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Effect of Replacing Inorganic Copper, Zinc, and Selenium with Chelated Minerals on Productive Performance, Nutrient Utilization, Tibia Morphology, and Intestinal Histology of Growing Japanese Quail (*Coturnix japonica*)

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This study evaluated the impact of replacing inorganic mineral sources of Cu, Zn, and Se with chelated organic minerals (OM) on performance, nutrient and mineral utilization rates, and intestinal morphometry in growing Japanese quails (*Coturnix japonica*). A total of 150 nine-day-old quails were randomly assigned to receive one of the following diets over 4 weeks: CTRL (100% inorganic minerals), OM33 (replacement of 33% inorganic minerals), OM67 (replacement of 67% inorganic minerals), and OM100 (100% organic minerals). Quails fed the OM67 diet exhibited higher ($P < 0.05$) viability, daily weight gain, and live weight than the other groups, with no significant difference in feed intake or feed efficiency across treatments. The utilization rates of Cu and Fe were lower in the OM33 group. The CTRL group presented the lowest tibial weight ($P < 0.05$). Growing quails fed the OM67 diet contained the highest intestinal villi in the duodenum, jejunum, and ileum. In conclusion, the partial replacement (up to 67%) of inorganic mineral with OM in the diet of growing quails can enhance their productive performance and intestinal histological traits.

Key words: chelated minerals, growth performance, histology, quail, viability.

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Introduction

Providing appropriate amounts of trace minerals, such as copper, zinc, and selenium is essential to ensure optimal health and performance in poultry. These minerals play crucial roles in energy metabolism, immunity, antioxidant defense, and bone mineralization. They influence intestinal morphology and enhancing nutrient absorption capacity[1,2].

To prevent deficiencies and promote growth, inexpensive inorganic sources of trace minerals are added to diets in amounts that exceed recommendations[3]. However, these minerals can interact with and antagonize other dietary components, thereby reducing their bioavailability[4]. Furthermore, excess trace elements can result in elevated residual concentrations in manure, which is potentially harmful to the environment[3].

Organic and chelated minerals have been suggested as an alternative to inorganic ones[5]. They are stable complexes formed by the covalent binding of a metal ion to an organic molecule,

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such as an amino acid, fatty acid, or carbohydrate[6]. A recent review[7] suggested that organic minerals led to greater bioavailability and absorption potential owing to their reduced antagonism and uptake mechanisms. Consequently, they may improve productive performance, nutrient utilization rates, and bone health. Bone health has been assessed through bone morphology, such as increased bone weight, height, and width, which is associated with greater rigidity and skeletal stability in the legs, crucial for the growth and health of birds[8]. In addition, organic minerals improve intestinal health, as suggested by longer intestinal villi, which are associated with more active cell mitosis, greater nutrient absorption, and overall development of birds[9].

Numerous studies have reported the beneficial effects of organic or chelated minerals on laying hens and broiler chickens[10–12]. However, limited information exist on the Japanese quail (*Coturnix japonica*), which are popular meat and egg producing bird, owing to its size, rapid growth, and high reproductive potential[13,14].

Therefore, the objective of this study was to evaluate the effect of different levels of substitution of inorganic Cu, Zn, and Se with chelated mineral sources on the productive performance, nutrient utilization rate, tibial morphology, and morphometry of the intestine in growing Japanese quails.

Materials and Methods

Location and Experimental Design

The experiment was conducted at the Agronomy Department of the Universidad Autónoma de Nuevo León in Gral. Escobedo, NL, Mexico. A complete randomized block design was employed to evaluate the effect of substituting 0%, 33%, 67%, and 100% of inorganic Cu, Zn, and Se (copper sulfate [$CuSO_4$], zinc sulfate [$ZnSO_4$], and sodium selenite [Na_2SeO_3], respectively) with chelated mineral sources of Cu (Optimin® Copper 15%; hydrated amino acid copper chelate), Zn (Optimin® Zinc 15%; hydrated amino acid zinc chelate), and Se (Optimin® SeY 3000 ROW25; L(+) selenomethionine derived from inactivated *Saccharomyces cerevisiae*). All three products were obtained from Selko (Nutreco, Amersfoort, The Netherlands).

Ethical Approval

The protocols and procedures for the care, feeding, housing, and management of the 150 quails used in this study, as well as for euthanasia by cervical dislocation of 32 animals, were conducted in accordance with the Mexican Official Standard NOM-062-ZOO-1999 and approved by the Ethics and Animal Welfare Committees as well as the Biosafety and Hygiene Committee of the Faculty of Veterinary Medicine and Animal Science at the Universidad Autónoma de Nuevo León (File 33/2022; Approval Statement 44/2022, dated April 29, 2022).

Management of the Birds and Experimental Design

A total of 150 nine-day-old quails were used in this study. Approximately 50% were male and 50% female. The sex ratio was similar ($P > 0.05$) across treatments, ranging from 44.4%–48.6% males to 51.4%–55.6% females, with both sexes randomly assigned to each of four treatments: CTRL (basal diet with 100% inorganic minerals), OM33 (33% inorganic minerals substituted by organic ones), OM67 (67% inorganic minerals substituted by organic ones), OM100 (100% organic minerals). Each treatment included four replicates, consisting of 8–10 birds per replicate, housed in 16 experimental cages measuring 33 cm (height) × 80 cm (width) × 60 cm (depth).

Prior to the start of the experiment, the birds were reared for 8 days for environmental acclimation purposes. The evaluation phase began when the birds were 9 days old and continued for 4 weeks. Birds were fed different diets during the starter (from 9 to 24 days of age) and grower (from 25 to 39 days of age) stages (Table 1).

Determination of Productive Performance

The productive variables assessed during each of the four weeks and for the entire experimental period were live weight (g, weekly weighing of the birds) and daily weight gain (DWG, g/day, calculated as the daily weight increment between the corresponding week and the previous one).

To determine feed consumption (g/bird/day), the amount of feed added to the feeders was recorded daily, and the amount of uneaten feed was recorded weekly. Feed efficiency was calculated as DWG / actual feed consumption[15]. Bird viability (% survival) was estimated as final number of birds / initial number of birds × 100[16].

Determination of the Nutrient and Energy Utilization Coefficient

Excreta were collected from each quail cage over a 96-h period during the fifth experimental week and analyzed to determine their dry matter and protein content as described elsewhere[17]. The gross energy contents of the diets and excreta were measured using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA) with benzoic acid as standard[18]. Zn^+, Cu^{2+}, Mn^{2+}, and Fe^{2+} content was measured in samples (diets, excreta, and tibias) incinerated at 550 °C for 4 h to obtain ash. The ash was dissolved in 20 mL distilled water, 5 mL HCl (37.1% v/v), and 10 drops HNO_3 (70% v/v), after which it was digested at 200 °C to reduce the volume to 10 mL. The extract was filtered into a volumetric flask and the volume was adjusted to 100 mL with distilled water. The extract was loaded on an atomic absorption spectrophotometer (PinAAcle 900F AA; PerkinElmer, Waltham, MA, USA) and the above ions were measured at 324.7 nm, 279.5 nm, 213.8 nm, and 248.3 nm, respectively[19]. The apparent utilization coefficient of dry matter, protein, energy, and trace minerals were calculated using the following formula[20]: apparent digestibility of nutrient (%) = (ingested nutrient − excreted nutrient in feces) / ingested nutrient × 100.

Morphological Analysis of Tibiae

At the end of the 4th week of the evaluation phase, eight male birds were sacrificed per treatment. Their left and right tibiae were extracted and cleaned of soft tissues (muscles, ligaments, nerves, and vessels) as previously described[21]. The length (cm) and width (cm) of the tibia were measured using a caliper (Stainless Hardened® Gns 150, Fujian, China)[22]. The tibial length was defined as the distance from the upper end of the proximal

Table 1. **Starter and grower diets (g/kg), in which 0%, 33%, 67%, and 100% inorganic Cu, Zn, and Se were replaced with chelated minerals.** Diets were fed to chicks between 9–24 days of age (starter) and 25–39 days of age (grower).

	Starter				Grower			
Ingredients, g	CTRL	OM33	OM67	OM100	CTRL	OM33	OM67	OM100
Yellow Corn	522	522	522	522	610	610	610	610
Soybean meal, 48% CP	414	414	414	414	340	340	340	340
Vegetable oil	35	35	35	35	20	19	19	19
Premix Exp[1]	28.8	28.6	28.6	28.4	29.1	29.2	29.1	29.0
$CuSO_4$ (25% Cu)	0.04	0.02	0.01	0.00	0.04	0.02	0.01	0.00
$ZnSO_4$ (36% Zn)	0.20	0.13	0.07	0.00	0.20	0.13	0.07	0.00
Na_2SeO_3 (1% Se)	0.02	0.01	0.01	0.00	0.02	0.01	0.01	0.00
Optimin® Copper	0.00	0.02	0.04	0.06	0.00	0.02	0.04	0.06
Optimin® Zinc	0.00	0.16	0.32	0.48	0.00	0.16	0.32	0.48
Optimin® SeY	0.00	0.02	0.04	0.07	0.00	0.02	0.04	0.07
Chemical composition analyzed (dry matter basis)								
Dry matter, %	88.8	89.1	89.0	88.9	88.6	88.6	88.0	87.6
Crude protein, %	24.4	24.4	24.2	24.1	22.7	23.7	22.1	21.5
Energy, kcal/kg	4472	4432	4435	4411	4672	4467	4168	4511
Fat, %	2.70	2.33	2.44	2.32	1.98	1.96	1.81	1.82
Ash, %	6.46	6.53	7.04	5.99	5.77	5.74	5.86	5.99
NDF, %	11.9	14.2	11.5	12.0	11.9	11.1	12.0	12.0
ADF, %	2.26	2.94	2.20	2.82	2.36	1.93	2.00	2.30
Lignin, %	1.09	1.07	0.95	1.32	0.99	1.08	1.09	0.97
Hemicellulose, %	9.62	11.32	9.28	9.17	9.61	9.22	10.01	9.74
Cellulose, %	1.1	1.8	1.2	1.5	1.37	0.86	0.91	1.33

[1] Experimental Premix of amino acids, macronutrients, vitamins, and trace minerals, providing (per kg of diet): 2.70 g methionine, 0.9 g choline chloride, 1 g $CaHPO_4$, 19.6 g $CaCO_3$, 4 g NaCl, 0.14 g $FeSO_4$, 0.20 g MnO, 0.01 g EDDI, and 0.25 g avian vitamin core.

epiphysis to the lower end of the distal epiphysis. The diaphysis length was measured by subtracting the measurements of the proximal and distal epiphyses from the total tibial length[23]. Tibial width was measured as the distance between the external and internal edges in the central portion of the tibia. The tibial weight (g) was recorded using an analytical balance (HR-200; A&D Co. Ltd., Tokyo, Japan) with a minimum discrimination of 0.1 mg[22].

Morphometric Analysis of the Intestine

Immediately after euthanasia, intestinal samples (9 to 14 cm) were collected from the duodenum, jejunum, and ileum and preserved in 10% buffered formalin in phosphate-buffered saline. The samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and mounted on slides for observation[24]. Observations were performed using an optical microscope (Primo Star; Carl Zeiss, Jena, Germany) equipped with a digital camera. Photomicrographs and measurements were taken using Zen 3.2 software (blue edition) (Carl Zeiss) to obtain the following histological parameters: villus height, villus width, crypt depth, and villus height to crypt depth ratio.

Villus height (µm) was measured from the base of the villus, where it joins the top of the crypt, to the apical tip (Fig. 1). Villus width (µm) was measured at the midsection, between the most

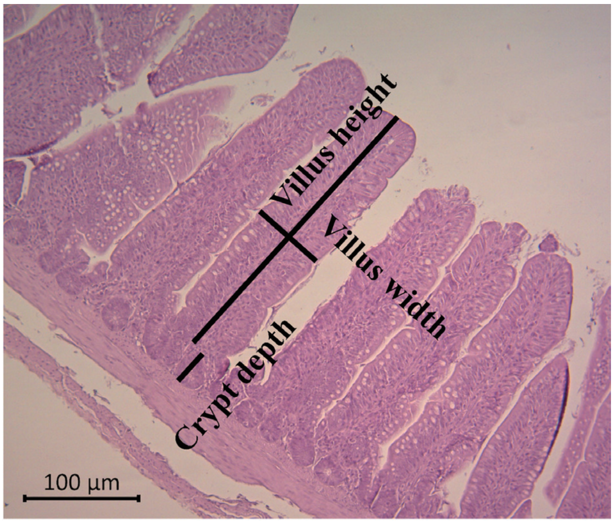

Fig. 1. **Cross-sectional view of the duodenum in growing quails, stained with hematoxylin and eosin.**

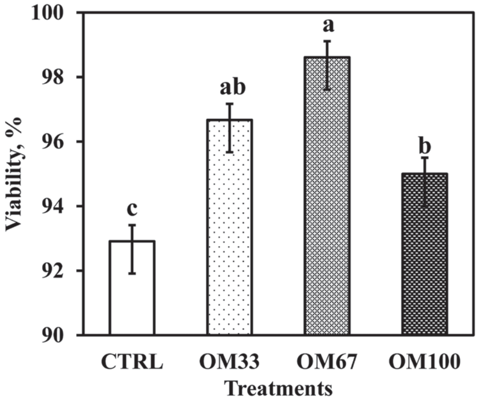

Fig. 2. **Viability of growing quails, whose inorganic Cu, Zn, and Se in feed was replaced by four different percentages (0%, 33%, 67%, and 100%) of chelated mineral sources. Superscript letters indicate statistically significant differences ($P \leq 0.05$).**

distant points of its lateral edges. Crypt depth (μm) was measured from its base to where it connects to the base of the villus (Fig. 1). The criterion for the selection of the measured villi (n = 613 in total) was villus integrity.

The villus height to crypt depth ratio was calculated as described previously[25]. In addition, the villus surface absorption area was calculated as 2π × (mean villus width / 2) × villus height[9].

Statistical Analysis

Analysis of variance was performed for each continuous variable using a completely randomized block design with four treatments and four replicates per treatment. Mean comparisons were conducted using Tukey's test, with a 95% confidence level, employing SPSS version 25 (IBM, Chicago, IL, USA). Regression analysis was performed with continuous variables as dependent variables and the linear and quadratic effects of the treatments as independent variables. The optimal physiological dose was estimated when treatment effects were significant. Viability was assessed using the Kruskal-Wallis test. Differences were considered significant when $P < 0.05$.

Results

Productive Performance

Performance parameters were independent of sex, as sex ratios were similar across treatments ($P > 0.05$). Viability was higher ($P \leq 0.05$) in the OM67 group (98.61%) compared to the OM100 (95.00%) and CTRL (92.91%) groups (Fig. 2).

Table 2 shows the productive parameters measured each week, as well as the averages calculated for the 4-week period. The average body weight for the 4-week period did not differ across treatments. In contrast, the average DWG calculated over this period was higher for birds fed the OM67 diet (4.3 g/day) than the CTRL diet (3.8 g/day) ($P = 0.037$). All treatments resulted in similar mean values for feed consumption ($P = 0.378$) and feed efficiency ($P = 0.631$). However, in 3 out of 4 weeks, daily feed intake was lower in the CTRL group ($P < 0.05$) than in birds fed a diet with 100% chelated minerals (OM100).

Nutrient, Energy, and Trace Mineral Utilization Rate

The four substitution levels of Cu, Zn, and Se attained similar outcomes ($P > 0.05$) regarding the utilization rates of dry matter, protein, and energy in week 5 (Table 3). The apparent utilization rate of Cu was lower ($P = 0.001$) in OM33 (26.12%) compared to CTRL (48.27%), OM67 (60.04%), and OM100 (58.38%) groups. The utilization rate of Fe was higher ($P < 0.05$) in the OM100 than in the OM33 group; whereas those of Zn and Mn were similar ($P > 0.05$) across groups. The trend for Mn indicated increased utilization with higher levels of substitution of inorganic minerals, albeit not in a significant way (Table 3).

Tibial Morphology

Table 4 shows that tibial weight was higher ($P = 0.045$) in the OM33 group (0.710 g) compared to other groups. The tibiae had also similar length ($P = 0.888$), width ($P = 0.344$), and diaphyseal diameter ($P = 0.482$).

Morphometrical Analysis of the Intestine

Figure 3 shows that average villus height was greater ($P < 0.01$) in the duodenum (520 μm) compared to the jejunum (430 μm) and ileum (304 μm).

In the duodenum and jejunum, villus height and crypt depth were higher ($P < 0.05$) in birds fed the OM67 diet than the OM33 diet (Table 5). The villus width and villus height to crypt depth ratio were similar ($P > 0.05$) across treatments (Table 5), along with the villus absorptive surface area in the duodenum ($P = 0.326$) and jejunum ($P = 0.131$). In the duodenum, the absorptive surface area ranged from 132,841 μm^2 to 155,082 μm^2; whereas in the jejunum, it ranged from 99,304 μm^2 to 124,177 μm^2.

In the ileum, villus height was higher ($P < 0.05$) for birds fed the OM33 diet (406 μm) than the CTRL diet (307 μm); the same was true also for crypt depth (57 μm vs. 38 μm; $P < 0.05$) and villus absorptive surface area (89,302 μm^2 vs. 43,692 μm^2; $P = 0.001$). Villus width in the ileum was significantly lower ($P < 0.05$) in birds fed the CTRL diet (62 μm) compared to other treatments. Instead, the villus height to crypt depth ratio was similar ($P > 0.05$) across treatments (Table 5).

Discussion

The results obtained in this study show that substituting 67% of inorganic sources of Cu, Zn, and Se with organic ones (OM67 diet) improved viability and DWG in quails. Even though birds fed the OM67 diet were not significantly heavier than those receiving the CTRL diet (100% inorganic minerals), a clear increase in body weight ($P = 0.052$) was observed between these two treatments in the 4th week (Table 2). A previous study[26] indicated that from 37 days of age onwards, there was a significant effect of sex on quail growth. In the present study, growth performance was evaluated on a cage basis for mixed sexes during 4 weeks, and the birds were 36 days old at the end of the experiment. To exclude any gender bias, the sex ratio was similar

Table 2. **Productive performance of growing quails fed a diet, in which 0%, 33%, 67%, and 100% of inorganic Cu, Zn, and Se were substituted with organic sources.**

Parameter	Week	CTRL	OM33	OM67	OM100	SEM	*P*
Body weight, g	Initial	31.3	32.2	33.1	31.7	2.69	0.916
	1st	54.1	57.1	57.6	57.1	4.40	0.845
	2nd	86.7	89.9	97.1	88.4	4.98	0.249
	3rd	113.6	118.5	127.0	118.4	5.05	0.139
	4th	139.8	143.8	152.9	141.8	4.09	0.052
	Mean	85.1	88.3	93.6	87.5	3.13	0.060
Daily weight gain, g	1st	3.2	3.5	3.5	3.6	0.27	0.583
	2nd	4.7^{b}	4.7^{b}	5.6^{a}	4.5^{b}	0.24	0.005
	3rd	3.8	4.1	4.3	4.3	0.17	0.104
	4th	3.7	3.6	3.7	3.3	0.32	0.606
	Mean	3.8^{b}	4.0^{ab}	4.3^{a}	3.9^{ab}	0.15	0.037
Daily feed intake, g	1st	8.8^{b}	11.2^{a}	11.4^{a}	12.9^{a}	0.70	0.003
	2nd	12.5^{b}	13.8^{ab}	14.6^{ab}	15.1^{a}	0.69	0.027
	3rd	15.31	16.0	18.9	18.21	0.92	0.056
	4th	17.7^{b}	18.7^{ab}	20.0^{a}	20.5^{a}	0.68	0.013
	Mean	14.8	15.5	15.9	15.1	0.60	0.378
Feed efficiency	1st	0.370^{a}	0.315^{ab}	0.305^{ab}	0.280^{b}	0.02	0.031
	2nd	0.373^{a}	0.339^{ab}	0.387^{a}	0.297^{b}	0.02	0.012
	3rd	0.242	0.240	0.244	0.254	0.01	0.493
	4th	0.211	0.195	0.184	0.162	0.01	0.180
	Mean	0.273	0.267	0.281	0.275	0.01	0.631

Means with different superscript letters are statistically different across diets ($P < 0.05$).
SEM = Standard error of the mean.

Table 3. **Utilization rate (%) of nutrients, energy, and trace minerals in growing quails fed diets, in which 0%, 33%, 67%, and 100% of inorganic Cu, Zn, and Se were replaced with chelated sources.**

Nutrient, %	CTRL	OM33	OM67	OM100	SEM	*P*
DM	73.85	73.39	73.34	74.36	1.887	0.942
Protein	49.66	50.53	45.99	42.08	4.855	0.330
Energy	68.13	66.59	70.98	70.65	2.221	0.204
Copper	48.27^{a}	26.12^{b}	60.04^{a}	58.38^{a}	6.830	0.001
Manganese	25.43	34.25	35.65	44.35	7.886	0.147
Iron	44.59^{ab}	36.83^{b}	51.65^{ab}	56.73^{a}	6.562	0.050
Zinc	79.78	73.56	79.60	86.34	4.306	0.076

Means with different superscript letters are statistically different across diets ($P < 0.05$).
SEM = Standard error of the mean.

Table 4. **Morphological traits of tibiae in quails fed diets, in which 0%, 33%, 67%, and 100% of inorganic Cu, Zn, and Se were substituted with organic ones.**

Trait	CTRL	OM33	OM67	OM100	SEM	*P*
Weight, g	0.648^{c}	0.710^{a}	0.662^{b}	0.663^{b}	0.022	0.045
Total length, cm	4.996	5.052	5.015	5.014	0.727	0.888
Diaphysis, cm	3.877	3.934	4.018	3.973	0.089	0.482
Width, cm	0.312	0.309	0.287	0.291	0.167	0.344

Means with different superscript letters are statistically different across diets ($P < 0.05$).
SEM = Standard error of the mean.

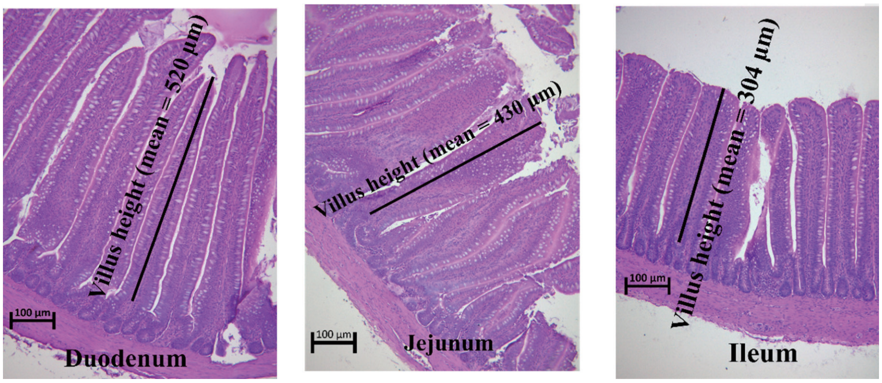

Fig. 3. **Photomicrographs showing villus height in each section of the small intestine of growing Japanese quails. Magnification 10×, stained with hematoxylin and eosin.**

Table 5. **Intestinal histological traits of quails fed diets, in which 0%, 33%, 67%, and 100% of inorganic Cu, Zn, and Se were replaced with organic minerals.**

Trait	CTRL	OM33	OM67	OM100	SEM	*P*
Duodenum (n = 40)						
Villus height, µm	607^{ab}	594^{b}	665^{a}	542^{c}	26.37	<0.001
Villus width, µm	91	95	103	100	7.05	0.208
Crypt depth, µm	65^{ab}	58^{b}	72^{a}	63^{ab}	5.01	0.024
Villus height: crypt depth	9.87	10.72	9.63	9.27	1.01	0.256
Villus absorption area, μm^2	135410	155082	146658	132841	15909	0.326
Jejunum (n = 40)						
Villus height, µm	495^{ab}	438^{b}	550^{a}	555^{a}	27.22	<0.001
Villus width, µm	83	96	95	87	6.68	0.118
Crypt depth, µm	53^{bc}	48^{c}	64^{a}	60^{ab}	4.35	<0.001
Villus height: crypt depth	9.97	9.54	9.14	9.68	0.91	0.670
Villus absorption area, μm^2	114367	105319	124177	99304	12778	0.131
Ileum (n = 50)						
Villus height, µm	307^{c}	406^{a}	326^{bc}	348^{b}	15.10	<0.001
Villus width, µm	62^{b}	83^{a}	87^{a}	96^{a}	6.77	<0.001
Crypt depth, µm	38^{c}	57^{a}	47^{bc}	49^{ab}	4.33	<0.001
Villus height: crypt depth	8.66	7.78	7.67	7.41	0.83	0.465
Villus absorption area, μm^2	43692^{c}	89302^{a}	83032^{ab}	69771^{b}	7772	<0.001

Means with different superscript letters are statistically different across diets ($P < 0.05$).
SEM = Standard error of the mean.

($P > 0.05$) across treatments.

Viability is a significant economic and animal welfare parameter[27]. The higher viability (98.6%) measured in the present study in the group fed the OM67 diet compared to the CTRL group is consistent with the findings of Vieira *et al.*[10], who reported higher viability (~1.67% more) in broiler chickens fed diets containing organic trace minerals of Zn, Cu, Mn, and Fe at 14 days, 32 days, and 48 days of age compared to birds receiving inorganic trace minerals ($P < 0.05$). In Japanese quails, Ray *et al.*[28] similarly reported higher viability (98.6% vs. 92.9%) in birds whose diet included a 50% substitution of inorganic Zn, Cu, and Mn compared to birds fed a control diet without organic minerals.

Substituting 100% of the inorganic Cu, Zn, and Se with chelated minerals (OM100) resulted in lower viability (95.0%) compared to partial substitution (OM67), but still more than no substitution (CTRL). Based on these results, the optimal dose was calculated by linear regression as 57.4% substitution of inorganic minerals with chelated Cu, Zn, and Se.

The higher feed intake in the groups fed diets containing chelated minerals may indicate the positive effect of greater trace mineral availability. In a previous study[29], supplementation with chelated Zn-glycine improved feed intake in broilers from 0 to 21 days of age, even at doses lower than those required when $ZnSO_4$ was used as supplement.

The observed improvement in this trait could be attributed to increased immunity and disease resistance, as suggested by previously reported beneficial effects of organic minerals on viability[27,30,31]. Recently, Mohammadizad *et al.*[32] reported that complete substitution of inorganic trace minerals with advanced chelated Fe, Zn, Cu, Mn, Se, Cr, and I protected against heat stress by downregulating the proinflammatory cytokines interleukin (IL)-1β, IL-6, and IFN-γ by nuclear factor kappa B, while upregulating the anti-inflammatory cytokine TGF-β. According to Feng *et al.*[29], adding 90 mg Zn as Zn-glycine to the diet during the later period (22–42 days) increased the IgA serum content of broilers.

In the present study, body weight and DWG were higher with the OM67 diet than with the CTRL diet. However, according to regression analysis, the optimal physiological dose to maximize these traits should be a 56.8% substitution of inorganic Cu, Zn, and Se with their chelated forms. The positive effect of using organic trace minerals on body weight and DWG in broiler chickens has been reported previously[33]. Indeed, broiler chickens fed diets containing organic and hydroxychloride trace mineral premixes exhibited a 1.34% higher weight gain compared to those given only inorganic minerals[34]. Similarly, broiler chickens fed diets containing 120 mg/kg of Zn-glycine presented higher DWG and better feed conversion than those fed an inorganic Zn source[29]. Previously reported quail weights at 4 weeks of age (136.5 ± 1.65 g)[35] coincided with those obtained in the present study. While some studies[36,37] documented improvements in productive performance of broilers fed diets containing organic minerals; others[38] contradict this observation, reporting no differences following diets containing 33%, 66%, and 100% chelated trace minerals of Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{+} compared to a diet composed solely of inorganic trace minerals.

There are several mechanisms through which Zn, Cu, and Se may promote chicken growth. Zn contributes to the proper functioning of the immune system, development of skeletal tissues, and maintenance of bone health[39]. Additionally, it plays a crucial role in antioxidant metalloenzymes such as superoxide dismutase[40]. Cu plays a key role in the development of bones and tissues, in addition to being a component of cytochrome oxidase and superoxide dismutase, as well as in forming antibodies and white blood cells[41]. Se maintains the antioxidant defense system in laying hens[42], as it is a cofactor in glutathione peroxidase (GSH-Px), which protects cells from oxidative damage[43].

Addition of 9 mg Cu, 72 mg Zn, and 0.200 mg Se per kg feed did not alter the utilization rates of dry matter, protein, energy, Mn, and Zn in the 5th week across treatments. These results are consistent with those reported by Zhao *et al.*[36], who found no significant effects of organic mineral supplementation on nutrient digestibility in broilers. However, a previous study in broilers[44] reported improved protein utilization when coated chelated minerals were added at 6 mg Cu, 60 mg Zn, and 0.345 mg Se per kg feed compared to inorganic minerals at 10 mg Cu, 100 mg Zn, and 0.575 Se. This level of Se was higher than the recommended dose (0.2–0.3 mg/kg diet) for broilers[45] and growing quails (0.28 mg/kg diet)[26]. According to Vieira *et al.*[10], feeding higher levels of trace minerals than recommended leads to higher mineral excretion in the litter and could reduce the utilization rate (bioavailability) of trace minerals and proteins in broilers.

In the present study, the utilization rates of Cu and Fe improved upon substitution with chelated mineral sources, suggesting they may influence the absorption of these and other nutrients[46]. Dietary inclusion of organic trace minerals, such as Fe, Zn, Mn, and Se, resulted in better standardized ileal digestibility coefficients in broilers[47], echoing a previous study[48], which reported higher bioavailability and absorption of organic minerals compared to inorganic forms in poultry.

Some studies report that organic minerals may have better absorption and utilization owing to fewer negative interactions between the minerals[46,48]. This phenomenon could explain the elevated Fe utilization observed in the MO100 treatment compared to the MO33 treatment[45]. Here, CTRL, OM67, and OM100 treatments resulted in higher Cu utilization rates. As reported previously[47], inorganic Cu can interfere with the absorption of Zn and Fe. Although the differences were not statistically significant, lower levels of Zn and Fe were recorded in the CTRL than in the OM100 treatment, suggesting that high doses of inorganic Cu in the diet could have a negative impact on absorption of other minerals.

Mineral sources can be used as an indicator of bone health[49], with Cu, Zn, Se, Mn, and Fe having a positive effect on bone development[50]. In the current study, the tibial weight was greater ($P < 0.05$) in the OM33 group than in the OM67 and OM100 groups, and all three groups had larger tibiae than the CTRL group. Body weight of animals receiving chelated minerals was also higher than that of animals in the CTRL group on the 4th week ($P = 0.052$). According to Nishimura *et al.*[51] bone and body growth are correlated.

The heavier tibiae of birds fed diets supplemented with chelated minerals were thinner (NS, $P = 0.344$) (Table 4), which could be associated with greater bone density. Bone density increases with body weight[51], and birds fed chelated minerals were heavier in the 4th week.

Broiler chickens fed with organic forms of Cu, Zn, and Mn[12] showed a 51.2% reduction in lameness conditions compared to the CTRL group. Trace minerals play a crucial role in bone health by preventing bone mass loss and fractures, reducing bone resorption, and stimulating the formation of new bone tissue[52]. Zn, Cu, and Se act together as substrates in bone formation and may improve tibial weight. Zn promotes osteoblast activity and collagen synthesis, while reducing—together with Cu—osteoclast-mediated bone resorption[53]. Se reduces the accumulation of reactive oxygen species, thereby inhibiting osteoclast activation and promoting osteoblast differentiation[46,53]. In the present study, the content of Se in the tibiae was not measured; however, it will be quantified in future investigations.

Histological measurements revealed that villus height was greater in the duodenum and decreased progressively caudally along the small intestine. Wilkinson *et al*.[54] observed that villus height in Japanese quails peaked in the jejunum, becoming shorter and shallower in the ileum. Here, villus height in the duodenum and jejunum was significantly higher ($P < 0.05$) in birds fed the OM67 diet. This increase could be related to Se serving as a component of intestinal GSH-Px[4], which could actively support enterocyte survival[55]. In birds weighing 139.8–152.9 g at the 4th week of age, jejunum villi reached 438–555 µm in height, which is comparable with the values (450–530 µm) reported by Rezaei *et al.*[56] in Japanese quails weighing 160–170 g, despite a different growth rate between the two studies. The OM100 diet significantly improved villus height in the jejunum (555 µm); while the OM33 diet did so in the ileum (406 µm).

The larger intestinal surface of the ileum observed in the present study is directly related to better digestion and absorption[57] and, therefore, to increased weight and resistance to intestinal disorders. Therefore, improvements in villi morphology in the OM67 group could partly explain the increase in live weight, DWG, overall health, and viability observed in this group of birds. The width of the villi in the ileum in this study was lower in the CTRL group; whereas treatments with any level of organic mineral substitution improved villus width and increased the surface area for absorption[58]. In the duodenum and jejunum segments, villus width was similar across treatments. In birds fed the OM33 diet, crypt depth was lower in the duodenum (58 µm) and jejunum (48 µm) compared to other treatments, suggesting a higher rate of cell renewal and migration of crypt cells to the epithelium[59]. This, in turn, would benefit nutrient absorption efficiency in the birds. Epithelial cells of the villi are in direct contact with the luminal content and are susceptible to damage, which often results in greater loss of epithelial cells in gut disorders[60].

In conclusion, replacing inorganic sources of Cu, Zn, and Se with chelated minerals in the quails' diet is an effective nutritional strategy for improving performance, viability, tibial bone development, and intestinal histology. Among the four evaluated substitution levels, a 67% replacement (OM67) showed the best productivity and health performance. These results were close to the calculated optimal physiological dose values (57.4%).

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Author contributions

Carlos Gaona conducted the experiments and analyses, interpreted the data, and drafted the manuscript. Hugo Bernal conceptualized and designed the experiment, interpreted the data, and critically reviewed and edited the draft. Nydia Vásquez supervised the chemical analyses. Cecilia Ramírez oversaw the processing of samples for histological image analysis. Humberto González supervised the quantification of trace minerals, and reviewed and edited the manuscript. Adriana Morales, Miguel Cervantes, and Jesús Escareño reviewed the results and contributed to the final draft. The statistical analyses, data interpretation, and figure preparation were performed by Carlos Gaona, Emilio Olivares Sáenz, and Hugo Bernal.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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