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# Feeding Zinc-Biofortified Wheat Improves Performance, Nutrient Digestibility, and Concentrations of Blood and Tissue Minerals in Quails

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## Abstract

The present study aimed to investigate the effects of feeding zinc (Zn)-biofortified wheat on performance, digestibility, and concentrations of minerals in quails. Zinc biofortification of wheat has been realized in the field by ergonomically applying Zn to foliar two and three times, which increased grain Zn from 18 mg/kg (control) to 34 and 64 mg/kg. A total of 180 quails were divided into six groups, each containing 30 birds, and fed diets containing wheat grain with either 18, 34, or 64 mg/kg with or without zinc picolinate (ZnPic) supplementation. Bodyweight, feed intake, feed efficiency, and cold carcass weights were greater ($P = 0.0001$) when the quails were fed a diet containing the biofortified wheat-containing 64 mg Zn/kg. Nitrogen, ash, Ca, P, Zn, Cu, and Fe retentions were greater with the Zn-biofortified wheat-containing 64 mg Zn/kg ($P \leq 0.026$). The nutrient excretions were low with feeding a diet containing biofortified wheat-containing 64 mg Zn/kg ($P \leq 0.023$). Serum, liver, and heart Zn concentrations increased with feeding biofortified wheat-containing 64 mg Zn/kg ($P \leq 0.002$). Thigh meat Fe concentrations increased with increasing Zn concentrations of the wheat samples used ($P = 0.0001$), whereas the liver Cu concentrations decreased with feeding the wheat-containing 64 mg Zn/kg ($P = 0.004$). The Zn-biofortified wheat-containing greater Zn concentrations, particularly 64 mg Zn/kg, is a good replacement for corn in the poultry diet as long as its availability and low cost for better performance, greater digestibility, and elevated tissue Zn and Fe concentrations.

**Keywords** Zn-biofortified wheat · Zinc picolinate · Performance · Digestibility · Quail

## Introduction

Zinc (Zn) is an essential trace element for humans and animals, including poultry. The importance of Zn in animal nutrition, particularly in poultry, comes from its necessity for growth (live performance), immunity, bone development, feathering, appetite, skin health, gene regulation, antioxidant defense, reproduction, and fast absorption [1–4]. Zinc has critical functions in immune response in the human body against various bacterial and viral attacks such as coronaviruses [5]. Zn also has particular structural and physiological processes in about 10% of the human proteins [6].

Cereals such as wheat, rice, and maize are relatively low in Zn contents (26–35 mg Zn/kg dry matter (DM) compared

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with animal protein sources [2]. The requirement of Zn for optimum poultry growth up to 21 days has been reported to be 40–50 mg Zn/kg DM [7, 8]. Therefore, a classical cereal-soybean meal-based diet of poultry requires a supplemental Zn. Dietary supplementation of Zn in poultry has been shown to provide better live performance and digestibility [9, 10].

Wheat (*Triticum* spp.) represents a significant part of the diet consumed in many countries, providing an ample proportion of energy and protein despite its low concentrations of minerals such as Zn and Fe [11, 12]. Therefore, Zn intake is restricted when a substantial part of the diet contains wheat which has a naturally low Zn concentration (20–40 mg Zn/kg DM) with poor bioavailability because of phytate-bound Zn, resulting in deficiency symptoms including retarded growth and depressed immunity in humans [13] as well as in poultry [14, 15].

Zinc deficiency is probably the most common micronutrient deficiency in arable soils. Although soils are sufficiently rich in Zn, the Zn in the soils is not chemically available for root absorption due to several adverse chemical and physical soil properties, including high soil pH, low organic matter, and low soil moisture [16]. Previously, it has been reported that about 50% of the cultivated soils globally have a low amount of plant-available Zn [17]. Consequently, plants grown on these soils contain a low amount of Zn in edible parts. Recently, Hill et al. [18] reported that grazing animals are at high risk of reduced Zn intake because the pastures contain very low Zn due to the low amount of plant-available Zn in soils. Today, there is an increasing trend to enrich (biofortify) pasture and fodder with Zn to ensure better Zn nutrition of animals. One strategy used for biofortification of pastures is agronomic biofortification (i.e., application of Zn fertilizers) [12].

Today agronomic biofortification of food and feed crops with micronutrients, especially Zn, is becoming very popular [16, 19, 20]. Compared to soil Zn application, foliar application of Zn fertilizers has been found to be highly effective in increasing grain Zn concentration [21]. In a recent human zinc absorption study, it has been shown that wheat biofortified with Zn by applying Zn had a distinct effect on dietary zinc absorption in humans, and this agronomic approach could help reduce the global burden of Zn deficiency [22].

Published reports show that among the interventions focusing on enrichment of foods or feeds with micronutrients, genetic biofortification (through classical breeding) and agronomic biofortification (through fertilizer applications) are similar or even more cost-effective than food fortification or supplementation for long term. In addition, there is also a high potential to improve the yield of plants in the field by applying micronutrient-containing fertilizers because soils are usually low in micronutrients, especially with Zn [16, 20, 23].

To our knowledge, the effect of agronomically biofortified wheat on the growth and development of poultry has not been studied. In the present study, we first produced wheat grains containing three different Zn concentrations by applying $ZnSO_4$ to foliar. These Zn-biofortified wheat grains were then used to study their effects on performance, carcass characteristics, nutrient digestibility, and serum and tissue mineral concentrations in broiler-type quails.

## Materials and Methods

### Wheat Grain Material

In order to obtain wheat grains differing in Zn concentrations, bread wheat (*Triticum aestivum*) cultivar (Bezostaja) has been grown in Eskisehir, Central Anatolia in Turkey, as described by Cakmak et al. [21]. Wheat plants were sprayed with ZnSO4 either two times at the booting and milk stages or three times at the booting, anthesis, and milk stages. The control plots (no Zn application) were treated with water (see Cakmak et al. [21] for further details). The grains collected from the plots with different Zn applications were analyzed for Zn. The analysis results showed the following grain Zn results: (i) 18 mg Zn/kg (no Zn spray), (ii) 34 mg Zn/kg (2 times Zn spray), and (iii) 64 mg Zn/kg (3 times Zn spray). These grains were then used in the feeding trials described below.

### Birds, Diets, Experimental Design

One hundred and eighty (one-day-old) Japanese quails (*Coturnix coturnix japonica*) were randomly divided into six equal groups, each containing 30 birds according to the body weights. Each group was subdivided into three replicates with ten birds. The birds were kept in cages with $20 \times 20$ cm dimensions, providing a space of 400 $cm^2$ per bird in a temperature-controlled room at $24 \pm 2$ °C with 60 to 75% relative humidity.

The brooding temperature was 37.5 °C during the first week of life, with a weekly decline of 3 °C until room temperature (24–27 °C) was achieved. The photoperiod was 14 h light and 10 h dark (06.00–20.00 h). The birds were fed a starter diet until 21 days of age, followed by feeding a grower diet from day 21 to day 42 (Table 1). Feed and water were offered ad libitum throughout the experiment. The experiment was conducted in accordance with animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey (011/5–2).

All the diets were formulated based on the wheat–maize-soybean meal to meet or exceed NRC [24] requirements for quails and supplemented with the enzyme xylanase (200 mg/kg of feed; Ronozyme® WX (CT), also known as BioFeed®

**Table 1** Ingredients and chemical composition of the starter and grower diets fed to quails

| Item | Starter 0–21 days | Grower 22–42 days |
|---|---|---|
| Ingredients, % | | |
| Wheat* | 30.00 | 30.00 |
| Maize | 28.22 | 34.35 |
| Soybean meal (48% CP) | 36.00 | 28.00 |
| Soybean oil | 2.30 | 4.00 |
| Calcium carbonate | 1.38 | 1.32 |
| Dicalcium phosphate | 0.65 | 0.83 |
| Salt, NaCl | 0.20 | 0.20 |
| Sodium bicarbonate | 0.15 | 0.15 |
| DL-methionine | 0.30 | 0.15 |
| L-lysine | 0.10 | 0.30 |
| L-threonine | 0.25 | 0.25 |
| Enzyme | 0.20 | 0.20 |
| Vitamin-minerals premix** | 0.25 | 0.25 |
| Chemical composition | | |
| Metabolizable energy (ME) MJ/kg | 12.27 | 12.98 |
| Crude protein (CP), % | 24.18 | 21.00 |
| Ash, % | 5.67 | 5.35 |
| Ether extract, % | 4.09 | 5.84 |
| Crude fiber, % | 2.92 | 2.75 |
| Methionine-cystine, % | 1.09 | 0.85 |
| Lysine, % | 1.33 | 1.27 |
| Ca, % | 0.80 | 0.80 |
| P, % | 0.30 | 0.30 |

*Wheat varieties with three different zinc concentrations (18 mg/kg, 34 mg/kg, and 64 mg/kg Zn) were used. Maize and soybean meal zinc concentrations were 15 mg/kg and 60 mg/kg

**Vitamin-minerals premix (free of Zn) provides the following per kg: all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-tocopherol acetate, 1.25 mg; menadione (menadione sodium bisulfate, 1.1 mg; riboflavin, 4 mg; thiamine (thiamine mononitrate), 1.1 mg; pyridoxine, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B12, 0.02 mg; folic acid, 0.55 mg; d-biotin, 0.1 mg; Mn (from MnO), 40 mg; Fe (from FeSO4), 12.5 mg; Cu (from CuSO4), 3.5 mg; I (from KI), 0.3 mg; Se (from NaSe), 0.15 mg; and choline-chloride, 175 mg

Wheat; DSM Nutritional Products, Kaiseraugst, Switzerland). The enzyme product contained a mono-component thermostable endo1,4–xylanase (EC 3.2.1.8) derived from *Thermomyces lanuginosus*, expressed in a genetically modified strain of *Aspergillus oryzae*. A coated dry product form of the enzyme was used with a minimum activity of 1000 FXU/g product (Farbe xylanase units).

The quails were fed regular diets including either (1) 18 mg Zn/kg DM-containing wheat along with no zinc picolinate (ZnPic) supplemented to the diet (W18), or (2) 18 mg Zn/kg DM-containing wheat along with 212.4 mg/kg ZnPic supplemented to the diet (W18 + Zn), or (3) 34 mg Zn/kg DM-containing wheat along with no ZnPic supplemented to the diet (W34), or (4) 34 mg Zn/kg DM-containing wheat along with 189.5 mg/kg ZnPic supplemented to the diet (W34 + Zn), or (5) 64 mg Zn/kg DM-containing wheat along with no ZnPic supplemented to the diet (W64), or (6) 64 mg Zn/kg DM-containing wheat along with 146.7 mg/kg ZnPic supplemented to the diet (W64 + Zn). In the zinc-supplemented groups, dietary zinc levels were adjusted to 50 mg Zn/kg of diet by supplementing an organic source of Zn as ZnPic based on recommended doses [3, 7, 8]. The concentrations of zinc in the ingredients and diets are shown in Table 2.

## Data Collection

Cumulative feed intakes and body weight gains were recorded weekly, and weight gains and feed efficiencies were calculated. At the end of the study (day 42), 12 birds (two per replicate) randomly chosen from each treatment group were slaughtered for carcass evaluations and analyses of sera and tissues, namely liver, heart, and thigh meat. The birds were not fed before slaughter, and carcasses were yielded by removing feathers (wet), feet, and visceral organs. Blood samples (5 mL) were centrifuged at $3000 \times g$ for 10 min for sera collection. The liver, heart, and left thigh were also removed for mineral analysis. The samples were stored at − 80 °C until analyses.

At the last 7 days of the experiment, 6 birds from each group were placed into individual battery cages for excreta collection to measure the digestibility of nitrogen, ash, Ca, P, Zn, Cu, and Fe. The excreta samples were oven-dried at 60 °C for 48 h and then ground to pass through a 40-mesh screen.

## Laboratory Analyses

Feed and excreta samples were analyzed for crude protein (#988.05) and crude ash (#936.07) in triplicates [25]. Nutrient excretion (g/bird) was determined according to analyzed nutrient concentration in the excreta produced during the 7 days. Nutrient retention (g/bird) was calculated as nutrient intake (g/bird) minus nutrient excretion (g/bird). Nutrient retention (%) indicates the percentage of nutrients retained by the bird as a function of nutrient intake, and it was calculated as follows:

$$\textit{Nutrient retention}\ (\%) = \left[\textit{nutrient retention}\ (g/bird)\ /\ \textit{nutrient intake}\ (g/bird)\right] * 100$$

For detection of the concentrations of Ca, P, Zn, Cu, and Fe in feed, excreta, liver, heart, and thigh meat samples with 0.3 g each, along with 0.5 mL of serum samples, were weighed into a Teflon digestion vessel for digestion in 5 mL of 65% (v/v) spectra pure $HNO_3$ (Suprapur, Merck, Darmstadt, Germany) in the model Speedwave™ MWS-2 Microwave Digestion System (Berghof, Eningen, Germany) which

**Table 2** The levels of Zn (mg/kg) in the diets fed to quails*

| Treatment groups | Zn from wheat | Zn from ZnPic | Zn amount (calculated) | Zn amount (analyzed) | |
|---|---|---|---|---|---|
| | | | | 0–21 days | 22–42 days |
| W18 | 5.4 | 0.0 | 5.4 | 31.3 ± 1.1 | 26.5 ± 2.3 |
| W18 + ZnPic | 5.4 | 44.6 | 50.0 | 76.4 ± 2.5 | 72.0 ± 1.9 |
| W34 | 10.2 | 0.0 | 10.2 | 35.2 ± 2.7 | 33.5 ± 1.0 |
| W34 + ZnPic | 10.2 | 39.8 | 50.0 | 76.0 ± 2.4 | 71.3 ± 2.9 |
| W64 | 19.2 | 0.0 | 19.2 | 43.8 ± 2.1 | 42.6 ± 2.5 |
| W64 + ZnPic | 19.2 | 30.8 | 50.0 | 77.2 ± 1.6 | 73.1 ± 1.1 |

*The three different wheat varieties were included in the diet at 30%. The Zn levels were adjusted to 50 mg/kg in each Zn-supplemental diets by adding 212.4 mg/kg ZnPic (44.6 mg elemental Zn), 189.5 mg/kg ZnPic (39.8 elemental Zn), and 146.7 mg/kg ZnPic (30.8 elemental Zn) for W18+ZnPic, W34+ZnPic and W64+ZnPic groups, respectively. The supplemental ZnPic contains 21% Zn

was equipped with a temperature, time, and power according to the digestion program. Sample solutions were diluted with 0.2% (v/v) nitric acid plus 0.2% (v/v) Triton X-100. Ca, Zn, Cu, and Fe concentrations were determined by flame atomic absorption spectrometry (F-AAS, AAS, Perkin-Elmer, Analyst 800, Norwalk, CT). The accuracy of quantitative measurements of Ca, Zn, Cu, and Fe was assured by simultaneous analysis of certified reference materials (NCS ZC730016 Chicken) which was digested analogously to the samples. The detection limits of Ca, Zn, Cu, and Fe were 45.0, 3.8, 1.5, and 0.3 µg/L, respectively. The mean recoveries of the reference material for tissues were as follows: Ca 95%, Fe 98%, Zn 95%, and Cu 99%.

### Statistical Analyses

Data were analyzed by one-way ANOVA using SPSS, 2012 Version 21.0. In addition, post hoc Tukey procedure was run for multiple comparisons of the groups. The Shapiro–Wilk test was used to test the normality of the data. $P < 0.05$ was regarded statistically significant.

## Results

Initial body weights of the quails were intended to be similar among treatments ($P > 0.05$). However, final body weights of the quails increased with the inclusion of greater Zn concentration of the biofortified-wheat samples in the diet, and additional ZnPic supplementation to each diet resulted in further but equal increases in final body weights ($P = 0.0001$; Fig. 1A). Bodyweight gains, as a reflection of the net body weight changes, were greater with the inclusion of the biofortified wheat-containing 64 mg Zn/kg DM to the diet compared with the other wheat samples ($P = 0.0001$), and supplementing ZnPic to each diet equally but further increased the body weight gains ($P = 0.0001$, Fig. 1B). The quails consumed more of the diet containing the wheat sample with 64 mg Zn/kg DM compared with other diets ($P = 0.0001$), and supplementing ZnPic to each diet equally but further increased feed intake ($P = 0.0001$, Fig. 1C). Feed efficiency expressed as feed intake over body weight gain (g/g) was greater with the inclusion of the wheat-containing 64 mg Zn/kg DM in the diet compared with the other wheat samples ($P = 0.0001$, Fig. 1D). Interestingly, when supplemented with an extra 1.6 g ZnPic per kg diet, feed efficiency remained similar for the diet containing the wheat-containing 64 mg Zn/kg DM (Fig. 1D). Unlike the inclusion of the wheat-containing 64 mg Zn/kg DM, however, supplementing ZnPic to the diets containing the other Zn-biofortified wheat samples with either 18 or 34 mg Zn/kg DM equally increased the feed efficiency ($P = 0.0001$). Nevertheless, all ZnPic-supplemented groups of quails had similar feed efficiency.

Although the liver and heart weights of the quails were all similar among treatments ($P > 0.05$), cold carcass weights were greater with the inclusion of the wheat grains containing 64 mg Zn/kg DM to the diet compared with the other wheat grains ($P = 0.0001$, Fig. 1E–H), and supplementing ZnPic to each diet equally but further increased the cold carcass weights ($P = 0.0001$, Fig. 1E). However, all ZnPic-supplemented groups of quails had similar cold carcass weights. Cold carcass yields were similar among the quail-fed diets containing different wheat samples with different Zn but no supplementation. However, supplementing ZnPic to each diet increased the cold carcass yields, particularly the diet with the wheat-containing 34 mg Zn/kg DM ($P = 0.001$; Fig. 1F).

Nitrogen, Zn, and Fe retentions increased as the biofortified wheat samples had more Zn concentration ($P \leq 0.026$; Table 3). Interestingly, when supplemented with an extra 1.6 g ZnPic per kg diet, the retentions remained similar for the diet containing the wheat sample with 64 mg Zn/kg DM. However, supplementing ZnPic to the diets containing other Zn biofortified wheat grains with either 18 or

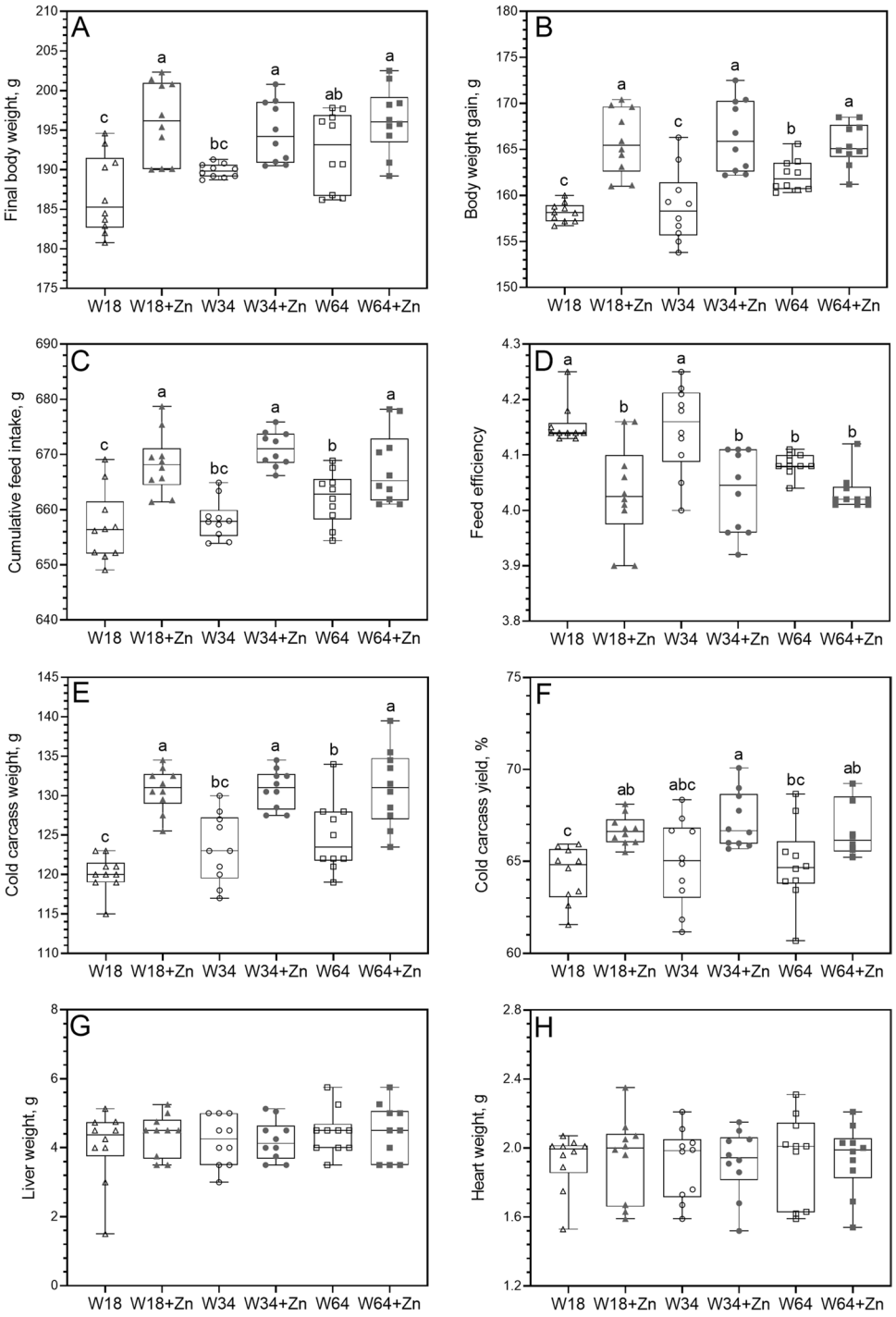


**Fig. 1** Final body weight (A), body weight gain (B), cumulative feed intake (C), feed efficiency: cumulative feed intake, g/body weight gain, g (D), cold carcass weight (E), cold carcass yield (F), liver weight (G) and heart weight(H) of quails fed different wheat varieties and supplemental organic Zn. Statistical comparisons are indicated with different superscript (a-c) located above the groups ($p < 0.05$; *ANOVA and Tukey's post-hoc test)

34 mg Zn/kg DM equally increased the retentions. Nevertheless, all ZnPic-supplemented groups of quails had similar nitrogen, Zn, and Fe retentions. Ash and copper retentions changed similarly, such that inclusion of the Zn biofortified wheat grains containing 64 mg Zn/kg DM to the diet compared with other wheat samples provided greater retentions ($P \leq 0.026$, Table 3) which were not further increased with an extra 1.6 g ZnPic per kg diet supplementation. Unlike the supplementation to the diet containing the wheat sample with 64 mg Zn/kg DM, supplementing ZnPic to the diets containing the other wheat samples with either 18 or 34 mg Zn/kg DM increased the retentions. Yet, ash and copper retentions remained similar in all ZnPic-supplemented groups of quails.

Calcium retention was greater with the inclusion of the wheat sample containing 64 mg Zn/kg to the diet compared with the other wheat grains with the lower amount of Zn ($P = 0.003$, Table 3), and supplementing ZnPic to each diet

**Table 3** Nutrient retention and excretion in quails fed different wheat varieties containing different zinc levels (dry matter basis)

| Dietary component | Treatments* | | | | | | –P– |
|---|---|---|---|---|---|---|---|
| | W18 | W18 + Zn | W34 | W34 + Zn | W64 | W64 + Zn | |
| Retention | | | | | | | |
| Nitrogen, g/bird/d | $1.94 \pm 0.04^{c}$ | $2.69 \pm 0.01^{a}$ | $2.19 \pm 0.06^{b}$ | $2.72 \pm 0.03^{a}$ | $2.65 \pm 0.07^{a}$ | $2.76 \pm 0.06^{a}$ | 0.012 |
| Ash, g/bird/d | $8.16 \pm 0.65^{b}$ | $11.57 \pm 0.26^{a}$ | $9.27 \pm 0.73^{b}$ | $11.12 \pm 0.33^{a}$ | $11.69 \pm 0.57^{a}$ | $11.63 \pm 0.69^{a}$ | 0.026 |
| Calcium, g/bird/d | $0.86 \pm 0.02^{c}$ | $1.72 \pm 0.03^{ab}$ | $0.96 \pm 0.06^{c}$ | $1.51 \pm 0.07^{ab}$ | $1.35 \pm 0.08^{b}$ | $1.84 \pm 0.04^{a}$ | 0.003 |
| Phosphorus, g/bird/d | $0.23 \pm 0.01^{c}$ | $0.57 \pm 0.02^{a}$ | $0.34 \pm 0.01^{b}$ | $0.55 \pm 0.02^{a}$ | $0.39 \pm 0.01^{b}$ | $0.59 \pm 0.02^{a}$ | 0.014 |
| Zinc, mg/bird/d | $10.49 \pm 1.14^{c}$ | $58.52 \pm 4.26^{a}$ | $23.58 \pm 2.03^{b}$ | $59.99 \pm 5.31^{a}$ | $51.24 \pm 4.75^{a}$ | $58.57 \pm 3.16^{a}$ | 0.0001 |
| Copper, mg/bird/d | $0.35 \pm 0.05^{b}$ | $0.78 \pm 0.11^{a}$ | $0.46 \pm 0.06^{b}$ | $0.77 \pm 0.09^{a}$ | $0.72 \pm 0.04^{a}$ | $0.75 \pm 0.09^{a}$ | 0.002 |
| Iron, mg/bird/d | $1.36 \pm 0.23^{c}$ | $3.74 \pm 0.22^{a}$ | $2.22 \pm 0.14^{b}$ | $3.76 \pm 0.23^{a}$ | $3.52 \pm 0.17^{a}$ | $3.73 \pm 0.15^{a}$ | 0.026 |
| Excretion | | | | | | | |
| Nitrogen, g/bird/d | $1.63 \pm 0.02^{a}$ | $1.31 \pm 0.03^{c}$ | $1.46 \pm 0.02^{b}$ | $1.36 \pm 0.01^{bc}$ | $1.42 \pm 0.03^{b}$ | $1.29 \pm 0.02^{c}$ | 0.001 |
| Ash, g/bird/d | $11.06 \pm 0.75^{a}$ | $8.37 \pm 0.52^{c}$ | $9.31 \pm 0.63^{b}$ | $8.82 \pm 0.34^{c}$ | $9.07 \pm 0.27^{b}$ | $8.41 \pm 0.39^{c}$ | 0.002 |
| Calcium, g/bird/d | $0.81 \pm 0.03^{a}$ | $0.63 \pm 0.02^{b}$ | $0.78 \pm 0.03^{a}$ | $0.65 \pm 0.03^{b}$ | $0.73 \pm 0.03^{a}$ | $0.66 \pm 0.02^{b}$ | 0.0001 |
| Phosphorus, g/bird/d | $0.67 \pm 0.12^{a}$ | $0.30 \pm 0.06^{c}$ | $0.39 \pm 0.09^{b}$ | $0.33 \pm 0.07^{c}$ | $0.35 \pm 0.06^{bc}$ | $0.32 \pm 0.07^{c}$ | 0.023 |
| Zinc, mg/bird/d | $10.83 \pm 1.01^{a}$ | $8.15 \pm 0.93^{c}$ | $9.46 \pm 0.67^{b}$ | $8.25 \pm 1.03^{c}$ | $8.96 \pm 1.07^{bc}$ | $8.18 \pm 0.46^{c}$ | 0.0001 |
| Copper, mg/bird/d | $2.88 \pm 0.11^{a}$ | $2.16 \pm 0.04^{c}$ | $2.65 \pm 0.08^{b}$ | $2.19 \pm 0.23^{c}$ | $2.23 \pm 0.17^{c}$ | $2.26 \pm 0.12^{c}$ | 0.0001 |
| Iron, mg/bird/d | $38.43 \pm 2.41^{a}$ | $32.21 \pm 3.34^{c}$ | $34.721 \pm 2.62^{b}$ | $34.82 \pm 3.18^{b}$ | $33.75 \pm 2.17^{bc}$ | $32,26 \pm 1.30^{c}$ | 0.001 |

*Dietary treatments are regular diets including either 18 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W18), or 18 mg/kg Zn-containing wheat along with 212.4 mg/kg ZnPic (44.6 elemental Zn) supplemented to the diet (W18 + Zn), or 34 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W34), or 34 mg/kg Zn-containing wheat along with 189.5 mg/kg ZnPic (39.8 elemental Zn) supplemented to the diet (W34 + Zn), or 64 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W64), or 64 mg/kg Zn-containing wheat along with 146.7 mg/kg ZnPic (30.8 elemental Zn) supplemented to the diet (W64 + Zn). Statistical comparisons are indicated with different superscript (a–c) in the same row ($P < 0.05$; *ANOVA and Tukey's post hoc test). Mean values are demonstrated with ± standard error of mean

increased the retention, particularly with the diet having wheat samples with 64 mg Zn/kg DM, providing the greatest Ca retention. The diets containing the wheat samples with 34 and 64 mg Zn/kg DM yielded greater phosphorous retention compared with the diet containing the wheat grain with 18 mg Zn/kg ($P = 0.014$). However, supplementing ZnPic to each diet equally increased the P retentions bringing the values to similar ranges.

Excretion of nitrogen and ash was lower for the diets, including the biofortified wheat grains with 34 and 64 mg Zn/kg DM compared with wheat grains with 18 mg Zn/kg DM ($P = 0.002$, Table 3). The excretions equally decreased even more, when additional ZnPic was supplemented to each diet containing different wheat samples. Calcium excretion remained similar among diets containing different wheat samples having 18, 34, or 64 mg Zn/kg DM ($P = 0.0001$). However, supplementing ZnPic to each diet equally decreased Ca excretions. Inclusion of the wheat samples containing either 34 or 64 mg Zn/kg DM decreased the P excretion ($P = 0.023$), but supplementing ZnPic to all diets containing all wheat samples further but equally decreased the P excretion.

Inclusion of wheat varieties with increasing Zn concentration in the diet resulted in decreases in Zn and Cu excretion ($P = 0.0001$, Table 3), and supplementing ZnPic to each diet equally but further decreased Zn and Cu excretions, except for Cu excretion with the diet containing the wheat with 64 mg Zn/kg DM which remained unchanged when ZnPic was supplemented. Excretion of iron was lower for the diets, including the wheat samples with 34 and 64 mg Zn/kg DM compared with that of wheat with 18 mg Zn/kg ($P = 0.001$). The excretions were even lower when additional ZnPic was supplemented to the diets, including 18 or 64 mg/kg Zn-containing wheat samples compared with the 34 mg/kg Zn-containing wheat.

Changes in serum and tissue Zn, Cu, and Fe concentrations upon feeding different Zn biofortified-wheat samples and dietary ZnPic supplementation are shown in Fig. 2 and Table 4. Serum Zn concentrations equally increased when the wheat sample with 18 mg Zn/kg DM was replaced with the other wheat samples in the diet and with supplementation of ZnPic in each diet ($P = 0.002$, Fig. 2A). Liver and heart Zn concentrations increased with increasing Zn concentration of wheat samples ($P = 0.001$, Fig. 2B–C). The liver Zn concentrations further increased with each dietary supplementation of ZnPic. Although ZnPic supplementation resulted in further but equal increases in heart tissue, Zn concentrations of quails fed a diet containing the wheat samples with 18 and 34 mg Zn/kg DM compared with

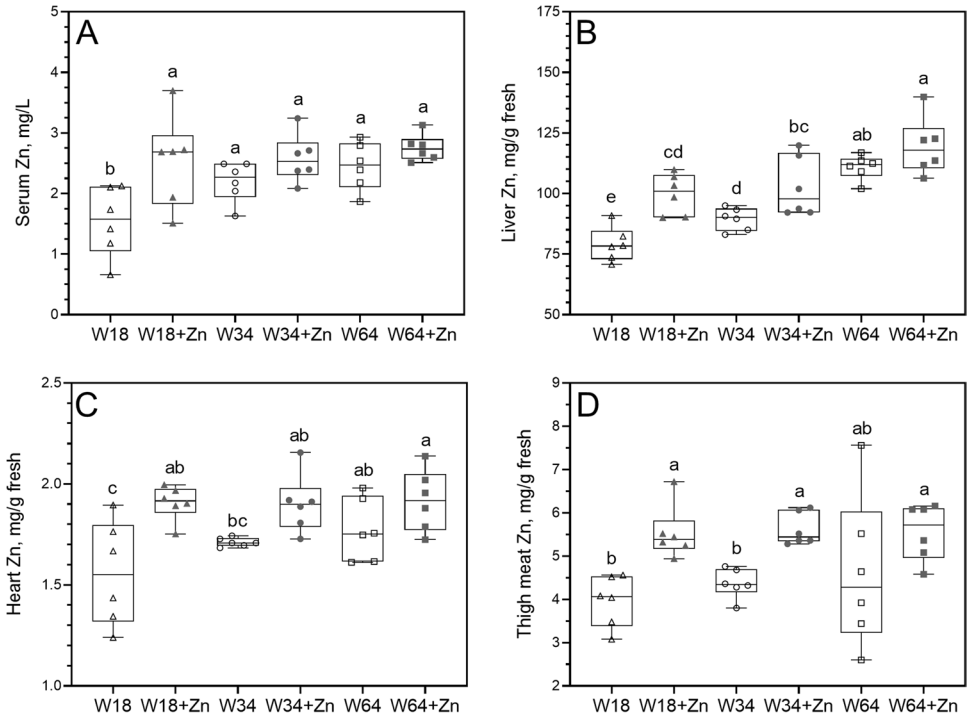


**Fig. 2** Zn levels of serum (A), liver (B), hearth (C), and tight meat (D) in quails fed different wheat varieties and supplemental organic Zn. Statistical comparisons are indicated with different superscript (a–e) located above the groups ($P < 0.05$; ANOVA and Tukey's post hoc test)

**Table 4** Copper and iron concentrations of serum and various tissues from quails fed different wheat varieties containing different zinc levels

| | Treatments* | | | | | | –*P*– |
|---|---|---|---|---|---|---|---|
| | W18 | W18 + Zn | W34 | W34 + Zn | W64 | W64 + Zn | |
| Copper | | | | | | | |
| Serum, mg/L | 0.141 ± 0.014 | 0.128 ± 0.010 | 0.129 ± 0.18 | 0.110 ± 0.13 | 0.117 ± 0.015 | 0.106 ± 0.016 | > 0.05 |
| Liver, mg/g fresh | 13.77 ± 1.10$^{a}$ | 11.44 ± 0.44$^{b}$ | 14.63 ± 1.20$^{a}$ | 11.13 ± 0.60$^{b}$ | 11.14 ± 0.41$^{b}$ | 10.68 ± 0.52$^{b}$ | 0.004 |
| Heart, mg/g fresh | 0.136 ± 0.009$^{a}$ | 0.118 ± 0.004$^{a}$ | 0.131 ± 0.006$^{a}$ | 0.108 ± 0.016$^{ab}$ | 0.115 ± 0.007$^{ab}$ | 0.089 ± 0.007$^{b}$ | 0.014 |
| Thigh meat, mg/g fresh | 0.125 ± 0.004 | 0.117 ± 0.003 | 0.125 ± 0.002 | 0.121 ± 0.005 | 0.127 ± 0.001 | 0.120 ± 0.002 | > 0.05 |
| Iron | | | | | | | |
| Serum, mg/L | 5.56 ± 0.71 | 4.05 ± 0.28 | 4.13 ± 0.30 | 4.72 ± 0.15 | 5.51 ± 0.72 | 6.50 ± 1.51 | > 0.05 |
| Liver, mg/g fresh | 118.25 ± 8.61$^{c}$ | 144.33 ± 13.39$^{ab}$ | 126.14 ± 3.52$^{bc}$ | 142.47 ± 7.82$^{abc}$ | 141.97 ± 4.18$^{abc}$ | 159.51 ± 6.02$^{a}$ | 0.016 |
| Heart, mg/g fresh | 7.14 ± 0.32 | 6.74 ± 0.23 | 7.45 ± 0.48 | 7.00 ± 0.21 | 6.29 ± 0.18 | 7.11 ± 0.45 | > 0.05 |
| Thigh meat, mg/g fresh | 1.62 ± 0.11$^{c}$ | 2.42 ± 0.34$^{ab}$ | 1.92 ± 0.14$^{bc}$ | 2.80 ± 0.22$^{a}$ | 2.16 ± 0.08$^{bc}$ | 2.83 ± 0.09$^{a}$ | 0.0001 |

*Dietary treatments are regular diets including either 18 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W18), or 18 mg/kg Zn-containing wheat along with 212.4 mg/kg ZnPic (44.6 elemental Zn) supplemented to the diet (W18 + Zn), or 34 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W34), or 34 mg/kg Zn-containing wheat along with 189.5 mg/kg ZnPic (39.8 elemental Zn) supplemented to the diet (W34 + Zn), or 64 mg/kg Zn-containing wheat along with no ZnPic supplemented to the diet (W64), or 64 mg/kg Zn-containing wheat along with 146.7 mg/kg ZnPic (30.8 elemental Zn) supplemented to the diet (W64 + Zn). Statistical comparisons are indicated with different superscript (a–c) in the same row ($P < 0.05$; *ANOVA and Tukey's post hoc test). Mean values are demonstrated with ± standard error of mean

non-supplemental groups, supplementing ZnPic to the diet containing the wheat with 64 mg Zn/kg, had the greatest heart Zn concentrations. Thigh meat Zn concentrations increased, although not statistically significant, with increasing Zn concentration of the wheat samples. However, supplementing ZnPic to each diet equally increased the thigh meat Zn concentrations ($P = 0.006$, Fig. 2D), bringing the values to similar ranges within supplemented groups.

Serum and thigh meat Cu concentrations remained unchanged among treatments ($P > 0.05$, Table 4). The liver Cu concentrations decreased only with feeding the wheat-containing 64 mg Zn/kg DM ($P = 0.004$), the measured level of which was similar for all supplemental treatments. Inclusion of wheat samples with different Zn concentrations to

the diets fed to quails decreased, although not significantly, the heart Cu concentrations. Supplementing ZnPic to the diets, particularly the wheat with the highest Zn, further reduced the heart Cu concentrations ($P = 0.014$).

Serum and heart Fe concentrations remained similar among treatments ($P > 0.05$, Table 4). Although not significantly, increasing Zn concentrations in the wheat grains resulted in increases in liver Fe concentrations. In addition, further increases with ZnPic supplementations were observed in liver Fe concentrations, particularly with ZnPic-supplemented wheat samples containing 64 mg Zn/kg DM as being greatest ($P = 0.016$). Thigh meat Fe concentrations increased with increasing Zn concentrations of the wheat ($P = 0.0001$). Further increases were observed with ZnPic supplementations to the diets, particularly with the diets containing wheat with 34 and 64 mg Zn/kg ($P = 0.0001$).

## Discussion

Biofortification of food and feed crops with micronutrients is a growing interest and acceptance with particular positive effects. In a recent study, enrichment of silage maize with Zn, Se, and Fe by using fertilizers (i.e., agronomic biofortification) has been shown as a useful strategy to improve the micronutrient nutrition of animals [26]. The present study also showed that wheat enriched with Zn using agronomic biofortification is a highly useful strategy to improve animal feedstuffs with Zn.

Live performance, measured as body weight gains, feed intake, and feed efficiency, were greater when the quails were fed a diet containing wheat with 64 mg Zn/kg DM. Supplementing proper amounts of ZnPic to balance the required Zn levels in each diet provided similar live performances. Therefore, the inclusion of the Zn-biofortified wheat-containing 64 mg Zn/kg DM in the diet itself provides better performance, compared with those of other wheat samples, without any additional Zn sources. Similar results were also obtained from the cold carcass weights of the present work.

Deficiency of Zn in diets containing the wheat samples biofortified with lower levels of Zn (i.e., either 18 or 34 mg Zn/kg DM) without supplementation resulted in a slowed growth (less weight gain) and feed efficiency as compared with the wheat samples biofortified with Zn at the highest level (i.e., 64 mg Zn/kg DM) at the present work. The wheat samples containing 64 mg Zn/kg DM were close to the required level, thus providing the greatest live performance in quails. Retardation in growth and development as a result of Zn deficiency has been well-documented in poultry [27]. In ducks fed a Zn-deficient diet, significant decreases were found in growth, reproductive performance, and plasma antioxidant pool [28]. Zinc supplementation has also been proved to support chick growth, immune system, and feathering along with accelerating live performance and digestibility in poultry [4, 9, 10, 14]. However, contrary to the present work results, Yogesh et al. [29] reported no differences in growth parameters in broiler chicks fed a diet containing various Zn levels from 40 to 100 mg/kg DM. Similarly, the Zn level above 40 mg/kg DM has been shown not to influence the performance of broiler chicks [30, 31].

As far as carcass performance was concerned, the results from the present work were in agreement with the results from Yogesh et al. [29] and Rossi et al. [32], who reported unchanged carcass characteristics in chicks fed a diet with increasing Zn concentrations. Similarly, increasing the Zn supplement to the diet of Pekin ducks caused no changes in dressing percentages [33]. However, increases in carcass yields have been observed in broiler chicks fed a diet supplemented with either Zn-sulfate or Zn-methionine at 80 mg/kg DM [34]. The differences in growth conditions, the composition of feeding diets, and the amount and form of Zn added are probably responsible for such controversial results. For example, Olukosi et al. [35] showed that Zn hydroxychloride, compared with those of Zn sulfate, had better effects on growth parameters and meat yield in broiler chickens. In a further study, Olukosi et al. [36] found that using hydroxychloride trace minerals such as Zn, Cu, and Mn, compared with those of sulfate salts, in the diet of broilers and laying hens provided a better growth performance, reducing egg loss along with alleviated oxidative stress. Hydroxychloride trace minerals are released slowly due to their crystalline structure during digestion, resulting in better absorption and decreased excretion [37]. Similarly, Ao et al. [38] measured greater growth rates and bone Zn accumulation in broiler chicks fed a diet supplemented with organic Zn (Zn proteinate), as compared with inorganic Zn ($ZnSO_4$). The supplemental Zn sources as ZnPic of the present work were used to minimize the interaction among other minerals and better bioavailability.

Improved digestibility of all nutrients (nitrogen, Ca, P, Zn, Cu, and Fe), measured as increased retentions and decreased excretions, were observed in quails fed a diet containing the wheat samples, particularly with 64 mg Zn/kg DM. Increased live performance of quails fed a diet with the wheat samples of 64 mg $Zn/kg^{-1}$ DM was supported by the improved digestibility of the nutrients from the present work. However, contrary to the current work results, Yogesh et al. [29] found decreased Zn retentions with increasing Zn levels from 40 to 100 mg/kg DM in a diet fed to broiler chicks. Similarly, chicks were reported to excrete more Zn when the diet contained increasing Zn levels up to 178 mg/kg DM [39]. However, broiler chicks fed a diet supplemented with organic Zn (Zn-amino acid) compared with zinc sulfate up to 80 mg/kg DM were observed to excrete less Zn [40].

Increasing the Zn concentration of the wheat samples resulted in increases, as expected, in serum and tissue Zn concentrations at the present work. These results were also in agreement with the retention of Zn with increasing Zn concentration of the wheat samples. Increased tissue concentrations of Zn would be desirable in combating Zn deficiency upon consumption of these tissues by humans. The same is also true for consuming the wheat samples themselves containing 64 mg Zn/kg DM. Therefore, consuming either the wheat samples containing greater Zn concentration or the tissues (meat or organs) from the animals fed the same wheat samples offers a two-sided benefit. Similar to the present work results, supplemental organic or inorganic Zn sources to the diet of broiler chicks, laying hens, and Pekin ducks have been reported to increase blood and liver Zn concentrations [10, 14, 33]. Liver Zn concentrations are considered an important indicator of the whole-body level [41].

Since Zn is also involved in bone calcification in broilers, [42] increased Zn concentration in the diet was expected to increase Ca and P retentions, as evidenced in the present study. Although bone calcification was not measured at the current work, the increased levels of Zn in the diet have been reported to increase Zn and Ca concentrations in the tibia of laying hens [43], broiler chicks [34], and Pekin ducks [33].

Increasing the Zn concentration of the wheat samples also resulted in increases in liver and thigh Fe concentrations but decreases in liver and heart Cu concentrations at the present work. As opposed to the current study results, supplementing either organic or inorganic Zn to the diet of laying hens was found not to influence the blood Cu or Fe concentrations [10]. However, the antagonist features of Zn and Cu, as shown in the present work, were also found by Ognik et al. [44], who observed decreased Zn absorption from the intestines of chicks after Cu administrations with no effects on Fe absorption. Similar to the present work results, Olukosi et al. [35] also found that increasing Zn level in the diet of broiler chicks caused increases in plasma Zn but not in plasma Cu concentrations with no respect to Zn sources (sulfate or hydroxychloride) supplemented in the diet. However, in the same study [35], the liver Cu levels were greater with the inclusion of hydroxychloride Zn sources as compared to that of sulfate into the diet, independent from the Zn levels of the diet.

Although antagonistic features of Zn, Fe, and Cu have been known in chicks [45], the present work proved that the natural source of Zn in wheat does not interfere with Fe absorption or retention. Ao and Pierce [3] also indicated that the Zn, Fe, and Cu antagonisms could be avoided by supplementing organic Zn sources instead of inorganic ones in the poultry diet. Therefore, feeding the different wheat samples containing greater Zn concentration, particularly 64 mg Zn/kg DM, to quails increased both Zn and Fe concentrations of the animal tissues (meat and organs), which upon consumption offer health benefits to humans.

In general, supplementing ZnPic to balance the total Zn concentrations of the diets provided similar effects in responses to measured parameters at the present work. The main effect detected for the most measured parameters was the wheat samples, particularly one containing 64 mg Zn/kg DM. The bioavailability of Zn in wheat samples was made possible through enzyme inclusions in the diets.

Wheat as a palatable feed for poultry can be included in poultry diets up to 60% or more along with soybean meal. Wheat has about 94–96% of corn's energy but greater protein, lysine, and tryptophan compared with those of corn [46]. Therefore, wheat is a good replacement for corn in the poultry diet due to its availability and low cost. This is also true for the wheat samples containing greater Zn concentrations. Although balanced and made ideal for human consumption, raising the Zn concentration of the wheat samples further, through biofortification, up to 50 mg/kg DM at the required level for the quail diets without compromising the balance of other nutrients needs be elucidated. Wheat, mostly used for human consumption, may not be appropriate for feeding animals due to its hardness and probably its starch type and concentration influencing the foregut digestibility of poultry, besides the cost issue. However, because of enzyme addition to the diet and limited inclusion (30%) of the wheat in the diet, these issues seem not a problem for feeding quails as evidenced at the present work, providing better performance, greater digestibility, and elevated serum and tissue Zn and Fe concentrations particularly with the wheat samples containing 64 mg Zn/kg DM. Although not meant to be designed particularly for animal consumption, the wheat samples with 64 mg Zn/kg DM are also a good choice as an animal feed, particularly for poultry.

**Author Contribution** Conceptualization, K.S. and I.C.; data curation, N.S. and C.O., methodology, N.S., O.K., C.O., and I.C.; formal analysis, N.S., E.S., C.O., K.S., and I.C.; writing—original draft preparation, O.K. and K.S.; and writing—review and editing, K.S. and I.C. The authors read and approved the final manuscript.

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**Data Availability** The datasets analyzed in the current study are available from the corresponding author on reasonable request.

**Code Availability** Not applicable.

## Declarations

**Ethics Approvals** The experiment was conducted in accordance with animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey.

**Consent to Participate** All participants provided written informed consent before participating in the study.

**Conflict of Interest** The authors declare no competing interests.

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