mg Zn-NPs/kg enhanced dressing percentage and meat quality.

The variability in the effects of dietary Zn on factors such as carcass characteristics in birds may be attributed to the initial Zn content in the base diet or the quantity and sources of Zn to be added.

Effect of zinc levels and sources on the weight of immune organs

Zinc plays a vital role in supporting cell proliferation, especially in the immune system, and influences both innate and acquired immune functions. Ebrahimnezhad *et al.* (2013) found that administering over 90 ppm of Zn from ZnO-NPs resulted in improved ($P<0.05$) bursa and thymus weights in broilers at 42 days of age compared to lower levels (30 and 60 ppm) and a control diet (Table 3).

Table 3: Effect of Zn on the weight of immune organs.

Dose (ppm)	Effect	Reference
40 and 60 (Nano-zinc)	Significantly increased the spleen, bursa, and thymus weights	(Hatab *et al.*, 2023)
40 (Nano zinc oxide and conventional zinc oxide)	Spleen and thymus index were higher in nano-zinc groups	(Qu *et al.*, 2023)
100 or 200 (Nanoparticles)	No difference among treatments on the weight of the spleen and bursa	(F. Mohammadi *et al.*, 2015)
15 (Inorganic), 7.5 and 15 (Organic) and (0.3, 0.06 and 0.03 (Nano-Zn)	The higher weight of the spleen	(Sahoo *et al.*, 2014)
Coccidiosis vaccine (With or without 40, 120, or 200 zinc)	Weights of the bursa and spleen were higher	(Sajadifar *et al.*, 2013)
30, 60 and 90 (Nanoparticles)	Improved the weights of the bursa and thymus	(Ebrahimnezhad *et al.*, 2013)
20, 40 or 60	The weight of the spleen and bursa were not influenced but liver weight was higher at 60 ppm in broiler breeders	(Soni *et al.*, 2013)
40, 120 and 200	Increase in weight of bursa and spleen	(Sajadifar *et al.*, 2011)
30, 60 and 90	Spleen, thymus, and bursa indexes were higher at 90 ppm of the starter diet	(Feng *et al.*, 2010)

Moreover, Soni *et al.* (2013) determined that dietary treatments (20, 40, or 60 ppm Zn methionine) had no significant impact on spleen and bursa weights (P>0.05) in broiler breeders aged 32–48 weeks. However, liver weight (P<0.01) was higher in birds fed a 60-ppm Zn diet compared to the control group (29.8 ppm dietary Zn + 10.2 ppm $ZnSO_4$) and lower supplementation levels. Similarly, Yusof *et al.* (2023) reported that supplementation with ZnO-NPs had no effect (P>0.05) on the barsa of Fabricius weight. However, an increase in the weight of the spleen and thymus was observed in groups supplemented with higher levels of ZnO-NPs (40, 70, and 100 mg/kg) compared to the control group (ZnO).

Qu *et al.* (2023) found that the Arbor Acres broilers fed diets containing 40 ppm ZnO-NPs and conventional ZnO had a higher (P<0.05) spleen index than the negative control group. The thymus index was higher (P<0.01) in the ZnO-NP-treated groups (82 and 21 nm) than in the negative control group, whereas the bursa of Fabricius index remained the same between the groups. Hatab *et al.* (2023) found that 40 and 60 ppm Zn from ZnO-NPs significantly increased the spleen, bursa, and thymus weights as compared to the un-supplemented group in the Ross-308 broiler chickens.

In a distinct study involving broiler chicks at 7 weeks of age, relatively higher bursa weights were achieved with 8 ppm of Zn supplementation than with higher Zn concentrations (up to 125 ppm). Mohammadi *et al.* (2015) reported no statistically significant differences (P>0.05) in weight indexes for the spleen and bursa of Fabricius among the experimental treatments during the broiler starter phase, which included two basal diets (one dry and one wet) supplemented with either 100 or 200 ppm of Zn from ZnO-NPs. The lack of distinction between dietary treatments may be attributed to the unique characteristics of ZnO-NPs, including high particle kinetics, reactivity, contact surface, and diet form (wet or dry).

El-Katcha *et al.* (2017) performed an experiment on broilers, supplemented the basal diet with organic zinc (60, 45, 30, or 15 ppm) and ZnO-NPs (60, 45, 30, or 15 ppm), and found significant improvement (P<0.05) in lymphoid organ weight. In contrast, Jain *et al.* (2021) found a non-significant (P>0.05) response in immune organs (bursa of Fabricius and spleen) when supplemented with organic (20 and 40 ppm) and inorganic Zn (40 ppm). However, the Zn treatments had higher weights of immune organs than the control.

Effect of zinc levels and sources on antibody titer

The reassessment of Zn requirements exceeds the growth-related criteria and encompasses immunity. Multiple studies have shown that Zn concentrations exceeding NRC (1994) recommendations are necessary to enhance both humoral and cellular immune responses. Ezzati *et al.* (2013) found that different Zn levels (ranging from 50 to 125 ppm) from $ZnSO_4$ improved humoral immune responses against ND virus (P<0.05). For broilers, methods such as hemagglutination inhibition (HA), hemagglutination inhibition (HI) (8.4 compared to 6.1), and enzyme-linked immunosorbent assay (ELISA) (14800 compared to 9863) did not exhibit improvements with Zn levels exceeding 100 ppm (Table 4). Similarly, Jain *et al.* (2021) found significant (P>0.05) results for antibody titers (IgG) between all treatment groups. A significantly

Table 4: Effect of Zn on antibody titer.

Dose (ppm)	Effect	Reference
40 (Zinc oxide, zinc lysine, and nano-zinc oxide)	Total antibody titer increased in the zinc lysine and nano-zinc oxide groups	(Alian *et al*., 2023)
40 and 60 (Nano-zinc)	Antibodies against Newcastle disease virus and Sheep red blood cells increased	(Hatab *et al*., 2023)
30 (Organic or nanoparticles)	The increase in antibody titers and cellular immune response was higher	(Sahoo *et al*., 2014)
3 or 6 (With or without prebiotic)	Better humoral response in Japanese quails	(Abd El-Samee *et al*., 2013)
50, 75, 100 or 125	Humoral immune response against NDV was improved	(Ezzati *et al*., 2013)
20, 40 or 60	Higher primary antibody titers and cellular immunity in broiler breeder	(Soni *et al*., 2013)
40, 120, and 200 (With and without vaccine)	Increase in the antibody titers against NDV	(Sajadifar *et al*., 2013)
10, 20 or 40	No effect on antibody titers against Bovine serum albumin	(Vieira *et al*., 2013)
40, 60, 80 or 100	No effect on cellular and humoral immune response	(Yogesh *et al*., 2013)
40, 120 and 200	Higher antibody titers against NDV	(Sajadifar *et al*., 2011)
30, 60, 90 or 120	Concentrations of Ig-G and IgM were increased in the starter phase	(Feng *et al*., 2010)

NDV: Newcastle disease virus, Ig: Immunoglobin.

(P <0.05) higher concentration of plasma IgG was reported in organic (20 and 40 ppm) and inorganic Zn (40 ppm) as compared to the control. In contrast, Rezapour *et al.* (2024) found non-significant results (P>0.05) of antibody titer against Infectious Bursal Disease (IBD) and lower antibody titer was observed (P<0.05) against bronchitis when supplemented with organic zinc (40 mg/kg feed).

In the study conducted by Soni *et al.* (2013), breeders fed a diet supplemented with Zn methionine displayed higher (P<0.05) primary antibody titers (6.4 compared to 4.3) against SRBC than those fed a control diet containing 40 ppm of Zn (29.8 dietary + 10.2 ppm from $ZnSO_4$). At 48 weeks of age, breeders consuming a diet containing 100 ppm Zn exhibited higher cellular immunity to PHA-P (291 compared to 166) than those consuming lower levels. According to Ma and Ali (2025), 90 mg/kg Zn methionine supplementation had a significant effect (P<0.05) on the immune response in broilers vaccinated against ND. In a study by Vieira *et al.* (2013), the supplementation of Zn (at levels of 10, 20, or 40 ppm) with either $ZnSO_4$ or Mintrex® Zn had no effect on antibody titers against BSA in broilers. This discrepancy in results might be attributed to the specific antigens used in the different immune protocol trials. However, Anoh *et al.* (2025) reported that the supplementation of ZnO in water at a dose of 200 mg/L with vaccine significantly (P<0.05) increased the HI antibody titer against ND and IBD in broiler chickens.

Hatab *et al.* (2023) found that the antibodies produced against ND virus and SRBC increased in the ZnO-NPs (40 and 60 ppm) groups than the un-supplemented control group in Ross-308 broilers at all test times. Sahoo *et al.* (2014) found that birds fed a basal diet containing 30 ppm of dietary Zn, along with supplementation of Zn methionine or ZnO-NPs, exhibited higher antibody titers (8.67 compared to 5.5) against SRBC compared to birds fed the basal diet or $ZnSO_4$. Additionally, at 42 d of age, the cellular immune response (P<0.05) to PHA-P injection was significantly greater (171 vs. 121) in birds fed diets supplemented with organic Zn and ZnO-NPs than in birds fed other diets. According to Alian *et al.* (2023), different dietary sources (Zn oxide, Zn lysine, and nano-Zn) at a concentration of 40 ppm did not affect (P=1.00) the antibody titer against the ND vaccine on day 23 compared to the control group. On day 35, the total antibody titer increased in the Zn-Lys and ZnO-NP groups.

The variations in immune responses observed in different studies could be attributed to the specific types of antigens (Pimentel *et al*., 1991) used in each study, such as sheep red blood cells, bovine serum albumin, ND, and PHA-P.

Effect of mutual supplementation of zinc with other minerals on growth, carcass characteristics, and immune functions

Growth performance

El-Husseiny *et al.* (2012) found that broilers fed diets containing 100 percent organic Zn and manganese (Mn) and 50 percent organic Cu exhibited higher (P<0.001) FI (3349 compared to 3125 g), BWG (1374 compared to 1179 g), and FCR (2.8 compared to 4.7) than those fed other combinations of organic minerals (Table 5). Similarly, Sunder *et al.* (2013) observed no effect on FI, BWG, or FCR

Table 5: Effect of interaction of Zn on growth, carcass characteristics, and immune functions when supplemented with other minerals.

Dose (ppm)	Effect	Reference
Growth performance		
24, 54, 84, or 114 (Zinc) and 100, 200, or 300 FTU/kg (Phytase)	No effect on feed intake and weight gain	(Zaghari *et al*., 2018)
40 or 80 (Zinc) and Cr (0.5 or 1)	Better feed conversion ratio and FI in quail	(Rouhalamini *et al*., 2014)
37, 70, or 175 (Zinc) and 200, 400, and 600 (Vitamin C)	Higher FI and weight gain but lower FCR in heat stress	(Al-Masad, 2012)
50 or 100 (Zinc), 60 or 120 (Manganese), and 8 or 16 (Copper)	Improved FI, weight gain, and FCR	(El-Husseiny *et al*., 2012)
Carcass characteristics		
50 or 100 (Zinc), 60 or 120 (Manganese), and 8 or 16 (Copper)	Improved dressing, liver, and breast percentages but decreased wings (%)	(El-Husseiny *et al*., 2012)
Zinc, Chromium and Selenium	Improved carcass characteristics	(Sobhi *et al*., 2020)
Immune functions		
40, 80 and 160 (Zinc) and 60, 120 and 240 (Manganese)	Better antibody production but cellular immune response was not influenced	(Sunder *et al*., 2013)
37, 70, or 175 (Zinc) and 200, 400, and 600 (Vitamin C)	Higher spleen and bursa weights and total antibodies	(Al-Masad, 2012)
40, 80, and 120 (Zinc) and 35, 70, and 105 (Copper)	No interaction on immune organ weights and antibody responses	(Arshami *et al*., 2010)

FI: Feed intake, FCR: Feed conversion ratio

in broilers up to 35 days of age when various combinations of organic Zn and Mn were added to a corn-SBM basal diet. It was presumed that the mineral concentrations in the basal diet were adequate for optimal growth. However, Sherif *et al*. (2024) conducted an experiment to determine the effect of dietary supplementation of *Chlorella vulgaris* (1 g) with ZnO-NPs (40 ppm) and selenium (Se) NPs (0.3 ppm) on broiler chicken performance. The results showed that the ZnO-NPs performed better (P<0.05) with *Chlorella vulgaris* than the synergistic supplementation with Se-NPs in terms of BWG. However, FCR was better in the treatment with ZnO-NPs and Se-NPs with *Chlorella vulgaris*. Similarly, Santos *et al*. (2021) added hydroxy-chloride Zn (80, 100, and 120 ppm) and Cu (15 and 150 ppm) to broiler chicken diets. These findings indicate that on day 42, the FCR was higher for birds supplemented with Cu (150 ppm) in diets containing Zn (100 ppm). The effect of feeding broilers sulfate or hydroxy-chloride forms of Zn and Cu at 15 and 80 ppm Zn levels was examined by Olukosi *et al*. (2018). None of the growth performance responses exhibited a significant source × mineral level relationship. On the other hand, broiler chickens that received hydroxy-chloride Zn and Cu had higher FCR (P<0.05), while those who received lower Zn levels had higher BWG (P<0.01).

Al-Masad (2012) found that broilers fed diets containing Zn and vitamin C at ratios of 35:600 and 70:200 ppm, respectively, exhibited higher FI (P<0.05) (773 compared to 637 g) and BWG (1992 compared to 1601 g) than other combinations when exposed to heat stress conditions (40°C) during the finisher phase. At the 4th, 5th, or 6th weeks of age, Zaghari *et al*. (2018) observed no effect on FI or BWG when Zn was supplemented with phytase in a diet containing 24 ppm of dietary Zn. The lack of reaction could be linked to the use of fiber and phytate sources and the lower bioavailability of ZnO compared to other Zn sources. Franklin *et al*. (2022) performed experiment to check the commercial premixes provide trace minerals such as Zn, Mn, and Cu (inorganic, organic and hydroxy) at 40, 60 and 8 ppm dose, respectively. The results revealed that the use of hydroxy trace minerals in broiler diets can maintain broiler performance and significantly reduce the negative impact on the environment (P<0.05). In broilers exposed to the Eimeria challenge, Chen *et al*. (2022) discovered that Zn:Cu:Mn in mineral methionine hydroxy-analog bis-chelate form (40:10:50 ppm) enhanced growth performance in contrast to high levels of inorganic trace minerals. These benefits may be attributable, in part, to an improved immunological response to coccidiosis as a result of increased bioavailability and antioxidant capacity.

Carcass characteristics

El-Husseiny *et al*. (2012) enhanced a diet with 100% inorganic Zn, Mn, and Cu, or 50% or 100% organic mineral sources. The diet containing 50% of each mineral (Zn, Mn, and Cu) resulted in enhancements in dressing percentage, liver weight, and breast meat percentage, but caused a decrease in wing meat percentage compared to the diet containing inorganic supplementation. This combined supplementation showed no significant effect (P<0.001) on the heart, gizzard, thigh meat, inedible parts, eviscerated yield, and giblet percentages. Sobhi *et al.* (2020) experimented on the Ross-308 broiler chickens and discovered that those fed yeast-based Zn, Se, and Cr had increased carcass weight, dressing %, and breast and thigh yield (P<0.05) compared to the control group. In the case of Zn, a low level (80 mg/kg) of supplementation was sufficient to attain good carcass traits.

Kwiecień *et al.* (2016) studied the effects of dietary ZnO (100 mg kg^{-1}) and Zn glycine chelate (25, 50, or 100 mg kg^{-1}) supplementation on male broilers (Ross 308) and found a positive trend in the weight of breast, thigh, and drumstick muscles when organic Zn was introduced, especially at doses of 50 and 25 mg. Similarly, Santos *et al.* (2021) concluded that the interaction between hydroxy-chloride Zn (80, 100, and 120 ppm) and Cu (15 and 150 ppm) had no effects on carcass characteristics, except the abdominal fat % in Cobb 500 broiler chickens. However, Olukosi *et al.* (2018) conducted an experiment in broilers to investigate the effect of supplementation with sulfate or hydroxy-chloride forms of Zn and Cu at 15 and 80 ppm Zn levels. The study found no significant association between the source and level of meat yield. Broiler chicks receiving hydroxy-chloride Zn and Cu exhibited a higher breast yield percentage (P < 0.05) than those receiving sulfate Zn and Cu.

Immune functions

Sunder *et al.* (2013) observed that the interaction between Zn and Mn had a significant impact on spleen weights (P<0.05), showing an increase in spleen weights when 120 ppm Mn was combined with all levels of Zn supplementation. They also noted that the combination of 80 ppm Zn and 240 ppm Mn resulted in higher antibody production (P<0.05) against SRBC than the other combinations. However, the Zn: Mn combination did not significantly affect the cellular immune response to PHA-P. In contrast, Arshami *et al.* (2010) found no interaction between different Zn (40, 80, and 120 ppm) and Cu (35, 70, and 105 ppm) levels on the weights of immune organs or the primary and secondary antibody responses against SRBC at 42 days of age. Similarly, Jain *et al.* (2021) supplemented inorganic and organic Zn (40 mg/kg), inorganic and organic Se (0.30 mg/kg), and inorganic and organic chromium (Cr) (2 mg/kg of feed). The results showed a non-significant change in the weight of the immune organs (bursa of Fabricius and spleen). Rezapour *et al.* (2024) found non-significant results (P>0.05) of antibody titer against IBD and higher antibody titer was observed (P<0.05) against bronchitis when supplemented with organic Zn, Mn, and Cr (40 mg, 30mg, and 2mg/Kg feed, respectively) than the other treatments.

Al-Masad (2012) discovered that supplementing combinations of Zn and vitamin C in the diet of heat-stressed birds (40 °C) during the finisher phase led to increased spleen and bursa weights (P<0.05), as well as higher primary antibody responses for IgG, IgM, and total antibodies (4.65 vs 2.05), and secondary antibody responses for IgG, IgM, and total antibodies. Similar results were reported by Faghih-Mohammadi *et al.* (2023), when chelated forms of Zn, Cu, iron, manganese, and Se were added at 110, 10, 50, 120, and 0.3 mg kg^{-1} in broiler breeder chicken diets. There was improvement in immune system of broilers fed experimental diets containing chelated forms of Zn, Fe, Cu, Se, and Mn and a basal diet containing 50% of the minerals Zn, Fe, Cu, Se, and Mn in the sulfate form and 50% in the chelate form. In contrast, Jankowski *et al.* (2019) examined the efficacy of feeding turkeys a combination of Cu (2 and 20 mg), Zn (10 and 100 mg), and Mn (10 and 10 mg) NPs. The study demonstrated that the antioxidant and immunological defense of turkeys was unaffected by the addition of Cu, Zn, and Mn to their diets in conventional forms and as NPs at 2, 10, and 10 mg Cu, Zn, and Mn, respectively. Likewise, a lack of additional Cu, Zn, and Mn in the turkey diet did not significantly influence immunity and even lowered the quantity of MDA in the small intestine and liver. Alterations in the redox status of turkeys whose diets were not supplemented with Cu, Zn, or Mn could imply decreased oxidation processes in the tissues.

Effect of zinc supplementation on growth, carcass characteristics, and immune functions in a hot environment

Growth performance

The Zn was recognized for its potential to alleviate the negative effects of heat stress in animals, although there are inconsistencies in the available evidence supporting this particular role of Zn. Salabi *et al.* (2011) observed that broilers provided with a diet containing 90 ppm of Zn displayed higher levels of FI (4481 vs 4417 g), BWG (2459 vs 2370 g), and an improved FCR of 1.82 compared to broilers on a diet with adequate Zn (45 ppm of Zn) at 42 days of age (Table 6). Similarly, Zhu *et al.* (2017) reported that supplementation of 110 mg/kg of Zn as either inorganic or organic Zn in the maternal di*et al*leviated the negative effect of maternal heat stress (32 ± 1°C) on the growth performance of offspring during the starter period. In a study, Waeothong *et al.* (2021) found that the supplementation of ZnO (40 ppm) and $ZnSO_4$ (40, 500 and 1000 ppm) improved the performance of the broiler chickens under heat stress (30 °C), and suggested 40 ppm Zn, an optimal dose for the supplementation.

Table 6: Effect of Zn supplementation on growth, carcass characteristics, and immune functions in a hot environment.

Dose (ppm)	Effect	Reference
Growth performance		
40 or 80	Linearly improved the feed conversion ratio in quails	(Rouhalamini *et al*., 2014)
45, 90 and 135	Improved feed intake, weight gain, and feed conversion ratio	(Salabi *et al*., 2011)
Carcass characteristics		
40 or 80	Increased the pH and water-holding capacity of meats in quails	(Rouhalamini *et al*., 2014)
3 or 6 (Organic with or without prebiotic)	No influence on the carcass, liver, gizzard, alimentary canal, and heart weights in quails	(Abd EL Samee *et al*., 2013)
45, 90 or 135	No influence on breast and thigh meat yields	(Salabi *et al*., 2011)
Immune functions		
40 or 80	No effect on the weights of the spleen, bursa, and thymus in quails	(Rouhalamini *et al*., 2014)
3 or 6 (With or without prebiotic)	No influence on the bursa of Fabricius, thymus, spleen, and thyroid gland weights in quails	(Abd EL Samee *et al*., 2013)
45, 90 and 135	Heavier bursa but liver weight was decreased and spleen weight was not affected	(Salabi *et al*., 2011)

Conversely, Zaghari *et al.* (2022) revealed that supplementing the diet with HiZox® (75, 100, and 125 ppm of Zn) or ZnO (100 ppm of Zn) had no significant effect on BWG and FI in broilers under heat stress. However, heat-stressed broilers receiving different dosages of HiZox® had 2.85% lower mortality than the normal ZnO group (P<0.06).

Rouhalamini *et al*. (2014) discovered that quails receiving diets supplemented with 40 ppm of Zn and 1 ppm of Cr had an improved FCR (2.6 vs 3.2) compared to other combinations. Moreover, when both minerals were supplemented together, quails exhibited better intake than when each mineral was fed separately. They also found that providing Zn to Japanese quails in a heat-stressed environment led to an improved FCR compared to diets without Zn supplementation. Al-Masad (2012) also reported that heat-stressed broilers fed diets with Zn: vitamin C ratios of 70:200 ppm and 35:400 ppm had a lower FCR (2.4 vs. 3.1) than those fed control diets and other combinations during the finisher phase. Ramiah *et al*. (2019) found lower FI and FCR in the broiler chickens fed 100 ppm ZnO-NPs under heat stress (34 ± 1°C) than the control from 1 to 42 day of experimental trial. Shah *et al*. (2018) performed an experiment on the broiler chicken and observed that the supplementation of Zn (30 and 60 mg $ZnSO_4$) singly or in combination with probiotic improved (P<0.05) the BWG and FCR in the heat stressed birds. In a study by Saleh *et al*. (2018), Zn methionine (25, 50, and 100 mg/kg) supplementation considerably improved broiler growth performance by increasing BWG and FCR (P<0.05) at high ambient temperature. One possible explanation for such benefits could be that Zn is crucial in maintaining the structure of metalloproteins such as IGF-1 (Khan *et al*., 2014; Saleh *et al*., 2012).

Carcass characteristics

Rouhalamini *et al*. (2014) noted that supplementing Zn (40 or 80 ppm) resulted in an elevation of pH (5.50 vs 5.25) and WHC (65.6 vs 63.1 percent) in the meat of Japanese quails in the heat-stressed environment. Conversely, Akhavan-Salamat and Ghasemi (2019) reported that the ZnO, Zn methionine, and ZnO-NPs at 20, 40, and 80 mg/kg had no effect on the carcass traits of the male broiler chickens (Ross 308) under heat stress. De Grande *et al*. (2022) conducted an experiment to examine the effect of Zn (60 ppm organic or inorganic) and vitamin E (50 or 100 IU/kg) supplementation on broilers under heat stress. The results revealed that organic Zn can improve breast meat yield and increase the WHC of broiler breast meat under chronic cyclic heat stress compared to inorganic Zn. Furthermore, the beneficial effects of organic Zn on breast meat yield and quality appear to be independent of vitamin E. Abd El-Samee *et al*. (2013) also found that supplementing organic Zn with or without prebiotics in the diet of heat-stressed Japanese quails (heat-stressed) did not influence carcass, liver, gizzard, alimentary canal, and heart weights. It is worth noting that in this study, both the temperature and humidity levels were higher, which could explain the absence of a performance response. Similarly, Zhu *et al*. (2017) observed non-significant results for carcass characteristics in broiler offspring when breeder hens were supplemented with 110 mg/kg Zn (inorganic and organic Zn). However, progeny meat quality improved due to organic Zn supplementation in the maternal diet. The non-significant results in carcass characteristics showed that Zn supplementation reduced the heat stress effect in the progeny.

Immune functions

Salabi *et al*. (2011) noted that broilers receiving diets containing 135 ppm of Zn from $ZnSO_4$ had a higher bursa weight (0.15 vs 0.12 percent of body weight) compared to heat-stressed broilers fed diets with 45 and 90 ppm of Zn. As Zn levels in the diet increased, liver weight decreased ($P<0.05$), whereas spleen weight remained unchanged. The reduction in liver weight might be attributed to decreased FI, leading to fewer nutrients available for liver and spleen growth. Abd El-Samee *et al.* (2013) reported that supplementing organic Zn with or without prebiotic in a basal diet during the growing phase of Japanese quails under heat stress did not affect the weights of the bursa of Fabricius, thyroid gland, spleen, and thymus. Notably, both the humidity and temperature were higher in this study, which could explain the absence of a performance response. However, Akhavan-Salamat and Ghasemi (2019) observed that delivering Zn in the form of Zn methionine and ZnO-NP at a dose of 40 mg/kg of feed could improve the immunological response and antioxidant status of broiler chickens grown under high ambient temperatures.

Hu *et al.* (2024) the author stated that supplementation with organic and inorganic Zn (30, 60, and 90 ppm) significantly enhanced serum antioxidant levels in Xueshan hens, with better superoxide dismutase and glutathione peroxidase activities, and that organic Zn was more efficient than inorganic Zn. However, Rouhalamini *et al.* (2014) found that Zn supplementation (40 or 80 ppm) had no effect on the weights of the thymus, bursa, and spleen in Japanese quails under heat stress. However, Shah *et al.* (2018) observed that that the supplementation of Zn (30 and 60 mg $ZnSO_4$) singly or in combination with probiotic increased ($P<0.05$) the area of cecal tonsils and bursa of Fabricius in the heat stressed birds (35± 1 °C). Ramiah *et al.* (2019) conducted a heat stress experiment on broiler chickens and proposed supplementation with 40 and 60 ppm ZnO-NPs to reduce corticosterone levels, which could be linked to Zn's antioxidant and antistress ability.

Effect of zinc supplementation on gene expression

Zn is a trace element that plays crucial roles in gene transcription, DNA synthesis, gene expression, RNA synthesis, and cellular division (Prasad, 1991). Sahin *et al.* (2009) emphasized its significance in poultry diets for mitigating the effects of heat stress. There is limited scientific literature on the impact of ZnO-NPs on poultry performance under heat stress conditions. Ramiah *et al.* (2020) found that supplementation of Zn-NPs increased mRNA levels of ghrelin and cholecystokinin in the gut (Table 7). In comparison to regular ZnO, dietary ZnO-NPs significantly elevated IgG and ghrelin levels in aged layers (Mao and Lien, 2017). Hu *et al.* (2016) reported that broiler chickens receiving Zn supplements exhibited

Table 7: Effect of Zn supplementation on gene expression.

Dose (ppm)	Effect	Reference
40 (Zinc oxide, zinc lysine, and nano-zinc oxide)	Nano-zinc increased mRNA expression of insulin-like growth factor-I in the liver tissues	(Alian *et al.*, 2023)
100 and 130	Improved the tight junction protein expression	(Barzegar *et al.*, 2022)
15, 30 and 60	Upregulated the levels of antioxidant enzymes and pro-inflammatory cytokines in quails	(El-Bahr *et al.*, 2020b)
40 and 80	Increased the activity of superoxide dismutase and catalase but decreased the concentration of malondialdehyde	(Hafez *et al.*, 2020)
40, 60 and 100	Altered the gene expression of ghrelin, heat shock protein-70, heat shock protein-90, and cholecystokinin	(Ramiah *et al.*, 2020)
40, 60 and 100	Raised the serum corticosterone levels	(Ramiah *et al.*, 2019)
15, 30, 60, 120, and 240	Increased the tight junction protein and mRNA levels of barrier-related genes in pekin ducks	(Wen *et al.*, 2018)
50 (Organic and nano-ZnO)	Influenced the levels of MDA, SOD, and mRNA expression of growth hormones and insulin	(Ibrahim *et al.*, 2017)
80 (Organic, inorganic and nanosized)	Nano-zinc increased immunoglobin-G and ghrelin level in brown laying hens	(Mao and Lien, 2017)
120	Reduced ghrelin mRNA expression	(Hu *et al.*, 2016)
20, 70 and 320	Decreased tumor necrosis factor-α and active A20 abundance in broiler breeder	(Li *et al.*, 2015)
30, 60, 90 or 120 (Nanosized)	Lower levels of MDA and higher levels of SOD and CAT	(Ahmadi *et al.*, 2014)
20, 60 and 100 (Nanosized)	Lower levels of MDA and higher levels of SOD but no effect on CAT	(Zhao *et al.*, 2014)

SOD: Superoxide dismutase, MDA: Malondialdehyde, CAT: Catalase reduced ghrelin mRNA expression in hypothalamic tissue. Broiler chickens receiving the highest levels of ZnO-NPs showed increased levels of heat shock protein 90 mRNA expression in the glandular stomach and duodenal tissue samples (Ramiah *et al.*, 2020). Same findings were reported by Ramiah *et al.* (2019), who showed that a feed containing 100 ppm ZnO-NPs increased serum corticosterone levels. Dietary ZnO-NPs altered the expression of genes for heat shock protein 70 (in the jejunum and ileum), cholecystokinin (in the ileum), and heat shock protein 90 (duodenum, jejunum, and ileum). The interaction between temperature and ZnO-NPs concentration in the duodenum and stomach affected the expression of ghrelin (Ramiah *et al.*, 2020).

Chickens fed diets containing ZnO-NPs displayed decreased malondialdehyde levels and increased superoxide dismutase and catalase activity. Zn also influences the mRNA expression of growth hormone and insulin genes (Ahmadi *et al.*, 2014; Ibrahim *et al.*, 2017; Zhao *et al.*, 2014). In Japanese quails, all tested doses of ZnO-NPs significantly elevated the mRNA levels of superoxide dismutase 1, catalase, glutathione peroxidase 1, interleukin 6, and interferon alpha genes in the liver and brain tissues compared to the control. However, the expression of glutathione peroxidase-7 in the brain tissue remained unchanged in Japanese quails in the control group fed ZnO-NPs (El-Bahr *et al.*, 2020). The antioxidant impact of ZnO-NPs in Japanese quail liver and brain tissues was confirmed at the molecular level, with a notable increase in antioxidant enzyme genes (superoxide dismutase 1, catalase, and glutathione peroxidase 1). Broilers supplemented with ZnO-NPs exhibited significantly increased antioxidant enzymes, superoxide dismutase, and catalase activity, along with reduced malondialdehyde concentration (Hafez *et al.*, 2020).

Supplementation with either small or large ZnO (100 and 130 ppm) improved the expression of tight junction proteins zonula occludens-1 and occludin, reducing intestinal inflammation (decreased tumor necrosis factor) in breeder chickens (Ross-308) (Barzegar *et al.*, 2022). Wen *et al.* (2018) experimented on ducks and found that Zn added to diets increased (P<0.05) the transcription of tight junction proteins claudin1, occludin, zonula occludens-1, and zonula occludens-3 in the jejunum. The mRNA levels of claudin2 (leak protein) decreased with increasing dietary Zn levels at 14 and 35 days of age. Zn supplementation increased (P<0.05) the mRNA levels of the chemical barrier-related genes mucin2 and trefoil factor-2 in the jejunum at 14 and 35 days of age, as well as the transcription of immunological barrier-related genes immunoglobin A, polymeric immunoglobulin receptor, lysozyme, and avian β-defensin 2. Environmental conditions, such as intestinal infections, low-quality feed ingredients, and variations in feed formulation, can induce gut inflammation during commercial production (Kogut *et al.*, 2018). The innate immune system triggers inflammation in response to infection, potentially harmful chemicals, and host damage. This leads to the synthesis of various cells and pro-inflammatory molecules, such as tumor necrosis factor (Barton, 2008). Stress may increase the expression of pro-inflammatory cytokines, but the harmful effects of inflammation can be mitigated by anti-inflammatory substances such as Zn (Barzegar *et al.*, 2022). Zn has been shown to shield cells from tumor necrosis factor α-induced breakdown of the monolayer cell layer (Hennig *et al.*, 1993). Increased Zn levels in the diet reduce the number of inflammatory components in hens susceptible to intestinal inflammation due to once daily feeding (Barzegar *et al.*, 2022). Alian *et al.* (2023) discovered that the ZnO-NPs at the concentration of 40 ppm linearly increased (P<0.001) the mRNA expression of IGF-I in the liver tissues of Ross-308 broiler chickens as compared to the ZnO, Zn-Lys, and control (un-supplemented) groups.

Research suggests that adding Zn to the diet of broiler breeders can enhance the intestinal morphological traits of offspring by causing DNA hypomethylation and hyperacetylation of histone H3 at lysine 9 (H3K9) at the A20 promoter region. Organic Zn added to the mothers' diet showed greater attenuation of gut impairment but decreased tumor necrosis factor-α and active A20 abundance compared to inorganic Zn (Li *et al.*, 2015).

CONCLUSION

This review concludes that Zn supplementation in poultry diets can enhance growth performance, health status, and meat quality. The bioavailability of Zn-NPs form surpasses that of conventional Zn sources, both organic and inorganic, likely due to its size, availability, and absorption characteristics. Furthermore, Zn from organic sources exhibits greater bioavailability than Zn from inorganic sources. Dietary Zn sources significantly influence gene expression and the management of heat stress, and they can be effectively combined with other minerals to improve performance. Zinc supplementation bolsters the immune system of birds, potentially by increasing the weights of immune organs. Additionally, Zn enhances the absorptive capacity of the small intestine in poultry. This review also highlights Zn's role in protecting cells against free radical damage, suggesting its inclusion in poultry feed to mitigate oxidative stress. Although the exact way Zn benefits poultry is not fully understood, it is thought to improve gut metabolism and enzyme activity, support feed intake and nutrient use, strengthen gut immunity, and boost antioxidant enzyme production. However, existing literature presents conflicting findings regarding the effects of Zn supplementation on performance, health, and meat quality in birds, necessitating further investigation. These discrepancies may arise from variables such as chicken age, sex, breed, broiler strains, dietary Zn content, environmental factors, and rearing conditions.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad.

NOVELTY STATEMENT

This review highlights the supplemental effect of different sources and levels of Zinc in poultry birds. The importance of Zinc in growth performance, health, quality and immunity under stress conditions and at genetic level.

AUTHOR'S CONTRIBUTION

MA, MS and SH: Wrote the manuscript.
MMAH and WA: Edited the final version of the manuscript.
HT and MIS: Prepared the figures and tables of the manuscript.
SH: Correspondence.
All authors contributed to the revision of the manuscript, intellectual content, and approved the manuscript for publication.

Generative AI and AI-assisted technology statement

The authors declare the use of AI in enhancing the writeup of the content. AI was employed ethically to improve quality and transparency while respecting academic standards.

Conflict of interest

The authors have declared no conflict of interest.

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