

# Supplementation of different zinc sources to low-CP diets and its effect on performance, carcass traits, liver and kidney functions, immunological, and antioxidant parameters of quail chicks

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**ABSTRACT** The present study was performed to evaluate the influence of low crude protein (CP) levels, zinc sources (organic as zinc methionine-Zn-Met and inorganic as zinc oxide-ZnO) and their interactions on growth performance, carcass traits, and blood components of growing Japanese quail. A total of 450 one-wk-old Japanese quail with the same body weights were randomly distributed into 9 groups of 50 birds. The 9-diet treatments comprised 3 levels of CP (20, 22, and 24%) and 3 Zn source (0 g of Zn/kg diet, 0.1 g ZnO/kg diet, and 0.1 g Zn-Met/kg diet). The results obtained from

this study showed that there were no significant differences among the groups, except for differences in body weight (BW) and body weight gain (BWG) at 3 to 5 – and 1 to 5 wk of age for quail supplemented with 24% and 20% CP. All the studied biochemical parameters were significantly influenced by different levels of CP and Zn, except urea and creatinine, which were affected by CP levels only. In conclusion, dietary protein level for growing Japanese quails could be reduced to 20% without negative effects on their performance, carcass traits, and blood metabolites.

**Key words:** quails, protein, zinc, performance, blood

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## INTRODUCTION

Feeding costs constitute about 65 to 70% of the total production costs of animal production projects (FAO, 2006; Attia et al., 2020, 2021), and crude protein (CP) has a substantial influence on feed costs, as it is one of the most expensive nutrients in poultry feeds (Alagawany et al., 2016). This has resulted in a focus on reducing the levels of CP in the diets of poultry species, and the use of different strategies that enhance the efficiency of protein and can decrease ammonia pollution and reduce the total costs of production in the poultry industry. Different studies on broiler chickens and quails have shown that productivity and growth are adversely influenced by a low-CP diet and have been reported as reasons for decreasing growth and production (Malomo et al., 2013; Folorunso et al., 2014;

Soomro et al., 2017). However, Dowarah and Sethi (2014) reported that using different dietary CP levels during the periods (starter-23, 25, and 27%) and (finisher-18, 20, and 22%) had no effect on the growth performance of quails. A low protein diet supplemented with some amino acids improves the growth performance of quails, and it is a good strategy for reducing ammonia emissions and nutritional costs (Alagawany et al., 2014, 2016, 2020; Attia et al., 2017). The use of synthetic or natural feed additives in diets containing low CP enhanced the feed conversion rate (FCR), growth, carcass traits, economic feasibility, and nitrogen retention of poultry (Rehman et al., 2018).

Zinc (Zn) plays a critical role in animal metabolism. It serves as both an essential element and feed supplement for increasing poultry productivity, immunity, appetite, normal growth, and the forming of feathers. Generally, minerals, including Zn, are added to poultry feeds because several feed ingredients are marginally Zn-deficient (Attia et al., 2013a,b; Zaghari et al., 2015; Abedini et al., 2017). Furthermore, Zn plays an important role in the transportation of vitamin A and the enhancement of the immune response (Ibs and

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Rink, 2003). Additionally, Zn is an important component of more than 300 enzymes that participate in the metabolism of several nutrients, that is, carbohydrates, proteins, and nucleic acids (Abd El-Hack et al., 2017, 2018). ZnO (Zinc oxide) is an inorganic element, which takes the form of a white powder that is insoluble in water and it is widely utilized in different animal nutrition propose (Attia et al., 2019).

The recommended level of Zn in poultry diets (40 mg/kg of diet) is primarily based on the criteria of broiler performance (NRC 1994). However, commercial poultry diets often contain double the recommended level of Zn: 80 mg/kg. This element can be obtained from organic and inorganic sources. The primary difference between these sources is that organic sources contain carbon. However, most inorganic sources do not have carbon atoms (Jahanian and Yaghoubi, 2010). Inorganic sources, such as oxides, are usually added to poultry diets at higher quantities than the recommended requirement of the National Research Council (NRC) to improve performance, feed utilization, and production (Leeson and Caston, 2008). Organic compounds, such as Zn-propionate (Zn-Pro) and Zn-methionine (Zn-Met), are more effective and available than inorganic compounds (Rahman et al., 2002; Attia et al., 2013b), and they provide better results for the health and performance of poultry. It is hypothesized that the use of different Zn sources in low-CP quail diets is expected to have beneficial impacts on growth, carcass and edible parts, feed utilization, and public health. Therefore, this study aimed to determine the effects of different dietary CP levels and Zn sources on growth performance, carcass traits, and blood biochemical indices in growing Japanese quails.

## MATERIALS AND METHODS

A total of 450 one-week-old unsexed Japanese quails were randomly divided into 9 groups of 50 birds, and each group was contained 5 replicates. Birds were fed 3 levels of CP (20, 22, and 24%) and 3 sources of Zn (0 g of Zn/kg diet, 0.1 g of ZnO/kg diet, and 0.1 g of Zn-Met/kg diet) throughout the experimental period of 1 to 5 wk of age. Each replicate was housed in a cage with dimensions of 90 × 40 × 40 cm. The basal diet was formulated according to the NRC (1994) (Table 1). All quails were subjected to the same conditions (managerial and hygienic aspects). Feed (mash) and water were available during the experiment. A clean, well-ventilated chamber with heaters was given for the growing quails, and the temperature was maintained at a comfortable level according to the quails' age. Apart from the first 3 d, the quails were subjected to a 17-h light/7-h dark during the experiment. The procedures of this experiment were performed according to the guidelines set out by the Local Experimental Animal Care Committee. ZU-IACUC/2/F/56/2021 is the ethical approval code.

Weekly, quails were individually weighed to calculate their **LBW** (live body weight), and different

**Table 1.** Ingredients and nutrient contents of experimental diets of growing Japanese quail.

Items	Crude protein levels (%)		
	20	22	24
Ingredient (%)			
Maize 8.5%	64.46	57.94	51.80
Soybean meal 44%	27.60	32.32	36.70
Maize gluten meal 62 %	3.00	4.00	5.21
Soybean oil	1.08	2.10	2.90
Limestone	0.70	0.72	0.70
Di-calcium phosphate	1.75	1.70	1.65
Salt	0.30	0.30	0.30
Premix <sup>1</sup>	0.30	0.30	0.30
L-Lysine	0.36	0.24	0.13
DL-Methionine	0.25	0.18	0.11
Choline chloride (50%)	0.20	0.20	0.20
Total	100	100	100
Calculated composition <sup>2</sup> , %			
ME, Kcal /Kg	3000	3000	2995
Crude protein	20.06	22.02	24.00
Calcium	0.80	0.80	0.80
Nonphytate P	0.45	0.45	0.45
Lysine	1.30	1.30	1.30
TSAA	0.90	0.92	0.92
Ether extract	3.82	4.66	5.29

<sup>1</sup>Provides per kg of diet: Vitamin A, 12,000 I.U.; Vitamin D3, 5,000 I.U.; Vitamin E, 130.0 mg; Vitamin K3, 3,605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4,950 mg; Vitamin B12, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18,333 mg; Folic acid, 2,083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

<sup>2</sup>Calculated according to NRC (1994).

performance parameters, including **FI** (feed intake) was measured, **BWG** (body weight gain), and **FCR**, were also calculated. At wk 5, five birds from each group (n = 45) were randomly selected for carcass examination, weighed, and manually slaughtered. All carcass parts, including carcass, liver, gizzard, heart, and giblets were weighed, and their weights were recorded and expressed as the percentage of final body weight. Dressing percentage was also calculated.

Blood samples were randomly collected from the jugular vein of all the slaughtered birds into heparinized tubes. Then, the samples were centrifuged (G force rate = 2,146.56 × g) for 15 min to obtain plasma, and the samples of plasma were kept at -20°C until they were analyzed. We determined albumin (g/dL), urea, creatinine, triglyceride, cholesterol, superoxide dismutase (**SOD**), reduced glutathione (**GSH**), alanine aminotransferase (**ALT**, U/L), malondialdehyde (**MDA**), immunoglobulin (IgG and IGM), high-density lipoprotein (**HDL**), and aspartate aminotransferase (**AST**, U/L) by commercial kits as described by the manufacturers (spectrum, Diagnostics, Egypt, Co. for Biotechnology, SAE) as mentioned by Akiba et al. (1982).

## Statistics

Data were analyzed using analysis of variance in SPSS version 17.0 using a 3 × 3 factorial arrangement. The statistical model included the impacts of protein, Zn source, and the combined effects of protein and Zn:

$$Y_{ij} = \mu + A_i + E_j + AE_{ij} + e_{ij}$$

where  $Y$  is an observation,  $\mu$  is the overall mean,  $A_i$  is the effect of dietary protein (20, 22, and 24%),  $E_j$  is the effect of Zn source (0 g/kg diet, 1 g of ZnO/kg diet, or 1 g of Zn-Met/kg diet),  $AE_{ij}$  is the dietary protein  $\times$  Zn source interactions (=1, 2... and 9), and  $e_{ij}$  is the random error. The cage (replicate) was the experimental unit for the performance, while the single quail was the experimental unit for carcass, and blood biochemistry. Differences among means were investigated using the post hoc Tukey's test.

## RESULTS AND DISCUSSION

### LBW and BWG

The effect of low-CP levels and Zn sources on the LBW and BWG of growing Japanese quail is presented in Table 2. Apart from the effect of Zn on BW at 5 wk of age, no significant differences were noticed due to the main effect of low-CP levels or Zn sources on LBW at all ages. However, the LBW and BWG values were higher for the treatment with 0.1 g of Zn-Met/kg diet than the other treatments at 5 and 1 to 5 wk of age. The lowest CP (20%) value achieved the highest BWG during the periods of 3 to 5 and 1 to 5 wk of age. No significant differences were found due to the interaction between CP levels and Zn sources on BW and BWG at all experimental periods, except for LBW at 5 wk of age, with the best value recorded for the treatment with 24% CP and 0.1 g of Zn-Met/kg of diet. These results agree with those of Rehman et al. (2018), who stated that chicks fed low protein (19.3%) levels had the heaviest body weight when compared with those fed the negative control (18.8% protein). While Gheisari et al. (2011) found that diet containing 24% CP improved BW when compared

with quail fed 21% CP. The positive impact of high dietary CP levels on growth rate could be due to increasing protein intake. Our results agree with previous findings that partially used Zn-Met led to higher BWG in broilers than inorganic Zn (Jahani and Rasouli, 2015). However, Sunder et al. (2012) showed that the use of organic Zn in broiler diets did not affect their performance during the fattening period. Additionally, Zhao et al. (2014) reported that ZnO supplementation (20 mg/kg diet) in broiler diets improved growth rate, Fawzy et al. (2016) found that the addition of ZnO to broiler rations caused a significant improvement in LBW. These results could indicate the essential role of Zn in the synthesis of DNA and metabolism of protein and carbohydrates. Furthermore, these findings could be attributed to the significance of Zn in good growth through its impacts on metalloproteins, such as insulin-like growth factor-1 and growth hormone, which drives the absorption of glucose (Attia et al., 2013b; Martin, 2016).

### FI and Feed Conversion Efficiency

The effect of dietary CP levels, Zn source, and their interactions on daily FI and FCR of growing Japanese quail is presented in Table 3. Both FI and FCR were significantly improved with 20% CP supplementation of quail diets at 3 wk and 1 to 3 wk, respectively, and FI and FCR showed no significant response to Zn sources in the diet. The highest FCR was obtained for the interaction between 20% CP and 0.1 g of Zn-Met/kg of diet at 1 to 3 wk of age. However, no significant differences were found for the interaction between CP levels and Zn sources on FI during the experiment. The obtained results for CP levels agree with those of Abd-

**Table 2.** Live body weight and body weight gain of growing Japanese quails as affected by dietary protein levels, zinc sources and their interaction during the growing periods (1–5 wk of age).

	Live body weight			Body weight gain		
	1 wk	3 wk	5 wk	1–3 wk	3–5 wk	1–5 wk
Dietary protein level (%)						
20% CP	39.14	99.97	197.0	60.84	97.05 <sup>a</sup>	157.9 <sup>a</sup>
22% CP	39.86	110.3	190.9	70.46	80.59 <sup>b</sup>	151.0 <sup>ab</sup>
24% CP	41.01	107.1	186.1	66.07	79.06 <sup>b</sup>	145.1 <sup>b</sup>
<i>P</i> -value	0.404	0.054	0.063	0.076	0.020	0.030
Zinc source (g/kg diet)						
0	39.98	106.2	187.2 <sup>b</sup>	66.24	81.01	147.2 <sup>b</sup>
0.1 g ZnO	40.32	101.4	182.3 <sup>b</sup>	61.05	80.97	142.0 <sup>b</sup>
0.1 g Zn-Met	39.71	109.8	204.5 <sup>a</sup>	70.07	94.72	164.8 <sup>a</sup>
<i>P</i> -value	0.463	0.141	0.001	0.099	0.070	<0.001
Interaction effect						
20% CP						
0	38.74	101.8	189.7 <sup>bc</sup>	63.10	87.84	150.9
0.1 g ZnO	39.90	95.10	197.9 <sup>ab</sup>	55.20	102.8	158.0
0.1 g Zn-Met	38.76	103.0	203.5 <sup>ab</sup>	64.21	100.5	164.7
22% CP						
0	40.47	116.0	193.3 <sup>abc</sup>	75.49	77.37	152.9
0.1 g ZnO	39.67	102.7	178.0 <sup>cd</sup>	63.00	75.37	138.4
0.1 g Zn-Met	39.43	112.3	201.3 <sup>ab</sup>	72.88	89.02	161.9
24% CP						
0	40.73	100.9	178.7 <sup>cd</sup>	60.14	77.80	137.9
0.1 g ZnO	41.39	106.4	171.1 <sup>d</sup>	64.96	64.71	129.7
0.1 g Zn-Met	40.92	114.0	208.7 <sup>a</sup>	73.12	94.67	167.8
SEM	1.11	7.83	7.50	3.45	9.50	6.50
<i>P</i> -value	0.538	0.369	0.037	0.406	0.313	0.054

<sup>abcd</sup>Means in the same column within each classification bearing different letters are significantly different. Abbreviation: CP, crude protein.

**Table 3.** Feed intake and feed conversion of growing Japanese quails as affected by dietary protein levels, zinc sources and their interaction during the growing periods (1–5 wk of age).

	Feed intake (g)			Feed conversion (g feed/ g gain)			
	1 wk	3 wk	5 wk	1–3 wk	3–5 wk	1–5 wk	
Dietary protein level (%)							
20% CP	20.14	28.46 <sup>a</sup>	24.30	4.656 <sup>a</sup>	6.264	5.391	
22% CP	20.16	24.52 <sup>b</sup>	22.34	4.043 <sup>b</sup>	6.557	5.227	
24% CP	22.43	25.91 <sup>ab</sup>	24.17	4.804 <sup>b</sup>	7.208	5.922	
<i>P</i> -value	0.203	0.019	0.225	0.013	0.360	0.103	
Zinc source (g/kg diet)							
0	20.71	25.21	22.96	4.438	6.750	5.514	
0.1 g ZnO	20.50	26.73	23.61	4.741	7.224	5.863	
0.1 g Zn-Met	21.52	26.94	24.23	4.324	6.054	5.162	
<i>P</i> -value	0.749	0.347	0.591	0.233	0.228	0.119	
Interaction effect							
20% CP	0	18.61	26.45	22.53	4.123 <sup>bcd</sup>	6.503	5.253
	0.1 g ZnO	17.62	30.29	23.95	4.580 <sup>abc</sup>	6.283	5.303
	0.1 g Zn-Met	24.19	28.63	26.41	5.263 <sup>a</sup>	6.007	5.617
22% CP	0	20.94	21.69	21.32	3.903 <sup>cd</sup>	6.160	4.903
	0.1 g ZnO	20.76	25.43	23.10	4.633 <sup>abc</sup>	7.153	5.847
	0.1 g Zn-Met	18.76	26.45	22.60	3.593 <sup>d</sup>	6.357	4.930
24% CP	0	22.58	27.50	25.04	5.287 <sup>a</sup>	7.587	6.387
	0.1 g ZnO	23.10	24.48	23.79	5.010 <sup>ab</sup>	8.237	6.440
	0.1 g Zn-Met	21.60	25.74	23.67	4.117 <sup>bcd</sup>	5.800	4.940
SEM	1.05	2.35	2.32	0.37	0.97	0.69	
<i>P</i> -value	0.098	0.135	0.476	0.008	0.608	0.107	

<sup>abcd</sup>Means in the same column within each classification bearing different letters are significantly different. Abbreviation: CP, crude protein.

Elsamee et al. (2014) who pointed out that birds fed 20% CP (low protein diets) consumed more feed than those receiving high protein diet (22%), but diets containing 22% CP resulted in better FCR than those with 20% CP. These increments could increase FI to meet their CP requirement. In contrast, Mosaad and Iben (2009) found that FI improved for Japanese quails fed diets containing 24 and 27% CP compared with those fed diets containing 21% CP. Additionally, Dowarah and Sethi (2014) reported that the daily FI of quails was higher significantly for 27 and 25% CP than diets contained 23% CP. These results for Zn are in accordance with those reported by Kaya et al. (2000) who found that the FCR of layers was not significantly affected by different levels of Zn up to 200 mg/kg of diet. In female broilers, Hess et al. (2001) found that adding 40 mg of Zn/kg diet from different sources (Zn-Met, Zn-Lys, or their mixture) significantly improved the feed conversion when Zn-amino acid mixtures were used. However, El-Husseiny et al. (2008) showed that high levels (175 mg/kg diet) of zinc oxide decreased FI and feed efficiency in laying hens when compared with low levels (75–140 mg/kg diet). Additionally, Ao et al. (2011) found that feed conversion was not improved with higher addition of organic or inorganic Zn. The effects of Zn on animal performance are related to Zn source/level, animal species and age of animals, and phytase (Attia et al., 2013a, b; 2019).

### Carcass Traits

Data in Table 4 summarize the impact of dietary CP levels and Zn source and their interaction on the carcass traits of Japanese quails at 5 wk of age. No significant effects were noticed due to the main effect of CP levels

or Zn source on carcass percentage, dressing percentage, and gizzard, while liver percentage, heart percentage, and giblets percentage. There were no significant changes in carcass traits due to adding Zn sources. Carcass and dressing percentages were significantly decreased by the interaction effect when compared with the control group, while heart% was only increased significantly by using 20% CP with 0.1 ZnO. Our results for the effect of CP levels did not agree with those of Sharifi et al. (2011) who did not find any significant impact on the carcass traits of quails due to dietary protein levels (22 and 24%). Additionally, Malomo et al. (2013) concluded that dressing percentage decreased with decreasing CP in broiler diets. However, Abd-Elsamee et al. (2014) found that 22% CP increased carcass percentage and giblets percentage ( $P \leq 0.05$ ) compared to 20% CP levels, and the highest value for giblets percentage was recorded for birds fed 22% CP. Our results for dietary supplementation of Zn sources did not agree with El-Husseiny et al. (2012), who found that using organic minerals such as Zn, Mn, and Cu up to 50% of recommended levels showed an improvement in carcass parameters, while Yalcinkaya et al. (2012) recommended that broilers fed diets enriched with organic Zn improved their pancreas, liver, and spleen. On the other hand, Jahanian and Rasouli (2015) stated that using Zn-Met instead of inorganic Zn increased carcass yield and liver weight, but decreased weight of abdominal fat.

### Blood Biochemical Parameters

The effect of dietary CP levels, ZN source, and their interactions on HDL, triglyceride (TG), cholesterol (CHO), albumin (ALB), AST, ALT, urea, and

**Table 4.** Carcass traits of growing Japanese quails as affected by dietary protein levels, zinc sources and their interaction during the growing periods (1–5 wk of age).

	Carcass traits (%)					
	Carcass	Dressing	Liver	Heart	Gizzard	Giblets
Dietary protein level (%)						
20% CP	75.89	81.89	2.47 <sup>a</sup>	1.07 <sup>a</sup>	4.21	6.14 <sup>a</sup>
22% CP	74.89	80.44	2.37 <sup>a</sup>	1.00 <sup>ab</sup>	3.98	5.78 <sup>a</sup>
24% CP	75.44	80.56	1.90 <sup>b</sup>	0.88 <sup>b</sup>	3.89	5.18 <sup>b</sup>
<i>P</i> -value	0.004	0.889	0.76	0.006	0.016	0.294
Zinc source (g/kg diet)						
0	76.67	82.11	2.29	0.94	4.23	5.60
0.1 g ZnO	75.33	80.89	2.25	1.02	4.01	5.72
0.1 g Zn-Met	74.22	79.89	2.21	1.00	3.84	5.78
<i>P</i> -value	0.508	0.600	0.87	0.40	0.176	0.800
Interaction effect						
20% CP 0	83.33 <sup>a</sup>	90.00 <sup>a</sup>	2.68	1.12 <sup>abc</sup>	4.81	6.63
0.1 g ZnO	74.00 <sup>b</sup>	80.00 <sup>b</sup>	2.42	1.21 <sup>a</sup>	4.17	6.16
0.1 g Zn-Met	70.33 <sup>b</sup>	75.67 <sup>b</sup>	2.30	0.88 <sup>d</sup>	3.65	5.62
22% CP 0	71.33 <sup>b</sup>	76.00 <sup>b</sup>	2.29	0.86 <sup>d</sup>	3.82	5.15
0.1 g ZnO	76.33 <sup>ab</sup>	82.00 <sup>ab</sup>	2.36	0.97 <sup>bcd</sup>	4.06	5.84
0.1 g Zn-Met	77.00 <sup>ab</sup>	83.33 <sup>ab</sup>	2.47	1.18 <sup>ab</sup>	4.08	6.35
24% CP 0	75.33 <sup>ab</sup>	80.33 <sup>b</sup>	1.90	0.84 <sup>d</sup>	4.08	5.02
0.1 g ZnO	75.67 <sup>ab</sup>	80.67 <sup>b</sup>	1.96	0.86 <sup>d</sup>	3.81	5.18
0.1 g Zn-Met	75.33 <sup>ab</sup>	80.67 <sup>b</sup>	1.85	0.95 <sup>cd</sup>	3.79	5.35
SEM	3.65	3.69	0.28	0.10	0.33	0.45
<i>P</i> -value	0.018	0.009	0.711	0.006	0.117	0.050

<sup>abcd</sup>Means in the same column within each classification bearing different letters are significantly different. Abbreviation: CP, crude protein.

creatinine of Japanese quails at 5 wk of age are presented in Table 5. The obtained results indicated that ALB was significantly increased by using 20 and 24% CP compared with the medium CP level, and urea and creatinine significantly increased with dietary supplements of 20 and 22% CP compared with the high CP level (24%). However, HDL, TG, CHO, AST, and ALT were not affected by using all dietary CP levels. Further, TG was significant decreased by adding 0.1 g of ZnO/kg of diet only and 0.1 g of Zn-Met/kg of diet only. However, CHO increased significantly with dietary supplements of 0.1 g

of ZnO/kg of diet only and 0.1g of Zn-Met/kg of diet only. Additionally, HDL was significantly increased due to interactions between 22% CP with 0.1 g of ZnO/kg of diet. However, CHO, AST, and ALT were significantly increased by the interaction between 22% CP and 0.1 g of Zn-Met/kg of diet, and ALB was increased by the interaction between 24% CP and 0.1g of ZnO/kg of diet. Additionally, TG, urea, and creatinine were not affected by the interaction between dietary supplementation of protein levels and Zn sources. The obtained results for protein partially agreed with Abou Zeid et al. (2000),

**Table 5.** Blood chemistry of growing Japanese quails as affected by dietary protein levels, zinc sources and their interaction during the growing periods (1–5 wk of age).

	Blood chemistry <sup>1</sup>							
	HDL	TG	CHO	ALB	AST	ALT	UREA	CREAT
Dietary protein level (%)								
20% CP	49.11	181	84.67	1.23 <sup>a</sup>	5.44	6.22	12.57 <sup>a</sup>	0.48 <sup>a</sup>
22% CP	48.89	176	83.44	1.11 <sup>b</sup>	6.77	8.44	11.46 <sup>a</sup>	0.45 <sup>a</sup>
24% CP	47.11	1743	88.22	1.28 <sup>a</sup>	4.50	6.05	9.356 <sup>b</sup>	0.20 <sup>b</sup>
<i>P</i> -value	0.778	0.384	0.300	<0.001	0.061	0.094	0.003	0.000
Zinc source (g/kg diet)								
0	46.11	165 <sup>b</sup>	79.89 <sup>b</sup>	1.23	6.22	7.44	12.62 <sup>a</sup>	0.43
0.1 g ZnO	50.78	184 <sup>a</sup>	87.89 <sup>a</sup>	1.23	5.55	7.11	10.13 <sup>b</sup>	0.34
0.1 g Zn-Met	48.22	182 <sup>a</sup>	88.56 <sup>a</sup>	1.16	4.94	6.16	10.62 <sup>b</sup>	0.36
<i>P</i> -value	0.337	0.007	0.020	0.110	0.379	0.525	0.015	0.205
Interaction effect								
20% CP 0	48.33 <sup>abc</sup>	168	83.00 <sup>ab</sup>	1.43 <sup>a</sup>	5.66 <sup>abcd</sup>	5.33 <sup>bc</sup>	14.13 <sup>a</sup>	0.60
0.1 g ZnO	48.67 <sup>abc</sup>	186	82.33 <sup>ab</sup>	1.18 <sup>bc</sup>	7.33 <sup>ab</sup>	9.00 <sup>ab</sup>	12.53 <sup>ab</sup>	0.46
0.1 g Zn-Met	50.33 <sup>ab</sup>	190	88.67 <sup>ab</sup>	1.08 <sup>c</sup>	3.33 <sup>cd</sup>	4.33 <sup>bc</sup>	11.03 <sup>ab</sup>	0.37
22% CP 0	37.33 <sup>c</sup>	155	64.00 <sup>c</sup>	1.09 <sup>c</sup>	6.33 <sup>abc</sup>	8.33 <sup>ab</sup>	10.90 <sup>ab</sup>	0.48
0.1 g ZnO	56.67 <sup>a</sup>	192	91.33 <sup>ab</sup>	1.10 <sup>c</sup>	5.00 <sup>bcd</sup>	6.33 <sup>abc</sup>	12.00 <sup>ab</sup>	0.38
0.1 g Zn-Met	52.67 <sup>ab</sup>	181	95.00 <sup>a</sup>	1.14 <sup>bc</sup>	9.00 <sup>a</sup>	10.67 <sup>a</sup>	11.47 <sup>ab</sup>	0.49
24% CP 0	52.67 <sup>ab</sup>	173	92.67 <sup>ab</sup>	1.17 <sup>bc</sup>	6.66 <sup>abc</sup>	8.66 <sup>ab</sup>	12.83 <sup>a</sup>	0.23
0.1 g ZnO	47.00 <sup>abc</sup>	174	90.00 <sup>ab</sup>	1.41 <sup>a</sup>	4.33 <sup>bcd</sup>	6.00 <sup>abc</sup>	5.86 <sup>c</sup>	0.18
0.1 g Zn-Met	41.67 <sup>bc</sup>	174	82.00 <sup>b</sup>	1.27 <sup>b</sup>	2.50 <sup>d</sup>	3.50 <sup>c</sup>	9.36 <sup>b</sup>	0.21
SEM	3.99	9.29	6.65	0.09	1.53	1.66	1.20	0.12
<i>P</i> -value	0.014	0.127	<0.001	<0.001	0.009	0.016	0.007	0.387

<sup>abcd</sup>Means in the same column within each classification bearing different letters are significantly different.

<sup>1</sup>Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CP, crude protein; CREAT, creatinine; HDL, high density lipoprotein; TG, triglycerides; TC, total cholesterol.

**Table 6.** Immunological and antioxidant parameters of growing Japanese quails as affected by dietary protein levels, zinc sources and their interaction during the growing periods (1–5 wk of age).

		Immunological and antioxidant parameters <sup>1</sup>				
		IGM	IGG	SOD	MDA	GSH
Dietary protein level (%)						
20% CP		150.9	1,133 <sup>c</sup>	0.333 <sup>a</sup>	0.108 <sup>b</sup>	0.346 <sup>a</sup>
22% CP		152.4	1,263 <sup>b</sup>	0.272 <sup>b</sup>	0.164 <sup>a</sup>	0.288 <sup>b</sup>
24% CP		143.8	1,362 <sup>a</sup>	0.338 <sup>a</sup>	0.144 <sup>a</sup>	0.358 <sup>a</sup>
<i>P</i> -value		0.468	<0.001	0.004	0.001	0.003
Zinc source (g/kg diet)						
0		136.7 <sup>b</sup>	1,225 <sup>b</sup>	0.331	0.131	0.346
0.1 g ZnO		155.8 <sup>a</sup>	1,230 <sup>b</sup>	0.293	0.137	0.306
0.1 g Zn-Met		154.7 <sup>a</sup>	1,304 <sup>a</sup>	0.319	0.149	0.340
<i>P</i> -value		0.030	0.001	0.145	0.398	0.089
Interaction effect						
20% CP	0	114.7 <sup>d</sup>	1,111 <sup>e</sup>	0.310 <sup>ab</sup>	0.123 <sup>bcd</sup>	0.317 <sup>abc</sup>
	0.1 g ZnO	160.3 <sup>ab</sup>	1,103 <sup>e</sup>	0.330 <sup>a</sup>	0.093 <sup>d</sup>	0.337 <sup>a</sup>
	0.1 g Zn-Met	177.7 <sup>a</sup>	1,186 <sup>d</sup>	0.360 <sup>a</sup>	0.107 <sup>cd</sup>	0.383 <sup>a</sup>
22% CP	0	172.3 <sup>a</sup>	1,224 <sup>cd</sup>	0.337 <sup>a</sup>	0.113 <sup>bcd</sup>	0.350 <sup>a</sup>
	0.1 g ZnO	148.0 <sup>abc</sup>	1,288 <sup>bc</sup>	0.240 <sup>b</sup>	0.163 <sup>b</sup>	0.253 <sup>c</sup>
	0.1 g Zn-Met	137.0 <sup>bcd</sup>	1,276 <sup>bc</sup>	0.240 <sup>b</sup>	0.217 <sup>a</sup>	0.260 <sup>bc</sup>
24% CP	0	123.0 <sup>cd</sup>	1,339 <sup>b</sup>	0.347 <sup>a</sup>	0.157 <sup>bc</sup>	0.370 <sup>a</sup>
	0.1 g ZnO	159.0 <sup>ab</sup>	1,299 <sup>b</sup>	0.310 <sup>ab</sup>	0.153 <sup>bc</sup>	0.327 <sup>ab</sup>
	0.1 g Zn-Met	149.3 <sup>abc</sup>	1,448 <sup>a</sup>	0.357 <sup>a</sup>	0.123 <sup>bcd</sup>	0.377 <sup>a</sup>
SEM		8.70	7.44	0.02	0.04	0.03
<i>P</i> -value		<0.001	0.032	0.041	0.003	0.032

<sup>abcd</sup>Means in the same column within each classification bearing different letters are significantly different.

<sup>1</sup>Abbreviations: CP, crude protein; GSH, reduced glutathione; IgM, immunoglobulin M; IgG, immunoglobulin G; MDA, malondialdehyde; SOD, superoxide dismutase.

who found that different dietary protein levels did not affect blood chemistry of quail and Jang et al. (2009), who showed that total protein and albumin were depressed for chickens subjected to qualitative and quantitative feed restriction compared with broilers fed a control ration, while AST levels increased in the restricted group. Mosaad and Iben (2009) reported that blood serum uric acid concentration in growing chicks increased significantly with higher protein diets. Additionally, our results are in agreement with Sharifi et al. (2011), who noticed that high dietary CP (24%) significantly decreased serum cholesterol and HDL levels but significantly increased serum triglyceride and LDL levels compared with low protein levels (22.08%), and this difference may be due to the authors using different protein diets than our diet. However, Saki et al. (2015) investigated the effect of 4 levels of crude protein (22.17, 24.2, 26.2, and 28%) on growing Japanese quail. They found that total protein, albumin, and globulin were increased significantly as CP increased to 24%. Additionally, Rabie and Abo El-Maaty (2015) studied the effect of 3 levels of CP (20, 22, and 24%) on blood parameters of growing Japanese quail, and they found that total CHO, triglycerides, LDL, HDL, and total lipids were not affected by any level of CP.

Our results for supplemented Zn sources disagreed with Amira (2009), who stated that blood total protein and globulin of chicks fed diets with Biozinc at levels of 40, 80, or 120 mg/kg were increased. However, Liu et al. (2013) found that increased Zn concentrations in plasma when birds fed a proteinate diet. Additionally, Bahakaim et al. (2014) showed that the addition of organic Zn increased Zn levels in plasma. Likewise El-

Kholy et al. (2017), Alagawany et al. (2018), and Farag and Alagawany (2018, 2019) observed that the blood parameters including LDH, ALT, urea, AST, TC, LDL, HDL, and Zn in the serum were increased with increasing dietary Zn level (Abd El-Hack et al., 2017, 2018, 2020).

### Immunological and Antioxidant Parameters

The data presented in Table 6 show the effect of dietary protein levels and Zn source and their interaction on SOD, GSH, MDA, IgG, and IGM of Japanese quails. A significant increase in SOD and GSH was recorded in birds fed on 20 and 24% CP only, MDA was increased significantly by feeding 22 and 24% CP only, and IgG increased with feeding 24% CP only and 0.1g ZnO only. However, IGM was significantly increased with feed on 0.1 g of ZnO/kg of diet only and 0.1 g of Zn-Met/kg of diet only. It was found that there were significant differences due to the interactions between CP levels and Zn sources on all immunity and antioxidant parameters, where SOD and GSH were significantly increased by the interaction between 20% CP and 0.1 g of ZnO/kg of diet and 0.1 g of Zn-Met/kg of diet. However, MDA was increased by the interaction between 22% CP and 0.1 g of Zn-Met/kg of diet, while IgG was increased by feeding 24% CP with 0.1 g of Zn-Met/kg of diet. However, IGM was increased by the interaction between 20% CP and 0.1 g of Zn-Met/kg of diet and also by the interaction between 24% CP and 0.1 g of Zn-Met/kg of diet.

The results for CP levels agree with those of Enting et al. (2007), who found substantial differences in IgG and IgM in broiler chickens at 35 days of age due

to dietary protein levels, which can play an important role in adaptive immune responses. Increasing dietary concentrations of protein could reduce or eliminate the adverse impacts on poultry performance resulting from vaccination through the starter stage, as lymphoid atrophy is a dramatic feature of protein-energy malnutrition. Additionally, a higher neutrophil percentage could reflect a higher immune response of quail chicks fed on high protein content compared with low protein feeding group (Lee et al., 2011; Mohamed et al., 2019). Rabie and Abo El-Maaty (2015) studied the effect of 3 levels of CP (20, 22, and 24%) on hematological parameters of quails during the fattening periods, and they reported that blood parameters were not affected by the addition of any level of crude protein.

Our results for the dietary supplementation of Zn sources agree with those of Powell (2000) and Zhao et al. (2014), who showed that Zn addition to diets can reinforce oxidative resistance and decrease the serum MDA. Zinc antioxidant activity reinforces the sensibility of poultry to some oxidative strain. Additionally, Abd El-Hack et al. (2018) demonstrated that Zn is a primary component in superoxide dismutase, and there is a strong relationship between dietary levels of Zn and superoxide dismutase activity. It has been reported that superoxide dismutase participates in the cellular scavenging of ROS.

The improvement in reduced glutathione in the groups enriched with Zn can be explained by removing radical O<sup>-</sup> (Reid and Tervit, 1999). Use of biological Nano particle Zn improved the antioxidant indices in blood, including Zn concentration, SOD and glutathione peroxidase as well as MDA. Also, supplemental biological Nano Zn up to 0.3 g/kg diet improved quail immunity including IgG and IgM (Abdelnour et al., 2021; Reda et al., 2021).

## CONCLUSIONS

From the obtained results, it can be concluded that dietary protein level for growing Japanese quails could be reduced to 20% without negative effects on their performance, carcass traits, and blood metabolites. We advise avoiding increasing the level of dietary protein, because using the low-CP diet with or without Zn source (ZnO or Zn-Met) gave similar results to high-CP levels (22 or 24%).

## DISCLOSURES

The authors have no conflicts of interest.

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