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Varied sources and levels of zinc in Ross 308 broiler rations: Its effect on performance, nutrient utilization, intestinal morphology and microbial composition

[*Fontes e níveis variados de zinco em rações para frangos de corte Ross 308: seu efeito no desempenho, na utilização de nutrientes, na morfologia intestinal e na composição microbiana*]

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ABSTRACT

Broilers (n=480) were used in an experiment designated for a 3×4 factorial arrangement to investigate the efficacy of three zinc (Zn) sources (organic Zn-Methionine, inorganic Zn sulphate and Zn oxide) and four inclusion rates (zero, 60, 120 and 180 mg/kg of feed). Growth performance was not affect by Zn source in the entire of experimental period, however Zn level at 180 mg/kg improved body weight gain and feed conversion ratio compared to other Zn levels (P<0.05). Inclusion of Zn-Methionine in the diet increased the population of *Lactobacillus* (P<0.05), while *E. coli* counts decreased in birds which received Zn sulphate compared with Zn oxide group (P<0.05). Inclusion 180 mg/kg of Zn decreased total intestinal aerobic bacteria in comparison to zero level (P<0.05). An improvement of intestinal villus length and width was found in treatments supplemented with organic Zn (P<0.05), Zn oxide increased intestinal crypt depth in birds as well (P<0.05). Ileal digestibility of crude protein increased in broilers received Zn at 120 and 180 mg/kg compared with zero level (P<0.05). Consequently, Zn-methionine supplement had an optimistic effect on the microbial population and intestinal morphometric variables. The level of 180mg/kg of supplemental Zn had beneficial influence on growth performance.

Keywords: broiler, performance, source, microbiota, zinc

RESUMO

Frangos de corte (n=480) foram usados em um experimento designado para um arranjo fatorial 3×4 para investigar a eficácia de três fontes de zinco (Zn) (Zn orgânico, sulfato de Zn inorgânico e óxido de Zn) e quatro taxas de inclusão (zero, 60, 120 e 180mg/kg de ração). O desempenho zootécnico não foi afetado pela fonte de Zn durante todo o período experimental, porém o nível de Zn na dose de 180 mg/kg melhorou o ganho de peso corporal e a conversão alimentar em relação aos outros níveis de Zn (P<0,05). A inclusão de Zn orgânico na dieta aumentou a população de Lactobacillus (P<0,05), enquanto as contagens de E. coli diminuíram nos frangos que receberam sulfato de Zn em comparação com o grupo óxido de Zn (P<0,05). A inclusão de 180 mg/kg de Zn diminuiu as bactérias aeróbicas intestinais totais em comparação ao controle (P<0,05). Uma melhora no comprimento e largura das vilosidades intestinais foi encontrada nos tratamentos suplementados com Zn orgânico (P<0,05), e o óxido de Zn aumentou a profundidade das criptas intestinais nas aves também (P<0,05). A digestibilidade ileal da proteína bruta aumentou em frangos de corte que receberam Zn nas doses de 120 e 180 mg/kg em comparação com o nível zero (P<0,05). Consequentemente, o suplemento orgânico de Zn teve um efeito positivo sobre a população microbiana e as variáveis morfométricas intestinais. O nível de 180 mg/kg de Zn suplementar teve influência benéfica no desempenho zootécnico.

Palavras-chave: frangos de corte, desempenho, fonte, microbiota, zinco

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INTRODUCTION

Trace elements similar to Zn play an important and effective role in maintaining healthy and productive birds. The beneficial influence of dietary Zn in various biological functions in animals, with particular importance for fast-growing broilers has been well documented. Moreover, it has been reported that Zn is an essential component in several biological activities in broilers, including enzymes functions, skeletal development and immune booster (Ogbuewu and Mbajiorgu, 2023; Zhu *et al*., 2022). In plant origin feed ingredients, a part of Zn is bonded with phytate complex so that its availability is poor for broiler chickens (Zarghi *et al*., 2022). Hence, supplemental Zn should be added to the most broiler chicken diets to meet the nutritional requirements. Different sources of inorganic supplemental Zn such as sulfate or oxide are added to the rations to overcome Zn deficiency in plant origin feed ingredients (Olukosi *et al*., 2018). It has been reported that inorganic forms of Zn such as Zn oxide (containing 72% Zn) and Zn sulfate monohydrate (36% Zn) are commercially utilized by the poultry feed producers (Abd El-Wahab *et al*., 2013). Furthermore, in many studies the beneficial effects of inorganic and organic Zn sources on the growth performance of broilers have been reported (Movahed *et al*., 2022; Ogbuewu and Mbajiorgu, 2023; Zarghi *et al*., 2022; Zhu *et al*., 2022). Organic supplemental Zn is more popular in broiler nutrition due to its greater bio-efficacy than inorganic forms (Zhu *et al*., 2022). Therefore, recently Zn from organic sources has been of interest to the poultry feed industry. In addition, it has been demonstrated that chelated trace mineral sources such as Zn-methionine supplements are more biologically available in the gastrointestinal tract of poultry than inorganic forms and may cause less mineral excretion and environmental pollution (Ogbuewu *et al*., 2023). However, there are conflicting results published regarding the effectiveness of various organic forms of Zn versus inorganic sources regarding the growth performance of broiler chickens. According to the National Research Council (Nutrient…, 1994), the optimum requirement of broiler chickens for Zn considered 40mg/kg of diet based on their growth performance criteria. However, this recommendation for Zn and most of the NRC recommended values for trace elements are based on elder strains of broilers and may be outdated for the modern strains of broilers used in commercial production today (Nessabian *et al*., 2021; Zarghi *et al*., 2022). Several reports showed improved growth performance and boosted immune system for broilers in response to higher inclusion rates of Zn in the diet (Mohamed *et al*., 2022; Movahed *et al*., 2022; Nessabian *et al*., 2021; Zarghi *et al*., 2022). Unfortunately, there is not perfect information on Zn requirements for growth performance, gut health, and blood biochemical metabolites of broilers yet. Therefore, this research aimed to compare bio-efficacy of different inorganic and organic supplemental Zn in combination with their inclusion rates in broiler diets on the growth performance, intestinal morphometric indices, nutrient utilization, and intestinal microbial population.

MATERIALS AND METHODS

This experiment was conducted at Bonyann Danesh poultry research farm (Qhaemshahr, north of Iran). All broiler chickens care and used methods were approved by the Department of Animal Science, Islamic Azad University (Chalous branch, Chalous, Iran).

All methods and animal care used in this experiment were approved by the Department of Animal Science, Chalous Branch, Islamic Azad University. This study was performed as a completely randomized design with 3×4 factorial arrangement. Factors included 3 sources of Zn (Zn Oxide, Zn Sulphate and Zn-methionine) and 4 levels of Zn inclusion (zero, 60, 120 and 180 mg/kg). A total number of 480 broiler chicks (mixed male and female Ross 308) were purchased from a commercial hatchery and were randomly distributed into 12 treatments and 10 replicates per each pen. All diets were formulated for starter (1 to 10d), grower (11 to 24d) and finisher (25 to 42d) to meet or exceed the nutrient requirements of Ross 308 broilers according to Aviagen handbook. All diets used in the present trial were provided in mash form during the entire 42 d period. The ingredients and calculated chemical composition of the experimental diets are presented in Table 1. The temperature was kept about 32 to 34°C for the first week, and was lowered 2°C weekly, until it reached 23°C. All birds were allowed feed and water *ad libitum* during the experiment.

Table 1. Ingredients and nutrient levels of the basal diets

Ingredients	Starter (d 1 to 10)	Grower (d 11 to 24)	Finisher (d 25 to 42)
Corn	54.21	60.49	65.95
Soybean meal	39.60	33.50	28.50
Soybean oil	1.80	2.00	1.90
Limestone	0.90	0.80	0.70
Dicalcium phosphate	2.20	1.95	1.70
Salt	0.225	0.20	0.20
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-Methionine	0.24	0.22	0.19
L- Lysine HCL	0.15	0.11	0.12
L-Threonine	0.025	0.03	0.04
Sodium bicarbonate	0.15	0.20	0.20
Total	100.00	100.00	100.00
Calculated composition (%)			
AME (kcal/kg)	2850	2940	3000
Crude protein	21.00	19.00	17.70
Lysine	1.23	1.04	0.92
Methionine	0.59	0.51	0.46
Methionine +Cysteine	0.88	0.78	0.70
Threonine	0.77	0.67	0.60
Calcium	1.02	0.89	0.80
Available Phosphorus	0.49	0.44	0.40
Zinc (mg/kg)	35.00	32.00	30.00

The vitamin / mineral premix provided per kg of diet: Vitamin A 11000 IU, Vitamin D3 5000IU, Vitamin E 75 IU, Vitamin K3mg, Thiamine 3mg, Riboflavin 3mg, Pyridoxine 4mg, Niacin 60 mg, Pantothenicacid 15mg, Biotin 0.15mg, Cyanocobalamin 0.016mg, Folic acid 2mg, Choline 700mg, Cu 16mg, I 1.25mg, Fe 40mg, Mn 120mg, Se 0.3mg, Antioxidant 10mg, Zn 0.0mg (mineral premix contained no zinc).

In the present experiment, productive performance variables including body weight gain, feed intake and feed conversion ratio were recorded (Palizdar *et al*., 2017). Briefly, at the end of starter, grower, and finisher phases and after 6h fasting, body weight gain (BWG) and feed intake (FI) of chicks were recorded. Then, ratio of feed to gain was calculated through dividing the feed intake by the body weight gain.

At the end of the trial period, five chicks from each pen were slaughtered by cervical dislocation for extraction of cecal digeta. After the removing of the intestinal tract, the fresh cecal contents of each bird were collected in sterile laboratory tubes for serial dilution (Palizdar *et al*., 2016). One gram of each cecal sample was then diluted (10^{-4} to$10^{-7)}$ in a sterilized physiological saline solution composed of 0.85g NaCl in 100 mL of solution. In the next step, 0.1mL of each diluted sample was inoculated onto Petri dishes with specific agar media. To measure the enumeration of *Escherichia coli*, *Lactobacillus* and total aerobic bacteria, appropriate agar media including eosin methylene blue agar (Merck, Darmstadt, Germany), de Man Rogosa, sharp agar (Merck, Darmstadt, Germany), and standard plate count agar (Merck) were used, respectively. Plates for *Lactobacillus* were incubated anaerobically at 37°C (for 48h) while *E. coli* was incubated aerobically at 37°C (for 24h). All results from microbiological study were expressed as log_{10} CFU/g of cecal fresh sample.

To determine the intestinal morphometric variables, five chicks per pen were euthanized on day 42 and tissue samples (3cm) were then gently collected from the middle of jejunum segment. Each jejunal sample was carefully flushed with buffered saline and fixed in 10 percent neutral buffered formalin overnight. Briefly, the tissue sample was serially dehydrated in graded ethanol solutions and embedded in paraffin wax. A section of 5cm was cut from each sample using a microtome and placed on a glass slide and stained with hematoxylin and eosin blue. In the present experiment the villus length, villus width and crypt depth as jejunal morphometric indices were measured by an optic microscope.

During 35 to 42 days of age, three grams of chromium oxide per each kilogram of feed was included into the experimental diets as an indigestible marker. On day 42, five birds per treatment were chosen, and then were euthanized. To determine the apparent ileal nutrient digestibility, the contents from the end half of ileum segment were gently collected. All ileal digesta were dried at 55°C for 72h, and then diets and died ileal samples were ground to pass through a 1-mm screen. Ground samples were stored at −20°C in airtight containers until further analysis. In the present experiment, ileal digestibility of crude protein, dry matter, and ether extract was calculated according to the following formula:

$D (\%) = 100 - (100 \times (A/B) \times (C/E))$
where D = Digestibility, A = chromium oxide in feed (%), B=chromium oxide in ileal digesta (%), C = nutrient concentration in ileal digesta (%), E = nutrient concentration in feed (%).

To assess the serum biochemical compound, five broiler chickens per treatment were randomly chosen and 3mL of blood were samples via wing vein by a syringe at 42 days of age. Taken samples were subjected to laboratory sterile test tubes. To obtain serum samples, test tubes containing blood samples were centrifuged at 3000×g for 10 min. Then, serum concentration of glucose, triglyceride, cholesterol, high density lipoprotein (HDL), total protein, albumin and uric acid were measured using an auto analyzer.

Collected data (with normal distribution) were analyzed as a completely randomized design with a 3×4 factorial arrangement using GLM procedure of SAS software (SAS, 1999). Data obtained from the SAS analysis were statistically evaluated for main effects of Zn sources and the levels of Zn and their interactions. Statistically significant differences among experimental groups were tested using Duncan multiple range test. An alpha level (probability value) of 0.05 was considered significant.

RESULTS

The effects of dietary treatments on feed consumption of broiler chickens are shown in Table 2. Different Zn sources did not have significant influence on feed intake. During starter and grower phases, the birds fed diets without supplemental Zn (control) had the greatest feed intake (P<0.05). Inclusion of 180mg/kg Zn in diet increased feed intake of broilers compared with control and 120mg/kg groups from 25-42 days of age (P<0.05). At the entire of the experimental period, feed intake increased in broilers which received control diet and control diet supplemented with 180 mg Zn/kg compared with 120mg/kg treatment (P<0.05).

Table 2. Effects of dietary treatments on feed intake of broiler chickens (g)

	Feed intake			
	Starter	Grower	Finisher	Total
Zinc sources				
Zinc oxide	205.92	901.15	3107.45	4214.80
Zinc sulphate	207.05	893.20	3065.60	4166.20
Zinc-methionine	206.41	914.25	3152.75	4273.60
P-Value	0.97	0.71	0.401	0.38
SEM	0.45	8.67	35.58	43.90
Levels (mg/kg)				
0	231.01 ^{a}	986.02 ^{a}	3083.60 ^{b}	4301.00 ^{a}
60	201.80 ^{b}	885.27 ^{b}	3108.07 ^{ab}	4195.50 ^{ab}
120	194.86 ^{b}	850.87 ^{b}	2993.50 ^{b}	4039.40 ^{b}
180	197.67 ^{b}	889.33 ^{b}	3249.20 ^{a}	4336.80 ^{a}
P-Value	0.00001	0.0003	0.011	0.006
SEM	14.42	50.25	91.70	115.50
Zinc sources×Levels				
P-Value	0.55	0.111	0.258	0.114
SEM	3.28	27.41	39.42	61.30

Means within the same column with no common superscripts differ significantly (*P* < 0.05).
SEM: standard error of the means.

The results of body weight gain of broilers in response to dietary treatments are presented in Table 3. No significant change was found in body weight gain of broilers in response to different dietary Zn sources. However, inclusion of 180mg Zn /kg decreased body weight gain of broilers compared with control group during starter phase (P<0.05). During grower period, the highest body weight gain was observed in broilers which received control diet (P<0.05). From 25-42 and 1-42 days of age, the greatest body weight gain was observed in the birds fed with 180mg Zn /kg (P<0.05).

Table 3. Effects of dietary treatments on body weight gain of broiler chickens (g)

	Body weight gain			
	Starter	Grower	Finisher	Total
Zinc sources				
Zinc oxide	173.40	544.50	1539.60	2258.00
Zinc sulphate	178.80	547.40	1497.60	2224.00
Zinc-methionine	175.80	565.70	1579.80	2221.00
P-Value	0.326	0.164	0.258	0.135
SEM	2.21	9.38	33.50	40.10
Levels (mg/kg)				
0	184.80 ^{a}	598.00 ^{a}	1467.60 ^{b}	2251.00 ^{b}
60	175.80 ^{ab}	544.80 ^{b}	1527.60 ^{b}	2249.00 ^{b}
120	175.04 ^{ab}	537.70 ^{b}	1449.30 ^{b}	2163.00 ^{b}
180	186.40 ^{b}	529.20 ^{b}	1711.60 ^{a}	2409.00 ^{a}
P-Value	0.036	0.0001	0.0001	0.0006
SEM	5.88	26.87	103.70	88.80
Zinc sources×Levels				
P-Value	0.507	0.268	0.535	0.310
SEM	3.43	12.73	48.51	49.20

Means within the same column with no common superscripts differ significantly (*P* < 0.05).
SEM: standard error of the means

The summarized results of feed conversion ratio of broilers are shown in Tab 4. During starter period, a tendency of improvement in feed conversion ratio was found in broilers which fed diets containing 60, 120, and 180mg Zn/kg compared with control group (P<0.05). From days of 11-24, addition of 60 and 120 mg Zn/kg in diets decreased feed conversion ratio of broilers (P<0.05). In finisher and total periods of the experiment, feed conversion ratio improved in broilers fed with diets containing 180mg/kg of supplemental Zn (P < 0.05).

In microbial population (Table 5.), the viable count of *Lactobacillus* increased in broilers which received diets supplemented with organic Zn (P<0.05). Besides, inclusion of Zn sulphate decreased the cecal enumeration of *E. coli* compared to Zn oxide (P < 0.05) while organic Zn was intermediate. In the main effect of different levels, 180 mg/kg supplemental Zn decreased total aerobic count of bacteria compared with control and 120mg/kg groups (P<0.05).

According to the results of Table 6 dietary organic Zn increased the jejunal villus length of broilers compared with Zn oxide (P<0.05) and Zn sulphate was intermediate. Addition of organic Zn also enhanced the villus width compared to Zn sulphate group (P<0.05). An increased crypt depth was observed in broilers fed with diet containing Zn oxide compared with Zn sulphate (P<0.05). The highest villus length was found in broilers which received 180mg/kg dietary Zn (P<0.05).

Table 4. Effects of dietary treatments on feed conversion ratio of broiler chickens (g/g)

	Feed conversion ratio			
	Starter	Grower	Finisher	Total
Zinc sources				
Zinc oxide	1.18	1.64	2.02	1.86
Zinc sulphate	1.15	1.62	2.04	1.86
Zinc-methionine	1.16	1.61	2.01	1.84
P-Value	0.342	0.563	0.662	0.526
SEM	0.003	0.042	0.22	0.038
Levels (mg/kg)				
0	1.24 ^{a}	1.74 ^{a}	2.10 ^{a}	1.96 ^{a}
60	1.14 ^{bc}	1.60 ^{b}	2.04 ^{a}	1.86 ^{a}
120	1.11 ^{c}	1.59 ^{b}	2.06 ^{a}	1.86 ^{a}
180	1.17 ^{b}	1.67 ^{a}	1.91 ^{b}	1.80 ^{b}
P-Value	0.0001	0.135	0.0002	0.004
SEM	0.044	0.050	0.064	0.054
Zinc sources×Levels				
P-Value	0.0211	0.516	0.405	0.577
SEM	0.017	0.041	0.037	0.041

Source × Level interactions for feed conversion ratio in starter period

zero	†ZO× 60	ZO×120	ZO×180	ZS×60	ZS×120	ZS×180	ZM×60	ZM×120	ZM×180
1.19 ^{a}	1.15 ^{b}	1.13 ^{bc}	1.17 ^{ab}	1.15 ^{b}	1.11 ^{c}	1.15 ^{b}	1.14 ^{b}	1.12 ^{bc}	1.18 ^{ab}

Means within the same column with no common superscripts differ significantly ($P < 0.05$).
SEM: standard error of the means.
† ZO: Zinc Oxide, ZS: Zinc Sulphate, ZM: Zinc-Methinone.

Table 5. Effects of dietary treatments on microbiota activity of broiler chickens

	Microbial count (cfu/g)		
	Lactobacillus	Coliforms	Total aerobic bacteria
Zinc sources			
Zinc oxide	0.895 ^{b}	0.901 ^{a}	0.913
Zinc sulphate	0.885 ^{b}	0.885 ^{b}	0.913
Zinc-methionine	0.906 ^{a}	0.892 ^{ab}	0.914
P-Value	0.001	0.028	0.981
SEM	0.04	0.01	0.028
Levels (mg/kg)		0.899	
0	0.890	0.890	0.915 ^{a}
60	0.898	0.893	0.913 ^{ab}
120	0.899	0.894	0.918 ^{a}
180	0.894	0.884	0.908 ^{b}
P-Value	0.111	0.114	0.018
SEM	0.063	0.007	0.043
Zinc sources×Levels			
P-Value	0.0003	0.261	0.677
SEM	0.005	0.016	0.039

Source × Level interactions for *Lactobacillus* count

zero	†ZO× 60	ZO×120	ZO×180	ZS×60	ZS×120	ZS×180	ZM×60	ZM×120	ZM×180
0.899 ^{d}	0.902 ^{c}	0.907 ^{b}	0.899 ^{d}	0.894 ^{d}	0.904 ^{b}	0.901 ^{c}	0.914 ^{a}	0.917 ^{a}	0.913 ^{a}

Means within the same column with no common superscripts differ significantly ($P < 0.05$).
SEM: standard error of the means
† ZO: Zinc Oxide, ZS: Zinc Sulphate, ZM: Zinc-Methinone.

Table 6. Effects of dietary treatments on intestinal morphology of broiler chickens

	Morphometric indices (µm)		
	Villus length	Villus width	Crypt depth
Zinc sources			
Zinc oxide	1339[b]	183 [ab]	192 [a]
Zinc sulphate	1347 [ab]	176[b]	175 [b]
Zinc-methionine	1350 [a]	185 [a]	185[ab]
P-Value	0.003	0.009	0.01
SEM	6.21	8.97	11.74
Levels (mg/kg)			
0	1346 [b]	185	182
60	1329 [c]	183	180
120	1347 [b]	182	187
180	1359 [a]	179	185
P-Value	0.03	0.121	0.008
SEM	23.04	7.61	2.47
Zinc sources×Levels			
P-Value	0.45	0.19	0.37
SEM	12.27	6.34	5.76

Means within the same column with no common superscripts differ significantly (P < 0.05).
SEM: standard error of the means.

In nutrient digestibility (Table 7.), ileal digestibility coefficient for dry matter increased in broilers which receive 180mg/kg supplemental Zn compared with control group (P<0.05). In addition, diets containing 120 and 180mg Zn/kg had greater crude protein digestibility compared with control group (P<0.05). The main effect of Zn sources did not have any significant impact on the ileal nutrient utilization. In the present experiment, except for albumin concentration, none of serum biochemical metabolites were affected by dietary treatments (Table 8). The birds fed with control diet had the greatest serum concentration of albumin (P < 0.05).

Table 7. Effects of dietary treatments on nutrient digestibility of broiler chickens

	Nutrient digestibility (%)		
	Dry matter	Crude protein	Total ash
Zinc sources			
Zinc oxide	80.29	70.79	61.11
Zinc sulphate	80.95	70.56	62.03
Zinc-methionine	85.57	71.69	65.38
P-Value	0.89	0.26	0.11
SEM	2.45	0.86	0.96
Levels (mg/kg)			
0	75.06 [b]	65.55 [b]	61.93
60	78.47 [ab]	69.21 [ab]	61.89
120	80.53 [ab]	71.20 [a]	61.95
180	83.38 [a]	71.86 [a]	60.99
P-Value	0.036	0.02	0.11
SEM	2.27	3.46	0.74
Zinc sources×Levels			
P-Value	0.48	0.56	0.26
SEM	1.68	0.41	0.67

Means within the same column with no common superscripts differ significantly (P < 0.05).
SEM: standard error of the means

Table 8. Effects of dietary treatments on blood biochemical parameters of broiler chickens

	Serum indices (mg/dl)					
	Uric acid	Cholesterol	TG	Albumin	Total protein	HDL
Zinc sources						
Zinc oxide	3.46	104.90	93.35	1.32	3.72	77.50
Zinc sulphate	3.73	109.85	100.75	1.49	4.15	74.85
Zinc-methionine	3.93	100.45	98.10	1.42	4.01	75.15
P-Value	0.51	0.335	0.70	0.307	0.298	0.587
SEM	0.197	3.84	3.06	0.063	0.178	1.18
Levels (mg/kg)						
0	3.48	93.00	93.80	1.18 [b]	3.56	79.40
60	4.30	108.13	103.53	1.46 [a]	4.03	74.86
120	3.44	110.93	90.27	1.53 [a]	4.17	72.66
180	3.61	108.20	102.00	1.46 [a]	4.08	76.40
P-Value	0.23	0.0609	0.509	0.037	0.246	0.218
SEM	0.344	7.05	5.53	0.134	0.230	2.45
Zinc sources×Levels						
P-Value	0.672	0.654	0.981	0.149	0.594	0.557
SEM	0.257	6.02	4.41	0.083	0.195	2.07

Means within the same column with no common superscripts differ significantly ($P < 0.05$).
SEM: standard error of the means

DISCUSSION

During the overall period of this trial, non-significant interaction between Zn sources and Zn inclusion rates were found in growth performance of broilers which is in line with earlier finding who (Yogesh *et al*., 2013) did not observe any significant interactions between Zn sources and levels on the productive performance of broilers. Besides, different Zn sources including organic or inorganic supplemental Zn had no significant effects on growth performance of broilers. Our results are in consistent with the observations of (Liu *et al*., 2011) who observed no differences in growth variables of broilers between different sources of supplemental Zn. However and in contrast with the present results, there are several reports of the beneficial effects of different Zn sources on the body weight gain and feed conversion ratio in broiler chickens (Liu *et al*., 2015). On the other hand, inclusion of 180 mg/kg of supplemental Zn improved body weight gain and feed conversion ratio in the present study. The improvement observed for growth performance in the birds supplemented with different inclusion rates of Zn may be related to Zn functions in the birds body (Santos *et al*., 2021). Zinc has several biological roles in broilers associated with growth rate; including growth hormone synthesis, and protein metabolism (Liu *et al*., 2011; Zhang *et al*., 2018). Therefore, Zn deficiency may primarily change the basal metabolism rate of fast-growing broiler chickens. It has been well documented that the growth performance parameters of broilers were improved at dietary levels of Zn exceeding the NRC recommendations of 40mg/kg diet (Ogbuewu *et al*., 2023).

Results from the present study showed Supplementation of organic Zn increased lactobacillus population, while viable count of E. coli decreased in broilers which received dietary Zn sulphate. Most of the *in vivo* studies about the efficacy of Zn element on the microbiota composition have been conducted on the Zn nanoparticles and few bioassay studies are found about the antimicrobial activities of different organic and inorganic Zn sources in poultry (Bami *et al*., 2020). Therefore, a direct comparison has not been made in this paper. In a study with piglets and by using DNA sequencing technique, high dietary Zn enhanced the diversity of intestinal microbiota composition (Vahjen *et al*., 2011). Recently, a study was performed on the possible efficacy of dietary Zn on the prevention of necrotic enteritis as well as the status of the intestinal microbial composition in broiler chickens (Bortoluzzi *et al*., 2019). According to these authors, inclusion of Zn proteinate decreased the ileal population of *Lactobacillus* in boilers.

In the present study, the organic Zn supplementation resulted in greater improvement in villus length and villus width of broilers than inorganic Zn sources. These results are in accordance with earlier findings (De Grande *et al*., 2020) who indicated that supplementation of experimental diets with organic Zn enhanced the intestinal villus length and villus length to crypt depth ratio of broiler chickens. It has been observed that in comparison with inorganic forms of Zn, organic sources are more absorbed in broilers which may influence the development of intestinal villi via increasing cell proliferation and differentiation (Nessabian *et al*., 2021). Moreover, results from several studies confirm the beneficial effects of dietary organic Zn on the development and function of digestive system (Ogbuewu and Mbajiorgu, 2023). Intestinal villus is the main site of the nutrient absorption, and the villus length may be an appropriate indicator for intestinal health and function in poultry. The mode of action of Zn on the intestinal morphology of broilers may be due to its efficacy on the intestinal cell proliferation and protein synthesis in the crypt base (Tako *et al*., 2005). However and in contrast with our results, it has been observed that the effectiveness of Zn sulphate as an inorganic source on the improvement of intestinal morphometric indices was more than Zn glycine as an organic source of Zn (Levkut *et al*., 2017). These contradictory results from different experiments may be due to the form of organic and inorganic Zn supplied in the broiler diets and more studies are needed to clarify the actual mechanism of action of Zn sources on the gut morphometric variables.

In the current study, no differences for nutrient utilization were observed between different sources of organic and inorganic Zn, while inclusion rates of dietary Zn altered ileal digestibility of dry matter and crude protein in broilers. Our observations are in parallel with other experiments (Saleh *et al*., 2018) which indicated that increasing Zn supplementation (zero, 30 and 60 mg/kg) in broiler and quail diets linearly enhanced the digestibility coefficients of dry matter, organic matter, crude protein and ether extract. The mode of action of dietary Zn on the improvement of nutrient utilization has been illustrated by (Saleh *et al*., 2018). According to these authors, Zn element has a protective function on pancreatic cells against oxidative damage which improves the pancreas to function properly and the production of more digestive endogenous enzymes and finally increased the process of digestion and absorption of nutrients.

Except for albumin, none of the serum biochemical parameters were significantly affected by the source or level of Zn in the diet. Inclusion of different rates of Zn interestingly decreased serum concentration of albumin in broilers. Our results are not supported by observations of (Saleh *et al*., 2018) who indicated that different inclusions of Zn in broiler diets had no significant effect on albumin concentration. It has been reported that elevated serum albumin mainly indicates a reversal of liver metabolic reactions to lead to nutrients and mainly carbohydrates and proteins to increase growth performance (De Neve *et al*., 2008). The results of studies on the effect of different levels of Zn supplementation on blood parameters in animals are contradictory. In general, it can be noted that the composition of blood biochemical variables in poultry is affected by several factors such as age, species and pathological factors (Hazrati *et al*., 2019). Therefore, further studies are needed to better understand the mechanism of action of mineral supplements such as organic or inorganic Zn sources on the blood parameters in birds.

CONCLUSIONS

It can be concluded that different organic and inorganic Zn had no significant effect on growth performance of broilers, while different inclusion rates of Zn had beneficial effects on productive performance. In addition, higher levels of dietary Zn enhanced intestinal morphology and total bacteria. Besides, organic Zn improved intestinal microbial composition in broilers.

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