Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 4, 448-455

Potential dietary of manganese, zinc and copper nanoparticles supplementation in improving growth performance, blood metabolites and cecal short-chain fatty acids of broilers

Samar M. Amein[1], Hassan A. Abdel-Raheem[2,3], Abdel-baset N. Ahmed[2,3], Mohamed Karmi[4], Fares A. Eldeeb[1*]

[1]*Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.*
[2]*Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71515, Egypt.*
[3]*Veterinary and Public Health Department, School of Veterinary Medicine, Badr University in Assiut, Assiut, Egypt.*
[4]*Food hygiene Department, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.*

ARTICLE INFO

Recieved: 16 July 2025

Accepted: 21 August 2025

*Correspondence:

Corresponding author: Fares A. Eldeeb
E-mail address: FaresAli@vet.aswu.edu.eg

Keywords:

Nano-minerals, Inorganic, Blood metabolites, SCFAs, Broilers.

ABSTRACT

A 35-day experiment was carried out to study the effect of replacement inorganic Mn, Zn and Cu by nanoparticles sources (either separately or in combination) in broilers diet on performance of growth, parameters of serum biochemistry and cecal short-chain fatty acids. A total number of 120 one day old, unsexed broiler chicks (Ross 308) were divided into five groups at random, with 24 chicks in each of the three replicates (8 per each). Group 1 was fed on the diet contained inorganic Mn, Zn and Cu sulfate while, group 2,3 and 4 fed diets containing Mn, Zn and Cu-sulfate nano particles, respectively). Chicks in group 5 receiving diet with a combination of nano- Mn, Zn and Cu. The results revealed that, inclusion of Mn, Zn and Cu nano particles had a positive effect on growth performance metrics, as seen by increased final weight of the body and total gaining in weight during the experimental period compared to control. The groups supplemented with nanoparticles had considerably ($p<0.01$) lower feed consumption than the inorganic-treated group (control). There was a significant improvement in the feed conversion ratio (FCR) and European performance efficiency factor (EPEF) of nano-trace elements supplemented group. In addition, all nano-treated groups exhibited a significant increase in serum total protein, globulin, serum minerals (Mn, Zn, Cu, Ca, and P), and cecal SCFAs as compared to the control. All groups supplemented with nano- minerals showed reduced levels of liver function test (ALT and AST), renal function test (creatinine, urea, and uric acid), lipid profile (Cholesterol, triglycerides, LDL, and VLDL). Conclusively, inclusion of nano-Mn, Zn and Cu in broiler diets (either single or in a combination) lead to appreciable enhancements in all assessed parameters experimented in this study.

Introduction

The creation of appropriate rations in terms of energy, protein, and micronutrients (vitamins and minerals) is crucial for the production, health, and well-being of poultry. A vital component of animal feeding systems, mineral nutrition guarantees the best possible health, productivity, and reproduction for both birds and animals. Despite being needed in smaller amounts than other nutrients like energy and protein, their imbalances and deficiencies are quickly manifested in alterations to the productivity and well-being of animals (Swain *et al*., 2021).

Traditionally, minerals are employed in diets through their inorganic salts; however, because of their low bioavailability, inorganic mineral salts must be used at higher levels to suit animal needs, which indirectly increases mineral pollution (Patra and Lalhriatpuii, 2020).

Better bioavailable mineral sources have been the focus of mineral research for many years, and these problems must be resolved. Numerous organic chelated minerals have been attempted to bridge the gap, with varying degrees of success reported in terms of their biological reactions, cost-effectiveness, and bioavailability. When compared to their inorganic counterparts, organic minerals supplemented with proteinate showed superior absorption and retention in poultry. Because they are more expensive, organic minerals are not as widely used as their inorganic equivalents, despite having higher bioavailability (Zhao *et al*., 2014). Better bioavailable sources must be employed immediately, especially in poultry production, to protect the environment without compromising the productivity of the animals or birds in an economical way.

Nano-sized minerals have been explored and evaluated in a variety of methods to confirm their improved bioavailability in a variety of mammals and birds, and they are now thought to be a viable alternative to close the gap. Materials with particle sizes in the nanometer (nm) range (<100 nm) are made possible by nanotechnology, and because of the nano-sized particles (NP), their structures have remarkably unique physical, chemical, and biological characteristics and capabilities (Mane *et al*., 2022). Among the many advantages of biogenic synthesis nanoparticles are their ease of use, simplicity, lack of hazardous chemical use, affordability, safety, cleanliness, controlled NP size, and environmental friendliness. They also help achieve the necessary high-yield and high-value NPs compounds (El-Saadony *et al*., 2021; Hatab *et al*., 2022; Hussein *et al*., 2022).

Because of their small size, they have a much larger surface area, which tends to result in a lot of positive reactions. Comparing NP's modified chemical and physical characteristics to those of its bulk components may change the biological reactions (Hassan *et al*., 2020). The promise of nanotechnology in the poultry business has not yet been completely realized due to a lack of information. Due to their unique properties, such as higher specific surface area, higher surface activity, higher catalytic efficiency, and stronger adsorbing ability, nanoparticles of mineral elements have a higher bioavailability in animal bodies than minerals, especially trace minerals (Albanese *et al*., 2012; Rajendran *et al*., 2013). They can also be more easily transported up the gastrointestinal tract and used in the animal system, reaching deeper tissues more effectively than larger particles (Liao *et al*., 2010). Mineral antagonism in the colon can be lessened by trace element nanoparticles, which will improve absorption and lessen environmental excretion. Additionally, they may help birds respond better to vaccinations and digest food more efficiently, which could lead to increased feed efficiency (MarappanGopi *et al*., 2017). The impact of adding nano-Mn, Zn, and Cu (either separately or in combination) to broiler feeds on growth performance, blood biochemical parameters, and cecal short-chain fatty acids was thus assessed in the field. Nanoparticles of trace elements can reduce mineral antagonism in the intestine leading to enhanced absorption, thereby reducing their excretion in the environment. These also have potential roles in improving bird's immune responses and digestive efficiency resulting in better feed efficiency (MarappanGopi *et al*., 2017). Thus, the current work evaluated under the field conditions, the effect of using nano-Mn, Zn and Cu (either separately or in combination) in broiler diets on performance of growth, parameters of serum biochemistry, liver and renal function test and cecal short-chain fatty acids.

Materials and methods

Ethical Approval

The Ethics Committee of the Faculty of Veterinary Medicine at Assiut University in Egypt granted ethical permission, and the prescribed ethical requirements were followed (Approval no. 06/2025/0350).

Experimental duration and location

The trial period was prolonged by 35 days (from February 1 to March 7, 2025). At the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt. The Nutrition and Clinical Nutrition Research Unit conducted the study.

Experimental protocol, chicks and housing

The experiment employed 120 one-day-old, unsexed broiler chicks (Ross 308), which obtained from local commercial source. The chicks' starting weights were almost identical (41.2–43.4 g). The birds were split into 5 equal groups of 24 chicks each, with three replicates of eight chicks each, at random. At the start of the trial and each week until the completion of the experimental period, each chick was individually weighed and had their legs banded. The chicks in the experimental groups were kept in ground-level enclosures with the same environmental conditions and management. At eight days of age, all chicks received ocular drops containing the Hitchner B1 strain vaccine to prevent Newcastle virus illness. Birds were given an eye drop of the infectious bursal disease vaccination when they were 14 days old. Lasota eye drops were used to vaccinate the birds against avian influenza and Newcastle virus illness at 18 days of age. Organic waste was disposed of hygienically.

Mn, Zn and Cu sources

Inorganic Mn, Zn and Cu

Anhydrous Mn sulfate (36% Mn), Zn sulfate (36.5% Zn) and Cu sulfate (39.8% Cu) were purchased from Chengdu Sunstar Feed Co., Ltd, China. Mn, Zn and Cu sulfate were added at level of 334, 301 and 40 mg/kg diets to meet recommended level of Mn (120 mg/kg), Zn (110 milligrams per kilogram) and Cu (16 milligrams per kilogram), respectively.

Nano Mn, Zn and Cu

Mn, Zn and Cu sulfate nanoparticles were purchased from Nano tech Egypt. Mn, Zn and Cu nano particles were added at level of 83.5 mg Nano Mn, 75.25 mg Nano Zn, and 10 mg Nano Cu / kg diet (25% amount of inorganic trace minerals).

Experimental diets and feeding

Chicks were distributed into 5 groups, control and 4 experimental groups according to the source of Mn, Zn and Cu used. Yellow maize, soybean meal (44% CP), sunflower oil, di-calcium phosphate, ground limestone, common salt, methionine, lysine, and premix were all mashed together to create the basal control experimental diet according to Ross nutrition specification (Avigan, 2022) to me*et al*l nutritional requirements including trace elements as illustrated in Table 1. Mn, Zn and Cu sulfate were included in the premix of the basal control diet as inorganic source to supply 120 mg Mn, 110 mg Zn and 16 mg Cu/kg diet (334 mg Mn sulfate, 301 mg Zn sulfate and 40 mg Cu sulfate/kg diet). Every feed ingredient and experimental diet formulation was examined in accordance with the guidelines set out by the Association of Official Analytical Chemists (AOAC, 2023). Birds were fed according to the three-phase feeding program: starter (0–14 days), grower (15–25 days), and finisher (26–35 days). The birds in the first group were given a basic control diet on an ad-libitum basis. They served as a control group against which the other treated groups were compared. Birds in the second group fed on basal control diet after replacement inorganic Mn sulfate with83.5mg Mn sulfate nanoparticles, while Zn sulfate in the diet of the third group was substituted by 75.25 mg Zn sulfate nanoparticles and 10 mg Cu sulfate nanoparticles were included in basal control diet of fourth group instead of Cu sulfate. In place of inorganic Mn, Zn, and Cu, the fifth group of birds were fed a basal control diet supplemented with a mixture of 83.5 mg Mn, 75.25 mg Zn, and 10 mg Cu sulfate nanoparticles (1/4 amount). All birds received ad libitum meals every morning and evening, and they were always permitted access to clean water.

Table 1. Composition (%) and energy value of the basic diets.

Ingredient	Starter (0-14 days)	Grower (15-25 days)	Finisher (26-35 days)
Physical Composition (%)			
Yellow corn, ground	53.97	57.37	63.02
Soybean meal (44% CP)	40.45	36.66	31.38
Sunflower oil	1.8	2.5	2.42
Di-calcium phosphate	1.3	1.2	1
Limestone, ground	1.48	1.34	1.29
Common salt	0.3	0.3	0.3
Methionine	0.2	0.18	0.14
Lysine	0.2	0.15	0.15
Premix*	0.3	0.3	0.3
Chemical Composition (%)			
Crude protein	23.05	21.5	19.5
Crude fiber	2.83	2.72	2.59
Calcium	0.99	0.9	0.82
Available phosphorus	0.48	0.43	0.39
Ether extract	4.07	4.84	64.9
Methionine	0.56	0.51	0.45
Lysine	1.43	1.29	1.16
ME Energy (Kcal/kg)	2977	3052	3100

*Each 3 kg contains: Vit. A, 12000000 IU; Vit. D3, 4000000 IU; Vit. E, 80000 mg; Vit. k3 4000 mg; Vit. B1, 5000 mg; Vit.B2, 8000mg; Vit. B6, 5000 mg; Vit. B12, 35 mg; Vit. B3, 70000 mg; selenium, 250 mg; Pantothenic acid, 20000 mg; Folic acid 1000mg; Biotin, 250 mg; Iron, 50000 mg; Cobalt, 250 mg; Iodine, 1500 mg. (Universal Animal Care Company).

Performance metrics

Throughout the experiment, the body's initial weight, ending weight, and cumulative feed consumption were recorded, and the cumulative body weight gain (BWG) and the feed to gain ratio (also known as the "FCR") and were computed. Bird viability and mortality were tracked every day, and the mortality percentage (%) for each replicate was computed by adding together all the deceased birds in each group and dividing that total by the number of live birds. The following formula, recommended by Huff *et al*. (2013), was used to determine the European performance efficiency factor (EPEF) following the computation of viability percentage and FCR.

EPEF= BW (kg)×% livability ×100/FCR×trial duration (days).

Where: BW= Body weight (kg), livability (100%-Mortality rate), FCR= Feed conversion ratio (kg feed / kg weight gain).

Collection and preparation of samples

Using a vacuum non-anticoagulant tube, three birds were chosen at random from each group (one from each replication) at the conclusion of the experiment to draw blood from the wing vein. The serum sample was separated and collected by centrifugation at 3000 rpm for 10 minutes after the blood samples had been left at room temperature for 30 minutes. The serum was then promptly stored at -18°C until it was further analyzed. Three slaughtered birds from each group (one from each replication) had their cecal contents taken at the conclusion of the experiment and kept at -20°C for additional examination.

Biochemical parameters of the serum

Using a spectrophotometer (Beckman DU-530, Germany) and a commercial kit from Sentinel CH (Milano, Italy), serum samples were assayed to estimate their protein profiles, including total protein (TP) and albumin (Alb). The serum globulin was computed by deducting the obtained serum albumin value from the corresponding total protein value for each sample, while the A/G ratio was computed based on the albumin and globulin results.

Using a spectrophotometer (SHIMADZU UV 1601), the serum levels of creatinine, urea, and uric acid as indicators of renal function and enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphates (ALP) as indicators of liver function were measured in accordance with the manufacturer's instructions.

A colorimetric reflectance spectrophotometric approach was used to measure the lipid profile, which included cholesterol, triglycerides, low density lipoprotein (LDL), and high-density lipoprotein (HDL). All parameters were measured with Biodiagnostic® kits from Cairo, Egypt. Using the relationship provided by Friedewald *et al.* (1972), the triglyceride value was divided by 5 (in mg/dL) to determine very low-density lipoprotein (VLDL).

Measurement of the serum's Mn, Zn, and Cu levels

Four milliliters of serum sample were wet-ashed in a tube with ten milliliters of nitric acid, and the solution was heated to a minimum volume (never allowed to dry) in order to measure the trace minerals (Mn, Zn, and Cu) in the serum. The solution was filtered into a 25 ml flask and diluted with 25 ml of deionized water once it had cooled. A double-beam atomic absorption spectrometer (AAS-7000 series, Shimadzu, Kyoto, Japan) was used to measure the amounts of manganese, zinc, and copper in serum.

Cecal short-chain fatty acids (SCFAs) analysis

In a sterile tube, 1 g of thawed cecal contents were suspended in 4 mL of distilling water. After homogenizing the samples, they were centrifuged for 15 minutes at 4000×g. After that, the 1 mL supernatant was put into an Eppendorf tube and combined with a 250 µL solution of metaphosphoric acid. For fifteen minutes, the solution was centrifuged once more at 4000×g. After that, 200 µL of crotonic acid solution was added to 1 mL of the supernatant in an Eppendorf tube, and the mixture was centrifuged for 15 minutes at 4000×g. Gas chromatography was used to evaluate the supernatant's concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate (Zhang *et al*., 2003).

Statistical analysis

GraphPad Prism Software, SAS (2009) (Version 2.02, 2009, USA) was used to do statistical analysis utilizing post hoc Tukey's multiple comparison tests and one-way analysis of variance (ANOVA). Group differences were deemed significant when P < 0.05. Means ± standard errors of the mean (SEM) are used to represent values in tables.

Results

Performance parameters and mortality

Comparing the groups fed diets containing nano Mn, Zn, and Cu either separately or in combination, no mortality was seen during the trial, in contrast to the control group (9.52%). This indicates that during the trial periods, broiler viability and survival rates were raised by nano forms of these minerals (Table 2).

The results obtained in Table 2 demonstrated that the ultimate body weight and weight gain in G2, G3, G4, and G5 were considerably higher than in control (G1) when broilers were fed nano-Mn, Zn, and Cu (either alone or in combination) rather than Mn, Zn, and Cu sulfate. When compared to the control group (1939.2 g and 1895.8 g, respectively), the fifth group receiving the basal diet supplemented with a mix of Mn, Zn, and Cu sulfate nanoparticles had the highest values among the treated groups (2100.5 g and 2059.2 g, respectively). Differences in overall feed consumption between the several experimental groups were significant (P<0.05). When compared to the control (3193 g), the total feed intake in groups 2, 3, 4, and 5 was considerably reduced by the addition of nano Mn, Zn, and Cu, either separately or in combination (3124, 3122, 3135, and 3126 g, respectively).

During the entire trial period, the feed conversion ratio (FCR) and European performance efficiency factor (EPEF) of treated groups supple-

Table 2. Performance metrics for broilers during the trial.

Parameters	Groups**					SEM	P value
	G1	G2	G3	G4	G5		
Initial body weight (g/chick)	43.4	42.1	41.2	41.3	41.3	0.67	0.01
Final body weight (g/chick)	1939.2^{b}	2038.6^{ab}	2051.2^{ab}	2092.4^{ab}	2100.5^{a}*	46.54	0.22
Weight gain (g/chick) (0-35 day)	1895.8^{b}	1996.5^{ab}	2010.0^{a}	2051.1^{a}	2059.2^{a}	44.00	0.07
Feed consumption (g/chick)	3193.0^{a}	3124.0^{c}	3122.0^{c}	3135.0^{c}	3126.0^{c}	0.58	<0.0001
Feed conversion ratio (FCR)	1.68^{a}	1.56^{b}	1.55^{b}	1.53^{b}	1.52^{b}	0.04	0.01
Mortality rate %	9.52	0	0	0	0	0	0
Survival rate (Livability %)	90.48	100	100	100	100	0	0
European performance efficiency factor (EPEF)	298.4^{b}	373.4^{ab}	378.1^{ab}	390.7^{a}	394.8^{a}	16.27	0

* Means in the same row having the same superscripts are not significantly different (P<0.0001).
**G1: Control: fed on basal diet (containing inorganic Mn, Zn and Cu); G2: fed on basal diet(containing Nano Mn +inorganic Zn and Cu); G3: fed on basal diet (containing Nano Zn+ inorganic Mn and Cu); G4: fed on basal diet (containing Nano Cu+ inorganic Mn and Zn); G5: fed on basal diet (containing Nano Mn, Zn and Cu instead of inorganic Manganese, Zinc, and Copper).

mented with nano Mn, Zn, and Cu, either separately or in combination, shown a significant improvement over the control. The fifth group had the highest FCR and EPEF (1.52 and 394.8, respectively), followed by G4, G3, and G2, in comparison to the control group (1.68 & 298.4, respectively).

Serum biochemical parameters

As indicated in Table 3, the addition of nano Mn, Zn, and Cu to broiler diets resulted in notable variations across various serum biochemical markers. According to the protein profile results, the fifth group had the greatest levels of serum total protein and globulin when compared to the other nano-treated groups and the control. Serum albumin not significantly affected by dietary Mn, Zn and Cu nanoparticles. Additionally, albumin /globulin ratio concentration was significantly different among various treated groups and the lowest ratio was recorded in G5.

The fifth group had the lowest levels of ALT and AST (19.08 & 26.51 U/L, respectively) when compared to the control group (28.15 & 30.92 U/L, respectively). ALP concentration significantly increased in nano treated groups (G2, G3, G4 and G5) in comparison with inorganic supplemented group (G1). The highest level of ALP was observed in G5 (130.02 U/L) in comparison with control (79.25 U/L). Creatinine, urea and uric acid concentrations were significantly decreased in nano treated groups (G2, G3, G4 and G5) in comparison with control. The lowest values were recorded in G5 (0.25, 2.83 & 5.31 mg/dl, respectively) compared with control (0.51, 4.70 and 8.40 mg/dl, respectively) as shown Table 3.

Concerning the lipid profile, the results in Table 3 demonstrated that broiler diets supplemented with nano Mn, Zn, and Cu (either separately or in combination) resulted in a significant drop in the serum total cholesterol, LDL, VLDL, and triglycerides and a significant increase in HDL levels as compared to the control (G1). The fifth group fed a mixture of nano Mn, Zn, and Cu had the greatest HDL value and the lowest serum total cholesterol, LDL, VLDL, and triglyceride levels.

Serum minerals concentrations

Table 4 revealed that replacement of inorganic Mn, Zn and Cu by nanoparticles Mn, Zn and Cu (either alone or in combination) increased numerically the serum mineral concentrations. Among the nano-treated groups, the highest values of Mn, Zn, Cu, Ca and P concentrations were recorded in the fifth group.

Short-chain fatty acids (SCFAs) in the cecum

As shown in Table 5, all experimental groups had considerably larger percentages of cecal SCFAs, including acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, isovaleric acid, and total SCFA, than the control group. The highest levels of cecal SCFAs were recorded in the fifth group.

Regarding the cecal percentages of SCFAs in relation to total SCFAs, Table 5's findings showed that adding nano Mn, Zn, and Cu (either separately or in combination) to broiler diets significantly reduced the percentage of acetic acid and significantly increased the percentage of propionic acid and butyric acid when compared to the control. The fifth group had the highest percentages of propionic acid and butyric acid and the lowest percentage of acetic acid.

Table 3. Parameters of serum biochemistry in broilers fed various experimental diets.

Parameters	Groups					SEM	P value
	G1	G2	G3	G4	G5		
Total Protein (g/dl)	3.88^{b}	3.91^{b}	4.10^{b}	4.18^{b}	5.35^{a}	0.12	<0.0001
Albumin (g/dl)	1.83	1.84	1.93	1.97	1.98	0.09	0.57
Globulin (g/dl)	2.05^{b}	2.07^{b}	2.17^{b}	2.21^{b}	3.37^{a}	0.16	<0.0001
A/G ratio	0.89^{a}	0.89^{a}	0.89^{a}	0.89^{a}	0.59^{b}	0.09	0.01
ALT (U/L)	28.15^{a}	25.25^{ab}	23.82^{ab}	22.98^{ab}	19.08^{b}	0.42	<0.0001
AST (U/L)	30.92^{a}	30.71^{ab}	29.14^{ab}	28.51^{ab}	26.51^{b}	0.79	<0.0001
ALP (U/L)	79.25^{d}	100.50^{c}	113.34^{b}	114.96^{b}	130.02^{a}	42.05	<0.0001
Creatinine (mg/dl)	0.51^{a}	0.41^{ab}	0.38^{b}	0.40^{ab}	0.25^{c}	0.04	0.00
Urea (mg/dl)	4.70^{a}	3.45^{ab}	3.40^{ab}	3.22^{ab}	2.83^{b}	0.22	<0.0001
Uric acid (mg/dl)	8.40^{a}	7.89^{ab}	6.51^{b}	7.60^{ab}	5.31^{c}	0.26	<0.0001
Total cholesterol (mg/dl)	150^{a}	133^{b}	135^{b}	138^{b}	125^{c}	3.91	<0.0001
HDL (mg/dl)	70.75^{c}	74.83^{b}	86.65^{ab}	93.23^{a}	84.98^{ab}	3.15	<0.0001
LDL (mg/dl)	58.23^{a}	37.37^{ab}	29.95^{b}	25.97^{bc}	23.42^{c}	0.90	<0.0001
VLDL (mg/dl)	21.00^{a}	20.80^{ab}	18.40^{b}	18.80^{b}	16.60^{c}	0.68	<0.0001
Triglycerides (mg/dl)	105^{a}	104^{ab}	92^{b}	94^{b}	83^{c}	3.41	<0.0001

*Means in the same row having the same superscripts are not significantly different (P<0.0001).

Table 4. Serum mineral concentrations of broilers fed different treated diets.

Parameters	Groups					SEM	P value
	G1	G2	G3	G4	G5		
Manganese (μg/L)	3.25^{b}	3.27^{b}	3.44^{ab}	3.50^{a}	3.52^{a}	0.03	<0.0001
Zinc (mg/L)	2.10^{b}	2.11^{b}	2.52^{a}	2.03^{b}	2.27^{ab}	0.04	<0.0001
Copper (mg/L)	0.23^{b}	0.28^{b}	0.20^{b}	0.43^{a}	0.32^{ab}	0.03	<0.0001
Calcium (mg/dl)	6.21^{b}	6.25^{b}	6.57^{ab}	6.69^{ab}	6.72^{a}	0.03	<0.0001
Phosphorus (mg/dl)	7.49^{b}	7.54^{b}	7.92^{ab}	8.07^{ab}	8.12^{a}	0.04	<0.0001

*Means in the same row having the same superscripts are not significantly different (P<0.0001).

Table 5. Cecal short chain fatty acids (SCFA) in broilers given various treatment diets (μmol/g cecal digesta).

Parameters	Groups					SEM	P Value
	G1	G6	G7	G8	G9		
Acetic acid (%)	66.75 ^{b}	81.12^{ab}	83.11^{ab}	84.15^{ab}	91.30^{a}	0.30	<0.0001
Propionic acid (%)	11.48 ^{b}	20.15^{ab}	23.12^{ab}	23.23^{ab}	29.40^{a}	0.27	<0.0001
Butyric acid (%)	14.15^{b}	23.22^{ab}	24.85^{ab}	25.18^{ab}	29.50^{a}	0.31	<0.0001
Isobutyric acid (%)	0.68^{c}	1.05^{bc}	1.11^{b}	1.25^{ab}	1.75^{a}	0.04	<0.0001
Valeric acid (%)	0.56^{c}	1.05^{b}	1.09 ^{b}	1.40^{ab}	1.63^{a}	0.03	<0.0001
Isovaleric acid (%)	0.85^{c}	1.62^{b}	1.78^{b}	2.15^{ab}	2.50^{a}	0.04	<0.0001
Total SCFA (%)	94.47^{c}	128.21^{b}	135.06^{ab}	137.36^{ab}	156.08^{a}	0.96	<0.0001
Profile (% of SCFA to total SCFA)							
Acetic acid	70.66^{a}	63.27^{ab}	61.54^{ab}	61.26^{ab}	58.50^{b}	0.3	<0.0001
Propionic acid	12.15^{b}	15.72^{ab}	17.12^{ab}	16.91^{ab}	18.84^{a}	0.10	<0.0001
Butyric acid	14.98 ^{b}	18.11^{a}	18.40^{a}	18.33^{a}	18.90^{a}	0.09	<0.0001

*Means in the same row having the same superscripts are not significantly different (P<0.0001).

Discussion

Within the first week of the trial, two birds died in the control group, which was fed an inorganic source of Mn, Zn, and Cu. However, no fatalities were reported in the other treated groups, which were administered nano Mn, Zn, and Cu. This means that nano forms of these minerals increased the viability and survival rate of broilers during the whole experimental periods. Zinc's role in intestinal permeability, intestinal development, immune system development, and the prevention of enteric disorders may be the reason for the increased survival rate (MacDonald, 2000; Bortoluzzi *et al.*, 2019). However, Pacheco *et al.* (2021) found that the mortality rate was unaffected by the supplies of Mn and Zn during any growth period. Furthermore, Ramiah *et al.* (2019) discovered that the mortality rate during the experimental periods was unaffected by the addition of Zn O-NPs to the broiler diet.

Because of their small size, good homogeneity, high surface area, and physical reactivity (Scott *et al.*, 2018a; Abdollahi *et al.*, 2020; Patra and Lalhriatpuii, 2020; Ouyang *et al.*, 2021), nano-forms of elements may increase manganese's bioavailability (Vijayakumar and Balakrishnan, 2014; Hill and Li, 2017; MohdYusof *et al.*, 2019; Hidayat *et al.*, 2021). This is why dietary nanoMn, Zn, and Cu alone or in combination may have a positive effect. Because Cu-NP was better absorbed in the GIT and the nanoparticles were more bioavailable, it had a more positive effect than CuSO4 (Abdullah *et al.*, 2022a; Mohamed *et al.*, 2022; Abdullah *et al.*, 2022b). Furthermore, the enhancement of Cu-NP's antimicrobial and antibacterial qualities is the mechanism responsible for the improvement in animal performance (Usman *et al.*, 2013; Arias and Koutsos, 2006). According to Shobha *et al.* (2014), copper nanoparticles can inhibit the growth of several bacteria, including S. aureus, B. subtilis, and E. coli. This could have reduced the bacterial burden in broilers, allowing them to make better use of their diet and resulting in improved growth performance. According to earlier research (Shubhnish *et al.*, 2021; Larasati *et al.*, 2022; Samy *et al.*, 2022; Abdel-Wareth *et al.*, 2024; Mozhiarasi *et al.*, 2024), Nano-ZnO considerably boosted body weight gain. Our findings are consistent with those findings. In a similar vein, Qady *et al.* (2022) found that supplementing broilers with Cu-NPs at the same amount of Cu2SO4 or at a lower level enhanced their body weight and weight gain. Furthermore, Wang *et al.* (2011) observed that the growth performance of broilers was enhanced using copper-loaded chitosan nanoparticles at 50 and 100 mg/kg. However, according to an earlier research, supplementing broilers with ZnO-NPs had no effect (P >0.05) on their body weight gain (Ramiah *et al.*, 2019; Abd El-Haliem *et al.*, 2020; Mahmoud *et al.*, 2020).

When compared to the inorganic-tread group (control), feed consumption was considerably lower in the nano-treated groups. The higher bioavailability of minerals, which are more easily absorbed due to their larger surface area, is one of the reasons why broilers supplemented with nano-minerals have lower feed consumption. The bird's mineral needs may be satiated more quickly as a result of the enhanced bioavailability, which could eliminate the need for additional meal (Liu *et al.*, 2011). It's possible that NP's larger surface area promotes improved biological interface interaction, longer intestinal retention time, less impact from intestinal clearance mechanisms, and efficient delivery of functional compounds to target sites, all of which improve bioavailability and functionality. Some nano-minerals may cause problems and might impact feed intake by interfering with the absorption of other vital nutrients (Chen *et al.*, 2006). The findings were supported by earlier research showing that Zn O-NPs considerably (P<0.05) reduced feed consumption when compared to control (Fathi *et al.*, 2016; Ramiah *et al.*, 2019). However, Mozhiarasi *et al.* (2024) and Mahmoud *et al.* (2020) found no significant (P>0.05) change in the total feed consumption of broilers fed a diet containing ZnO-NPs. According to Abdullah *et al.* (2022b), broiler feed consumption was not significantly impacted by the addition of Nano-Cu. Conversely, Shubhnish *et al.* (2021) and Larasati *et al.* (2022) discovered that birds fed a diet enhanced with zinc oxide nanoparticles (ZnO-NPs) had higher feed consumption.

Zinc has been identified as a crucial component of various digestive enzymes and is present in all six categories of enzymes, including oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase. This may explain why the FCR in the nano Zn supplemented group (G3) improved (Park *et al.*, 2004). Thus, adding nano-zinc to the diet may be improving weight increase and the digestion of minerals from the feed, which in turn may improve the feed conversion ratio (FCR) and European performance efficiency factor (EPEF). However, the negative effects of Cu_2SO_4 on the utilization of valuable feed components like certain vitamins (Marchetti *et al.*, 2000; Luo *et al.*, 2005), phosphorous, and the phytase enzyme (Banks *et al.*, 2004), or its poor absorbability (Scott *et al.*, 2018b) may be the reason why birds supplemented with Cu_2SO_4 had lower FCR and EPEF than birds supplemented with Cu-NPs at the same quantity. Our findings concur with earlier research that found that feed conversion ratios were improved by various forms of zinc (organic or nano) when compared to control fed inorganic forms of zinc (Ibrahim *et al.*, 2017; Abd El-Haliem *et al.*, 2020; Mahmoud *et al.*, 2020; Larasati *et al.*, 2022; Abdel-Wareth *et al.*, 2024). Additionally, groups treated with nano Mn showed an improvement in FCR when compared to control, according to Helbawi *et al.* (2024). When compared to inorganic form, Cu supplementation in nano form improved FCR (Qady *et al.*, 2022). However, when compared to inorganic Zn, Dosoky *et al.* (2022) and Mozhiarasi *et al.* (2024) found that adding Zn O-NPs to broiler diets had no influence on FCR among treatment groups. When compared to copper sulfate supplementation, El-Kazaz and Hafez (2019) demonstrated that the inclusion of Cu-NPs in broiler diets had no influence on FCR. Furthermore, dietary zinc treatments did not significantly (p>0.05) affect the total period EPEF, according to Eskandani *et al*. (2021).

A higher blood protein level could be a sign of either a slower rate of protein metabolism or a quicker rate of tissue protein production. The amino acid transport pathway, which is more readily absorbed by organisms, allows complexes of elements with proteins or amino acids to be consumed without altering the intestinal mucous membrane (Abd El-Hady, 2019). Attia *et al*. (2020) and Abdel-Monem *et al*. (2021) reported similar findings, indicating that nano Zn oxide raised plasma total protein. However, prior research found that Nano-Zn had no discernible impact on serum total protein (El-Katcha *et al*., 2017; Saleh *et al*., 2018; Tag-El Din., 2019; Hidayat *et al*., 2021; Dosoky *et al*., 2022; Abdel-Wareth *et al*., 2024). These findings are consistent with those of El-Katcha *et al*. (2017) and Tag-El Din (2019), who found that the concentration of serum albumin was not significantly impacted by Nano Mn, Zn, or Cu alone or in combination. However, Abdel-Monem *et al*. (2021) observed that nano Zn raised plasma albumin levels, which contradicted previous findings. Furthermore, adding copper nano particles to broiler diets lowered blood albumin levels, according to Ayoola *et al*. (2021).

Our findings regarding the liver function test are consistent with earlier research that discovered that broiler blood ALP concentrations were elevated by nano-Zn (Fathi *et al*., 2016; El-Katcha *et al*., 2017; Prabakar *et al*., 2018; Elashry, 2019). Furthermore, Abd El-Hady (2019) found that broiler diets containing nano-Cu had higher levels of ALP. The action of vitamin D3, which has multiple effects on the intestine, kidneys, and bones, including increasing calcium absorption into the extracellular fluid and potentially promoting the formation of ALP in the epithelial cells, may be responsible for the notable increase in serum ALP activity in birds supplemented with Nano-Zn (Guyton and Hall, 2006). The results were against those of Fathi *et al*. (2016) and Abdel-Monem *et al*. (2021), who showed no discernible change in ALT serum levels when broiler diets supplemented with Nano-Zn. When compared to the control, Nano-Zn did not significantly raise the ALT concentration, according to El-Katcha *et al*. (2017).

Reduced levels of creatine, urea, and uric acid in the blood of birds administered nano-Mn, Zn, and Cu supplements are consistent with findings from earlier research that showed dietary nano Zn inclusion diet dramatically reduced serum urea and uric acid concentrations (Attia *et al*., 2020; Dosoky *et al*., 2022; Abdel-Wareth *et al*., 2024). Groups given diets supplemented with Cu-NP showed a decrease in plasma uric acid concentration, indicating that amino acids were used more effectively than in other groups. When broilers were supplemented with 15 mg/kg of copper nanoparticles, their uric acid and alkaline phosphatase levels dropped (Abdullah *et al*., 2022a). According to Ayoola *et al*. (2021), birds treated with either CuSO4 or Cu-NP showed a significant decrease in uric acid. However, prior research found no discernible impact of adding Nano-Zn and Nano-Cu to broiler diets on the blood content of uric acid (El-Katcha *et al*., 2017; Abdel-Monem *et al*., 2021; Ibrahim *et al*., 2022).

According to earlier research (Ibrahim *et al*., 2017; Saleh *et al*., 2018; Tag-El Din, 2019; Dosoky *et al*., 2022; Abdel-Wareth *et al*., 2024), adding nano Zn to broiler diets significantly reduced total serum cholesterol. The results obtained regarding the lipid profile are consistent with these findings. In a similar vein, Tag-El Din (2019) and Attia *et al*. (2020) discovered that adding Nano-Zn to broiler meals raised HDL levels. Conversely, El-Katcha *et al*. (2017) and Prabakar *et al*. (2018) found that broiler chickens' plasma cholesterol levels were considerably raised when they were fed nano Zn supplements. According to Attia *et al*. (2020) and Dosoky *et al*. (2022), broiler LDL serum levels were elevated by ZnO-NPs.

Serum mineral (Mn, Zn, Cu, Ca, and P) concentrations were quantitatively raised when inorganic Mn, Zn, and Cu were replaced with nanoparticle sources, either separately or in combination. These outcomes were in line with those of earlier research that found that dietary substitution of organic or nano-zinc for inorganic zinc oxide raised blood serum levels of calcium, phosphorus, and zinc (Samanta *et al*., 2011; El-Katcha *et al*., 2017; Elashry, 2019; Mahmoud *et al*., 2020). The activity of copper nanoparticles on lysyl oxidase enzymes in groups supplemented with nanoparticles may be the cause of the elevated levels of calcium and phosphorus in treated groups (McNerny *et al*., 2015). However, when using copper nanoparticles in broiler diets, Wang *et al*. (2011) and Scott *et al*. (2018b) found no discernible changes in blood calcium and phosphorus contents.

A reduction in the dietary dosage of Cu nanoparticles was accompanied by an improvement in intestinal absorption of zinc. Metallothioneins (MTs) are low-molecular-weight proteins rich in cysteine residues that control the amounts of copper and zinc in cells. Up to 12 monovalent Cu ions and seven divalent Zn ions can be bound by a single MT molecule. Cu can push Zn away from the molecule by competing for Zn binding sites because of the two metals' comparable coordination chemistry (Gaetke and Chow, 2003).

The percentages of cecal SCFAs, such as acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, isovaleric acid, and total SCFA, were considerably raised when inorganic Mn, Zn, and Cu were substituted with nanoparticle sources. Regarding the cecal percentages of SCFAs in relation to total SCFAs, broiler diets supplemented with nano Mn, Zn, and Cu (either separately or in combination) showed a significant decrease in the percentage of acetic acid and a significant increase in the percentage of propionic acid and butyric acid when compared to the control.

These results are in line with the findings reported by Wang *et al*. (2023) noted that different sources and levels of trace minerals (inorganic and organic) had significant (p<0.05) increase in valeric acid %. Increased nutrient utilization and modifications to the gut flora are the main causes of the higher generation of short-chain fatty acids (SCFAs) in broilers fed diets enriched with nano-minerals. Nano-minerals, due to their increased surface area, improve mineral bioavailability, leading to a more favorable environment for beneficial gut bacteria. These bacteria then ferment dietary fiber and other undigested compounds in the hindgut, producing SCFAs as a byproduct.

Conclusion

Dietary substitution of inorganic manganese, zinc, and copper by nanoparticle sources (either separately or in combination) improve broiler growth metrics, blood metabolites, serum mineral concentrations, and cecal short chain fatty acids.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abd El-Hady, A.M., 2019. Effect of dietary sources and levels of copper supplementation on growth performance, blood parameters and slaughter traits of broiler chickens. Egyptian Poultry Science Journal 39, 897-912.

Abd El-Haliem, H.S., Attia, F.A., Saber, H.S., Hermes, I.H., 2020. Impacts of zinc oxide nano-particles supplementation in broiler diets on growth performance, some carcass characteristics and immune organs. Egyptian Journal of Nutrition and Feeds 23, 3-122.

Abdel-Monem, N., Elsebai, A., Abd El-Hady, A.M., 2021. Impact of dietary zinc oxide nano-particles on antioxidant status, liver and kidney functions in Alexandria chickens. Egyptian Poultry Science Journal 41, 675-690.

Abdel-Wareth, A.A.A., Ali, A.H., Amer, S.A., Younis, E.M., Abdel-Warith, A.W.A., Kassim, A.S.N., Salahuddin, M., Lohakare, J., 2024. Effects of dietary supplementation with zinc oxide or selenium nanoparticles and their combination on rabbit productive performance, nutritional and physiological responses. Italian Journal of Animal Science 23, 1258-1268.

Abdollahi, M., Rezaei, J., Fazaeli, H., 2020. Performance, rumen fermentation, blood minerals, leukocyte and antioxidant capacity of young Holstein calves receiving high-surface ZnO instead of common ZnO. Archives of Animal Nutrition 74, 189-205.

Abdullah, S.S., Masood, S., Zaneb, H., Rabbani, I., Akbar, J., Kuthu, Z.H., Masoodd, A., Vargas-Bello-Pérez, E., 2022a. Effects of copper nanoparticles on performance, muscle and bone characteristics and serum metabolites in broilers. Brazilian Journal of Biology 84, e261578.

Abdullah, S.S., Masood, S., Zaneb, H., Rabbani, I., Akbar, J., Kuthu, Z.H., Masood, A., Dhakal, R., Bello Pérez, E.V., 2022b. Effects of Copper nanoparticle supplementation on growth, intestinal morphology and antioxidant status in broilers exposed to cyclic cold stress. Emirates Journal of Food and Agriculture 34, 436-445.

Albanese, A., Tang, P.S., Chan, W.C., 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual Review of Biomedical Engineering 14, 1-16.

AOAC, 2023. Official Methods of Analysis. Association of Analytical Chemists, 22nd edition, AOAC International, Oxford University Press.

Arias, V.J., Koutsos, E.A., 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poultry Science 85, 999-1007.

Attia, F.M., 2020. Efficacy of dietary zinc oxide nanoparticles supplementation on serum biochemical, nutrients retention and chemical composition of meat and tibia in broiler chickens. Egyptian Poultry Science Journal 40, 29-46.

Aviagen, 2022. Ross 308. Broiler nutrition specification. In: Aviagen Inc., H, AL (Ed), USA.

Ayoola, A.A., Yusuf, A.O., Olatunbosun, O.B., Okanlawon, O.A., Yinusa, D.Q., 2021. Comparative antioxidative and hypocholesteremic properties of copper sulphate and copper nanoparticles supplemented in diet of broiler chickens. Thai Journal of Agricultural Science 54, 272-279.

Banks, K.M., Thompson, K.L., Rush, J.K., Applegate, T.J., 2004. Effects of copper source on phosphorus retention in broiler chicks and laying hens. Poultry Science 83, 990-996.

Bortoluzzi, C., Lumpkins, B., Mathis, G.F., França, M., King, W.D., Graugnard, D.E., Dawson, K.A., Applegate, T.J., 2019. Zinc source modulates intestinal inflammation and intestinal integrity of broiler chickens challenged with coccidia and Clostridium perfringens. Poultry Science 98, 2211-2219.

Chen, H., Weiss, J., Shahidi, F., 2006. Nanotechnology in nutraceuticals and functional foods. Food technology (Chicago) 60, 30-36.

Dosoky, W.M., Al-Banna, A.A., Zahran, S.M., Farag, S.A., Abdelsalam, N.R., Khafaga, A. F., 2022. Zinc oxide nanoparticles induce dose-dependent toxicosis in broiler chickens reared in summer season. Environmental Science and Pollution Research 29, 54088-54107.

Elashry, G.M., 2019. Effect of dietary zinc and manganese chelated with tryptophan and protein on growth performance and carcass traits of quail. Egyptian Poultry Science Journal 39, 579-597.

El-Katcha, M., Soltan, M.A., El-Badry, M., 2017. Effect of Dietary Replacement of Inorganic Zinc by Organic or Nanoparticles Sources on Growth Performance, Immune Response and Intestinal Histopathology of Broiler Chicken. Alexandria Journal of Veterinary Sciences 55, 129-145.

El-Kazaz, S.E., Hafez, M.H., 2019. Evaluation of copper nanoparticles and copper sulfate effect on immune status, behavior, and productive performance of broilers. Journal of Advanced Veterinary and Animal Research 7, 16-25.

El-Saadony, M.T., Alkhatib, F.M., Alzahrani, S.O., Shafi, M.E., Abdel-Hamid, S.E., Taha, T.F., Aboelenin, S.M., Soliman, M.M., Ahmed, N.H., 2021. Impact of mycogenic zinc nanoparticles on performance, behavior, immune response, and microbial load in Oreochromis niloticus. Saudi Journal of Biological Sciences 28, 4592-4604.

Eskandani, M., Janmohammadi, H., Mirghelenj, S.A., Ebrahimi, M., Kalanaky, S., 2021. Effects of Zinc Nanoparticles on Growth Performance, Carcass Characteristics, Immunity, and Meat Quality of Broiler Chickens. Iranian Journal of Applied Animal Science 11, 135-146.

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

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18, 499-502.

Gaetke, L.M., Chow, C.K., 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189, 147-163.

Guyton, A.C, Hall, J.E., 2006. Text-Book of Medical Physiology. 11thEdn, Elsevier Inc.

Hassan, S., Hassan, F.U., Rehman, M.S.U., 2020. Nano-particles of trace minerals in poultry nutrition: potential applications and future prospects. Biological Trace Element Research 195, 591-612.

Hatab, M.H., Rashad, E., Saleh, H.M., El-Sayed, E.S.R., Taleb, A.A., 2022. Effects of dietary supplementation of myco-fabricated zinc oxide nanoparticles on performance, histological changes, and tissues Zn concentration in broiler chicks. Scientific Reports 12, 18791.

Helbawi, E.S., Abd El-Latif, S.A., Toson, M.A., Banach, A., Mohany, M., Al-Rejaie, S.S., Elwan, H., 2024. Impacts of biosynthesized manganese dioxide nanoparticles on antioxidant capacity, hematological parameters, and antioxidant protein docking in broilers. ACS Omega 9, 9396-9409.

Hidayat, C., Sumiati, S., Jayanegara, A., Wina, E., 2021. Supplementation of dietary nano Zn-phytogenic on performance, antioxidant activity, and population of intestinal pathogenic bacteria in broiler chickens. Tropical Animal Science Journal 44, 90-99.

Hill, E.K., Li, J., 2017. Current and future prospects for nanotechnology in animal production. Journal of Animal Science and Biotechnology 8, 1-13.

Huff, G.R., Huff, W.E., Jalukar, S., Oppy, J., Rath, N.C., Packialakshmi, B., 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/Escherichia coli challenge. Poultry Science 92, 655-662.

Hussein, H.G., El-Sayed, E.S.R., Younis, N.A., Hamdy, A.E.H.A., Easa, S.M., 2022. Harnessing endophytic fungi for biosynthesis of selenium nanoparticles and exploring their bioactivities. AMB Express 12, 68.

Ibrahim, D., Ali, H.A., El-Mandrawy, S.A., 2017. Effects of different zinc sources on performance, bio distribution of minerals and expression of genes related to metabolism of broiler chickens. Zagazig Veterinary Journal 45, 292-304.

Ibrahim, I.A., EL-Gendi, G.M., Nihad, A.A., Okasha, H.M., El-Attrouny, M.M., 2022. Potential effects of different dietary copper sources to improve productive performance, plasma biochemical parameters and oxidative response activities of broiler chickens. Journal of Animal and Poultry Production 13, 111-118.

Larasati, N.H.D., Ariyadi, B., Dono, N.D., 2022. The Effect of Nano Zinc Dietary Supplementation on Growth Performance of KUB Chickens. In 9th International Seminar on Tropical Animal Production. Atlantis Press. pp. 88-91.

Liao, C.D., Hung, W.L., Jan, K.C., Yeh, A.I., Ho, C.T., Hwang, L.S., 2010. Nano/sub-microsized lignan glycosides from sesame meal exhibit higher transport and absorption efficiency in Caco-2 cell monolayer. Food Chemistry 119, 896-902.

Liu, Z.H., Lu, L., Li, S.F., Zhang, L.Y., Xi, L., Zhang, K.Y., Luo, X.G., 2011. Effects of supplemental zinc source and level on growth performance, carcass traits, and meat quality of broilers. Poultry Science 90, 1782-1790.

Luo, X.G., Ji, F., Lin, Y.X., Steward, F.A., Lu, L., Liu, B., Yu, S.X., 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. Poultry Science 84, 888-893.

MacDonald, R.S., 2000. The role of zinc in growth and cell proliferation. The Journal of Nutrition 130, 1500S-1508S.

Mahmoud, U.T., Abdel-Mohsein, H.S., Mahmoud, M.A., Amen, O.A., Hassan, R.I., Abd-El-Malek, A. M., Rageb, S.M.M., Waly, H.S.A., Othman, A.A., Osman, M.A. 2020. Effect of zinc oxide nanoparticles on broilers' performance and health status. Tropical Animal Health and Production 52, 2043-2054.

Mane, A.V., Khose, K.K., Nikam, M.G., Dhumal, M.V., Gangane, G.R., Munde, V.K., 2022. Effect of organic and nanoparticle trace minerals replacing inorganic trace minerals in diets on growth performance, immune response and economics of broilers. The Indian Journal of Animal Sciences 92, 465-470.

MarappanGopi, M.G., Beulah Pearlin, B.P., Kumar, R.D., MuthuvelShanmathy, M.S., GovindasamyPrabakar, G.P., 2017. Role of nanoparticles in animal and poultry nutrition: modes of action and applications in formulating feed additives and food processing. Int. J. Pharmacol. 13, 724–731.

Marchetti, M., Ashmead, H.D., Tossani, N., Marchetti, S., Ashmead, S.D., 2000. Comparison of the rates of vitamin degradation when mixed with metal sulphates or metal amino acid chelates. Journal of Food Composition and Analysis 13, 875-884.

McNerny, E.M., Gong, B., Morris, M.D., Kohn, D.H., 2015. Bone fracture toughness and strength correlate with collagen cross-link maturity in a dose-controlled lathyrism mouse model. Journal of Bone and Mineral Research 30, 455-464.

Mohamed, D.A., El-sadek, A., Abdel-Wareth, A.A.A., 2022. Effects of Copper oxide nanoparticles on productive performance of broiler chickens under climate change conditions. SVU-International Journal of Agricultural Sciences 4, 51-57.

MohdYusof, H., Mohamad, R., Zaidan, U.H., Abdul Rahman, N.A., 2019. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of Animal Science and Biotechnology 10, 1-22.

Mozhiarasi, V., Karunakaran, R., Raja, P., Radhakrishnan, L., 2024. Effects of zinc oxide nanoparticles supplementation on growth performance, meat quality and serum biochemical parameters in broiler chicks. Biological Trace Element Research 202, 1683-1698.

Ouyang, Z., Ren, P., Zheng, D.E., Huang, L., Wei, T., Yang, C., Kong, X., Yin, Y., He, S., He, Q., 2021. Hydrothermal synthesis of a new porous zinc oxide and its antimicrobial evaluation in weanling piglets. Livestock Science 248, 104499.

Pacheco, W.J., Patiño, D.B., Vargas, J.I., Gulizia, J.P., Macklin, K.S., Biggs, T.J., 2021. Effect of partial replacement of inorganic zinc and manganese with zinc methionine and manganese methionine on live performance and breast myopathies of broilers. Journal of Applied Poultry Research 30, 100204.

Park, S.Y., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., Ricke, S.C., 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biological Trace Element Research 101, 147-163.

Patra, A., Lalhriatpuii, M., 2020. Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding-a review. Biological Trace Element Research 197, 233-253.

Prabakar, G., Gopi, M., JJ, R., Beulah Pearlin, V., Tyagi, J. S., Mohan, J., 2018. Effect of supplementation of organic zinc on serum biochemistry in broiler breeders. IJCS 6, 342-344.

Qady, M., El-Banna, R., Farouk, F., Soliman, R., Gado, W., 2022. Influences of the Dietary Supplementation of Broiler Chickens with Copper Nanoparticles on Some Biometric Measurements and Transcription of Copper-allied Genes. Journal of Advanced Veterinary Research 12, 494-500.

Rajendran. D.; Kumar, G., Ramakrishnan, S., Thomas, K.S., 2013. Enhancing the milk production and immunity in Holstein Friesian crossbred cow by supplementing novel nano zinc oxide. Res. J. Biotechnol. 8, 11-17.

Ramiah, S.K., Awad, E.A., Mookiah, S., Idrus, Z., 2019. Effects of zinc oxide nanoparticles on growth performance and concentrations of malondialdehyde, zinc in tissues, and corticosterone in broiler chickens under heat stress conditions. Poultry Science 98, 3828-3838.

Saleh, A.A., Ragab, M.M., Ahmed, E.A., Abudabos, A.M., Ebeid, T.A., 2018. Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. Journal of Applied Animal Research 46, 820-827.

Samanta, B., Ghosh, P.R., Biswas, A., Das, S.K., 2011. The effects of copper supplementation on the performance and hematological parameters of broiler chickens. Asian-Australasian Journal of Animal Sciences 24, 1001-1006.

Samy, A., Hassan, H.M.A., Elsherif, H.M.R., 2022. Effect of nano zinc oxide and traditional zinc (oxide and sulphate) sources on performance, bone characteristics and physiological parameters of broiler chicks. Int. J. Vet. Sci. 11, 486-492.

SAS (Statistical Analysis System)., 2009. SAS/STAT® 9.2. User's Guide. SAS Institute Inc. Cary, North Carolina.

Scott, A., Vadalasetty, K. P., Chwalibog, A., Sawosz, E., 2018a. Copper nanoparticles as an alternative feed additive in poultry diet: a review. Nanotechnology Reviews 7, 69-93.

Scott, A., Vadalasetty, K.P., Łukasiewicz, M., Jaworski, S., Wierzbicki, M., Chwalibog, A., Sawosz, E., 2018b. Effect of different levels of copper nanoparticles and copper sulphate on performance, metabolism and blood biochemical profiles in broiler chicken. Journal of Animal Physiology and Animal Nutrition 102, 364-373.

Shobha, G., Moses, V., Ananda, S., 2014. Biological synthesis of copper nanoparticles

and its impact. Int. J. Pharm. Sci. Invent. 3, 6-28.
Shubhnish, V.S., Panwar, B.S., Tewatia, Jyotsana., 2021. Growth, feed conversion ratio and nutrient metabolizability of broiler chicks fed on inorganic, organic and nano zinc supplemented diets. International Journal of Chemical Studies 9, 12-16.
Swain, P.S., Prusty, S., Rao, S.B.N., Rajendran, D., Patra, A.K., 2021. Essential nanominerals and other nanomaterials in poultry nutrition and production. In: Advances in poultry nutrition research. IntechOpen. DOI: 10.5772/intechopen.96013
Tag-El Din, N.T., 2019. Effects of dietary nano-Zinc and nano-selenium addition on productive and physiological performance of growing rabbits at fattening period. Egyptian Journal of Nutrition and Feeds 22, 79-89.
Usman, M.S., Zowalaty, M.E.E., Shameli, K., Zainuddin, N., Salama, M., Ibrahim, N. A., 2013. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. International Journal of Nanomedicine 8, 4467-4479.
Vijayakumar, M., Balakrishnan, V., 2014. Nanoparticles supplementation on growth performance of broiler chicken. Indian J. Sci.Technol. 7, 1149-1154.
Wang, C., Wang, L., Chen, Q., Guo, X., Zhang, L., Liao, X., Huang Y., Lu L., Luo, X., 2023. Dietary trace mineral pattern influences gut microbiota and intestinal health of broilers. Journal of Animal Science 101:240.
Wang, C., Wang, M. Q., Ye, S.S., Tao, W.J., Du, Y.J., 2011. Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poultry Science 90, 2223-2228.
Zhang, W.F., Li, D.F., Lu, W.Q., Yi, G.F., 2003. Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. Poultry Science 82, 657-663.
Zhao, C.Y., Tan, S.X., Xiao, X.Y., Qiu, X.S., Pan, J.Q., Tang, Z.X., 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. Biological Trace Element Research 160, 361-367.