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Effects of Mineral Proteinate Mixture Inclusion Levels on *Oncorhynchus mykiss* Fry: Growth, Digestion, and Antioxidant Status

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Abstract

To improve growth, digestion, antioxidant status, and feed efficiency, this study sought to identify the ideal inclusion level of an organic mineral mixture (OMM®; proteinates of Zn, Cu, Mn, Fe, Co, Se) to partially replace inorganic mineral premixes in rainbow trout (*Oncorhynchus mykiss*) fry diet. Four isoproteic diets (0% control, 0.5% OMM-50, 0.75% OMM-75, 1.0% OMM-100) were subjected to triplicate testing using over 60 days. Final weight (13.55±0.03 g), SGR (3.99±0.02%/day), and trypsin activity (2.46±0.10 U/mg protein), were maximum in the OMM-50 group and lowest FCR (1.02±0.01). Both moderate OMM (0.5–0.75%) increased the SOD (8.79±1.9, 7.67±1.3 U/mg protein) and GPx (0.32±0.11, 0.17±0.06 U/mg protein) activities, decreasing MDA (1.60±0.87, 2.83±0.76 nmol/mg protein), suggesting enhanced antioxidant defense. Conversely, OMM-100 reduced growth (11.19±0.06 g), SGR (3.49±0.18%/day), and survival (84.29±0.82%), but increased feed intake (3.37±0.03%/day), indicating mineral imbalance. The peak of whole-body mineral contents was observed at OMM-50, validating higher bioavailability. Thus, 0.5% OMM replacement optimizes physiological outcomes and offers a sustainable alternative to inorganic minerals in aquafeeds.

Introduction

Minerals are critical micronutrients for the execution of numerous physiological and metabolic processes in aquatic organisms in which these functions encompass enzymatic catalysis, skeletal development, and antioxidant defense, which play a vital role in growth and health in juvenile fish such as rainbow trout fry (Akram et al., 2020; Lall, 2022). Although certain nutrients can be directly absorbed by fish via gills or skin, or water (drinking) as a direct origin of nutrients (Craig et al., 2017; Moraes & de Almeida, 2020), dietary sources are considered as the main source of mineral intake, particularly in intensive aquaculture production conditions where water quality and mineral levels are strictly controlled (Lall, 2022).

Mineral deficiencies may negatively affect the growth, skeletal integrity, and immune functioning in fish (Domínguez et al., 2017; Baeverfjord et al., 2019; Kazemi et al., 2020). As an example, the lack of zinc can lead to cataracts or growth retardation (Lall, 2022). Nevertheless, the absorption and use of minerals can be constrained by a number of reasons, even when minerals are present in adequate amounts in feeds, due to antagonistic nutrient interactions, the occurrence of antinutritional compounds, or the chemical form of the mineral (Apines-Amar et al., 2004; Watanabe et al., 1997).

Inorganic mineral salts are frequently used in aquafeeds, but they often show poor bioavailability and lead to more mineral excretion, which contributes to environmental pollution (Prabhu et al., 2018). It has

been proven that organic trace minerals, such as amino acid chelates and mineral proteinates are more stable and better absorbed (Byrne et al., 2021). Some of them involve the formation of mineral proteinates, which is the combination of trace minerals (zinc, copper, manganese, selenium, cobalt, and iron) with hydrolyzed proteins. The complexation increases resistance to dietary antagonists and pH variation, which can positively affect mineral absorption in juvenile fish (Cantwell et al., 2017; Shah et al., 2021). Special attention has been paid to the independent effects of organic Ze, Se, and Cu in fish, and the possibility of maintaining their concentrations in the organism and preventing oxidative stress (Meiler et al., 2021; Viegas et al., 2023; Nguyen et al., 2019). Nevertheless, there are only a few studies that evaluate the use of such minerals in combination, particularly using balanced organic mineral mixtures in early life stages of fish. Recently, it has been demonstrated that moderate levels of multi-element organic mineral supplementation can be used to boost growth, feed efficiency, and antioxidant measures of numerous aquaculture species. For instance, it was observed that turbot (*Scophthalmus maximus*) fed diets with an organic trace mineral mixture of Bioplex® at 0.075% had higher growth and antioxidant enzyme activity than those lower or higher concentrations (Yang et al., 2020). Similarly, Ramasamy et al. (2021) concluded that juvenile freshwater prawns (*Macrobrachium malcolmsonii*) fed an Aquamin®/Agrimin® mineral mixture at a dietary level of 1.0% showed optimal growth and carcass mineralization, while inclusion at higher levels (1.5% and above) resulted in reduced performance. Vijayan et al. (2025) also found that, in Indian major carps, feed efficiency, and hematological parameters were enhanced with a developed mineral premix at 1.5%, supporting the benefit of balanced mineral supplementation.

These studies, taken together across taxa reveal a pattern; moderate concentrations of multi-mineral supplementation have an optimal physiological and metabolic effect on organisms, but excessive mineral additions can disrupt mineral homeostasis and growth efficiency. There is however, limited evidence regarding cold-water salmonids, particularly at the early stages of development.

To address this gap, the current study was an attempt to determine the impact of a partial substitution of inorganic mineral premix with a commercial organic mineral mix, which is a proteinate of zinc, copper, manganese, selenium, cobalt, and iron, on the growth, enzymatic activities in the intestines, and antioxidant response of rainbow trout fry (*Oncorhynchus mykiss*). Using a combination of physiological and biochemical measurements, this paper aims not only to elucidate their biological implications of organic mineral supplementation but also provide practical insights for formulating more efficient and environmentally sustainable mineral strategies to employ more effective and ecologically facets of mineral approaches in aquafeed production.

Materials and Methods

Experimental Diet

Four isoproteic, isoenergetic, and isolipidic diets were developed to test the partial replacement of inorganic mineral sources with a commercial organic mineral mixture (OMM®, Tekinler Süt Ürünleri A.Ş., Çanakkale, Türkiye), which is composed of proteinates of zinc, copper, manganese, selenium, cobalt, and iron. The control diet (OMM-0) consisted of 2% inorganic mineral premix. In experimental diets, the replaceable 1% portion of the premix was substituted with OMM to obtain the following diets: OMM-50 (1.5% inorganic + 0.5% OMM), OMM-75 (1.25% inorganic + 0.75% OMM), and OMM-100 (1.0% inorganic + 1.0% OMM). Each diet was formulated to contain at least 1% inorganic minerals to ensure nutritional adequacy. The OMM® product consists of amino acid-chelated mineral proteinates, such as zinc proteinate, which are reported to have improved stability and bioavailability compared to inorganic counterparts.

Our version of the diet preparation was based on the protocol given by Şahin and Ergün (2021), with slight modifications. Concisely, dry ingredients were thoroughly mixed, fish oil was added, and water was then added to make a homogeneous dough. The dough was then cold-extruded (2 mm, LaMonferrina-P3, Italy), dried at 40°C to achieve a final moisture level of 9–10%, and ground and sieved to a particle size of 0.20–0.25 mm. All the prepared feeds were kept at −18°C until use. Table 1 contains the composition and detailed description of the diets.

Experimental System and Fish

Rainbow trout juveniles were purchased from a private fish farm (Keskin Alabalık Company, Çanakkale, Turkey) and moved to the experimental unit (Fish breeding unit of Çanakkale Onsekiz Mart University). Twelve fiberglass cylindrical tanks (150 L) were set up to form a triplicate design, and seventy fish at 1.24±0.04 g wet weight (mean±SD, n=840) were distributed into each experimental tank subsequently. Fish were adapted to the experimental system for two weeks prior to the feeding trial. Tanks were continuously supplied with atmospheric air by air-stones connected to a central blower. The system was illuminated for 12 hours, and fish were fed *ad libitum* three times daily at 08:30, 12:30, and 16:30 h for 60 days. The water temperature was set at 13±1°C and controlled by a thermostatic heater. Water quality was monitored regularly throughout the experiment to achieve stable rearing conditions. Dissolved oxygen and temperature were measured using a YSI® multiparameter device (Pro2030), while pH was determined using a HANNA®

benchtop pH meter (HI 2221). Total ammonia nitrogen (TAN), nitrite, and nitrate concentrations were analyzed spectrophotometrically by an Optizen® POP UV/VIS unit. The measured oxygen, temperature, pH, ammonia, nitrite, and nitrate values (mean±SE) were found to be as 7.99±0.6 mg/L, 14.4±0.7°C, 7.85±0.4, 0.01±0.00 mg/L, 0.02±0.00 mg/L, and 0.42±0.00 mg/L, respectively.

Growth Performances and Biometric Indices

The following formulas were used in order to determine growth parameters and biometric indices:

Feed Conversion Ratio (FCR)= Total Feed Consumed / Total Weight Gain

Feed Intake (FI, %)= 100 × Feed Consumed / [(Initial Weight + Final Weight) / 2 × Days]

Hepatosomatic Index (HSI, %)= (Liver Weight / Total Body Weight) × 100

Specific Growth Rate (SGR, %/day)= 100 × [ln (Final Weight) – ln (Initial Weight)] / Days

Viscerosomatic Index (VSI, %)= (Viscera + Fat Weight / Total Body Weight) × 100

Chemical Composition

For whole-body composition analysis, before the final sampling, the fish were deprived of feed for a 24-hour period to standardize digestive conditions. Five fish from each replicate (n=15 in total) were randomly collected and euthanized with an overdose of clove oil (200 mg/L). Moisture content was determined by drying samples at 105°C for 24 hours in a laboratory oven until a constant weight was achieved. Crude ash was measured by incinerating the samples at 525°C for 12 hours. Crude protein content was determined using the Kjeldahl method (AOAC, 2005), while crude fat was analyzed following the methanol/chloroform extraction protocol described by Folch et al. (1957).

Digestive Enzyme Assays

A high-dose clove oil (200 mg/L) was used to euthanize the fish samples (fifteen per treatment), and all samples were dissected on an ice-cold surface using sterile lancets. Samples of stomach and intestine tissues, but not the whole -body, were removed and taken from the same section. After placing the tissues into Eppendorf tubes, 1:10 (v:v) ice-cold distilled water was added into each tube, and the tissues were homogenized with a laboratory homogenizer. After homogenization, the samples were centrifuged at 21,400 g for 30 minutes using a centrifuge (Hettich® Micro 200R) cooled to 4°C. The pH of the supernatant was measured (Nya & Austin, 2011), after which the upper phase was collected and stored at −80 °C for enzyme activity determination. The activities of trypsin, alkaline phosphatase, lipase, amylase, and pepsin were determined as per Şahin & Ergün (2021). The protein concentration in the individual samples was quantified following the procedure described by Bradford (1976).

Antioxidant Enzyme Assays

Five fish per replicate were randomly sampled and dissected on an ice-cold surface to maintain enzymatic

Table 1. Ingredients (%) and proximate composition (% as-fed basis) of the experimental diets.

	Control OMM-0	OMM-50	OMM-75	OMM-100
Fish meal [1]	40	40	40	40
Soybean meal [2]	23	23	23	23
Wheat gluten [3]	5	5	5	5
Wheat flour [4]	11	11	11	11
Corn gluten [5]	5	5	5	5
Inorganic minerals [6]	2	1.5	1.25	1.0
OMM® [7]	0	0.5	0.75	1.0
BHT [8]	0.001	0.001	0.001	0.001
Vitamins [9]	1	1	1	1
Dicalcium phosphate	0.499	0.499	0.499	0.499
CMC [10]	0.5	0.5	0.5	0.5
Fish oil (anchovy)	12	12	12	12
Crude protein (CP)*	47.07	47.45	48.16	47.22
Crude fat (CF)*	15.54	14.77	15.48	16.14
Ash*	6.04	7.05	6.91	6.40

[1] Fishmeal (Anchovy: 68.85% CP, 6.61% CF) [2] Soybean meal (50.14% CP, 2.40% CF) [3] Wheat gluten (74.24% CP, 2.30% CF) [4] Wheat flour (9.99% CP, 1.00% CF) [5] Corn gluten (60.51% CP, 2.01% CF) [6] Inorganic minerals (per kg of diet: 25 mg Manganese, 160 mg Iron, 75 mg Zinc, 5 mg Copper, 1.5 mg Cobalt, 0.25 mg Selenium, 5 mg Iodine, and 65 mg Magnesium). [7] OMM® (Organic mineral mixture, per kg of diet: 25 mg Manganese-proteinate, 160 mg Iron-proteinate, 75 mg Zinc-proteinate, 5 mg Copper-proteinate, 1.5 mg Cobalt-proteinate, 0.25 mg Selenium-proteinate, 65 mg Magnesium-proteinate, 5 mg Iodine, and *Saccharomyces cerevisiae*). [8] BHT (Butylated-hydroxytoluene) [9] Vitamins (Per kg of diet: 10.000 IU Vitamin A, 7.000 IU Vitamin D3, 150 IU Vitamin E, 1.2 g Vitamin K3, 10 mg Vitamin B1, 10 mg Vitamin B2, 25.1 mg Vitamin B3, 46 mg Vitamin B5, 14.3 mg Vitamin B6, 0.35 mg Vitamin B7, 570 mg Vitamin B8, 2.4 mg Vitamin B9, 0.06 mg Vitamin B12, 200 mg Vitamin C and antioxidant matter). [10] CMC (Carboxymethyl cellulose) * Crude protein (CP), crude fat (CF), and ash contents are derived from laboratory proximate analysis, with calculated values for reference.

integrity. Liver tissues were immediately excised, frozen in liquid nitrogen, and stored at –45°C until enzyme activity analysis. Tissues were homogenized, cold-centrifuged (+4°C) twice at 10,000 g for 20 min, and the supernatants were diluted 1:5 with phosphate buffer. The activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were measured spectrophotometrically and expressed as U/mg protein, with one unit defined as the amount of enzyme that converts of 1 µmol of substrate per minute. Protein concentration was determined according to the Bradford (1976) method. The activity of SOD was determined based on the inhibition of nitro-blue tetrazolium (NBT) reduction by superoxide radicals generated via the xanthine oxidase/hypoxanthine system, in a 0.05 M Na_2HPO_4/NaH_2PO_4 buffer (pH 7.4) containing 48 µM xanthine, 0.2 units of xanthine oxidase, and 96 µM EDTA (Flohé & Ötting, 1984). Absorbance was read at 550 nm. The rate of H_2O_2 degradation at 240 nm was used to measure CAT activity, using a reaction mixture of 50 mM phosphate buffer (pH 7.0) and 30 mM (Claiborne, 1985). A reaction mixture composed of 100 mM K_2HPO_4, 1 mM GSH, 0.2 mM NADPH, 1 IU/mL glutathione reductase, 1 mM NaN_3, and 0.25 mM H_2O_2 was used for measurement of GPx activity, as explained by Wendel (1980). The decrease in the NADPH absorbance at 340 nm was used to calculate GPx activity.

Lipid Peroxidation Assay

Lipid peroxidation was measured by determining the amount of malondialdehydes (MDA) in liver tissue using thiobarbituric acid reactive substances (TBARS) method. The method was adapted with some modifications based on literature guidelines (Humam et al., 2020). Fifteen samples (five liver per replicate, n=15 per treatment) were used in each treatment group. Liver samples were homogenized at a 1:5 (w/v) ratio in 50 mM, pH 7.0 ice-cold phosphate buffer. The prepared homogenates were then centrifuged at 10,000 g for 15 minutes at 4°C. A 100 µL portion subsequently taken from the upper phase was mixed with 200 µL of 0.67% thiobarbituric acid (TBA) solution and 300 µL of 20% trichloroacetic acid (TCA) solution. The mixture was incubated at 95°C for 30 minutes, then cooled on ice and centrifuged again at 10,000 g for 10 minutes at 4°C. The absorbance of the upper phase was measured using a spectrophotometer at a wavelength of 532 nm. MDA levels were calculated according to a standard curve created using 1,1,3,3-tetramethoxypropane and expressed as nmol/mg protein. Protein concentration was determined according to Bradford (1976) method.

Whole-body Trace Mineral Concentrations

At the end of the trial period (60 days), whole-body samples were collected to determine trace mineral (Zn, Cu, Mn, Fe, Se, Co) levels in the fish. A total of 15 fish (5 fish per replicate) were used from each treatment group. Approximately 0.5 g samples were prepared according to the United States Environmental Protection Agency (EPA, 1994) Method 200.7 protocol. Samples were freeze-dried prior to analysis and then ground into a very fine powder. After this process, digestion was performed with concentrated nitric acid (HNO_3) at 180°C for 20 minutes. The resulting solutions have been analyzed through an ICP-OES device (PerkinElmer®, Optima 8000, USA), and according to the quality control procedure, blank and duplicate samples have been included to ensure analytical reliability and repeatability. Results have been expressed as micrograms per gram µg/g on a dry weight basis. Whole-body mineral analysis, which have been carried out to determine systemic mineral retention and to evaluate mineral bioavailability across different tissues, were performed in accordance with the methodological principles proposed by Prabhu et al. (2018).

Statistical Analysis

The data are expressed as means±standard error and were examined using the SPSS software (Version 25.0; IBM, Chicago, IL, USA). Tukey's test was used for pairwise comparisons following ANOVA when variances were homogeneous; otherwise, Tamhane's T2 test was applied. The normality of the data was tested using the Shapiro–Wilk test, and the homogeneity of variances using the Levene's test. In cases where the data did not meet the normality assumption, the Kruskal-Wallis's test was used to analyze group differences.

Results

Dietary organic mineral mixture (OMM) significantly influenced growth performance and feed efficiency in rainbow trout fry over the 60-day trial ($P<0.05$) (Table 2). According to the results, the OMM-50 group exhibited the highest final body weight (FW, 13.55±0.03 g) and specific growth rate (SGR, 3.99±0.02 %/day), alongside the lowest feed conversion ratio (FCR, 1.02±0.01) ($P<0.05$), indicating superior growth efficiency compared to the control (OMM-0: 11.17±0.06 g; 3.66±0.00 %/day; FCR=1.25±0.02) and OMM-100 (11.19±0.06 g; 3.49±0.18 %/day; FCR=1.29±0.01) groups, which showed similar performance. The OMM-75 (12.44±0.05 g; 3.85±0.00 %/day) group displayed moderate growth metrics, outperforming OMM-0 and OMM-100 but falling short of OMM-50 ($P<0.05$). Feed intake (FI) was lowest in the OMM-50 (2.83±0.02 %/day) and OMM-75 groups ($P<0.05$), suggesting an enhanced nutrient utilization, while OMM-0 (3.34±0.05%/day) and OMM-100 (3.37±0.03%/day) groups consumed more feed without proportional growth benefits. Survival rates were the highest in the OMM-50 (95.71±0.00%) group, followed by OMM-0 (92.86±0.83%) and OMM-75 (90.95±0.48%), with the OMM-100 (84.29±0.82%) group showing significantly lower survival ($P<0.05$).

Hepatosomatic (HSI=1.38–1.42%) and viscerosomatic (VSI=16.7–17.1%) indices remained unaffected across all treatments (P>0.05), indicating no significant impact on organ morphology.

Chemical Composition of Experimental Fish

Chemical composition (moisture=73.2–73.8%; protein=17.5–17.8%; lipid=5.4–5.7%; ash=3.06–3.13%) of fish whole-bodies did not differ significantly among treatment groups (P>0.05) (Table 3), suggesting that OMM substitution had no effect on nutrient storage or body composition over the trial duration.

Digestive Enzyme Activities of Fish

Dietary OMM significantly enhanced trypsin enzyme activity (P<0.001), with the OMM-50 group showing the highest levels (2.46±0.10 U/mg protein) compared to OMM-0 (1.49±0.09), OMM-75 (1.82±0.11), and OMM-100 (1.48±0.06) (P<0.001; Table 4). No significant differences were observed in pepsin (48.1–54.1 U/mg protein), lipase (0.22–0.26 U/mg protein), amylase (0.52–0.63 U/mg protein), or alkaline phosphatase (0.44–0.47 U/mg protein) (P>0.05), activities indicating that OMM primarily influences the protein digestion.

Antioxidant Enzyme Activities and Lipid Peroxidation Levels of Fish

Moderate OMM supplementation (0.5%–0.75%) significantly increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities (P<0.05) (Table 5), with OMM-50 showing the highest values (SOD=8.79±1.9; GPx=0.32±0.11 U/mg protein), followed by OMM-75 (7.67±1.3; 0.17±0.06). The OMM-0 (4.20±1.9; 0.10±0.00) and OMM-100 (3.95±1.4; 0.11±0.06) groups exhibited lower SOD and GPx activities (P<0.05). Catalase (CAT) activity (76–97 U/mg protein) remained unaffected across treatments (P>0.05). Lipid peroxidation, measured as malondialdehyde (MDA) levels, was lowest in the OMM-50 (1.60±0.87 nmol/mg protein), followed by OMM-75 (2.83±0.76), while OMM-0 (3.90±0.94) and OMM-100 (3.91±0.84) showed higher MDA levels (P<0.05), indicating greater oxidative damage.

Whole-body Mineral Concentrations

Whole-body concentrations of zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), selenium (Se), and cobalt (Co) were significantly influenced by OMM levels (Table 6). The OMM-50 group exhibited the highest mineral accumulation (Zn=59.35±0.86; Cu=6.69±0.10;

Table 2. Effects of OMM-supplemented diets on growth performance, biometric indices, and survival rate of rainbow trout fry

	OMM-0	OMM-50	OMM-75	OMM-100	P-value
FW	11.17±0.06 ^{c}	13.55±0.03 ^{a}	12.44±0.05 ^{b}	11.19±0.06 ^{c}	<0.01
FI	3.34±0.05 ^{a}	2.83±0.02 ^{b}	2.78±0.01 ^{b}	3.37±0.03 ^{a}	<0.01
SGR	3.66±0.00 ^{ab}	3.99±0.02 ^{a}	3.85±0.00 ^{ab}	3.49±0.18 ^{b}	0.02
FCR	1.25±0.02 ^{c}	1.02±0.01 ^{a}	1.12±0.01 ^{b}	1.29±0.01^{c}	<0.01
SUR	92.86±0.83 ^{b}	95.71±0.00 ^{a}	90.95±0.48 ^{b}	84.29±0.82 ^{c}	0.01
HSI	1.40±0.03 ^{a}	1.41±0.02 ^{a}	1.42±0.03 ^{a}	1.38±0.03 ^{a}	0.85
VSI	17.05±0.17 ^{a}	16.91±0.17 ^{a}	17.07±0.19 ^{a}	16.74±0.23 ^{a}	0.62

Values represent means ± standard error (n=15). Different superscripts within a row indicate significant differences (Tukey's test, P<0.05). FW: final body weight (g), FI: feed intake (%/day), SGR: specific growth rate (%/day), FCR: feed conversion ratio, SUR: survival rate (%), HSI: hepatosomatic index (%), VSI: viscerosomatic index (%).

Table 3. Whole-body chemical composition of fish fed experimental diets

	OMM-0	OMM-50	OMM-75	OMM-100	P-value
Moisture (%)	73.2±0.22	73.5±0.09	73.4±0.43	73.8±0.43	0.65
Protein (%)	17.7±0.18	17.5±0.27	17.8±0.31	17.6±0.10	0.72
Ash (%)	3.07±0.10	3.13±0.08	3.06±0.05	3.09±0.04	0.89
Lipid (%)	5.60±0.05	5.40±0.11	5.68±0.14	5.43±0.14	0.33

The values represent the means and standard error (n=15); the absence of superscripts within a row indicates statistical insignificance (P>0.05).

Table 4. Digestive enzyme activities of fish after 60 days of feeding.

	OMM-0	OMM-50	OMM-75	OMM-100	P-value
Pepsin	48.1±3.12	51.7±2.54	54.1±2.58	48.7±2.20	0.354
Trypsin	1.49±0.09 ^{b}	2.46±0.10 ^{a}	1.82±0.11 ^{b}	1.48±0.06 ^{b}	<.001
Lipase	0.23±0.02	0.26±0.02	0.23±0.01	0.22±0.01	0.331
Amylase	0.54±0.04	0.53±0.04	0.52±0.05	0.63±0.06	0.419
Alkaline phosphatase	0.47±0.06	0.46±0.07	0.47±0.08	0.44±0.09	0.993

The values represent the means and standard error (n=15); different superscripts within a row indicate statistically significant differences (P<0.05).

Mn=3.49±0.06; Fe=119.80±1.51; Se=1.07±0.02; Co=0.06±0.00 μg g^{-1}), followed by OMM-75 (Zn=56.51±0.80; Cu=5.97±0.07; Mn=3.24±0.06; Fe=107.35±1.32; Se=0.97±0.02; Co=0.05±0.00). Both OMM-0 (Zn=36.03±0.65; Fe=62.19±0.88) and OMM-100 (Zn=39.08±0.59; Fe=70.76±1.22) showed lower mineral retention (P<0.001).

Discussion

In the present study, effect of partially replacing the inorganic mineral premix with an organic mineral mixture (OMM) on growth, digestive enzyme activities, antioxidant defense and mineral retention of rainbow trout fry has been investigated. The parameters have been evaluated together to understand overall metabolic response of the fish. According to the obtained results, 0.5% OMM inclusion has excelled the other treatments and provided the most balanced performance in specific growth rate (SGR), feed conversion ratio (FCR), enzymatic activity and tissue mineral retention, while the 1.0% supplementation has caused a decline in beneficial effects and lowered feed utilization efficiency (P<0.05). Similar findings have been reported in different aquatic species. For instance, the organic trace mineral (OTM) supplementation around 0.075% in turbot (Yang et al., 2020) and 1.0% in freshwater prawn for two different commercial mixtures has been considered optimum (Ramasamy et al., 2021); however, performance declined when those inclusion levels were exceeded. Therefore, it is clear that the complex mineral sources are effective only within a narrow range, as evident here. According to the previous works, positive impact of the organic mineral complexes mainly arises from higher bioavailability and stability, which enable less interaction with antagonistic substances such as phytic acid and better solubility across various pH ranges. It has been demonstrated that chelated minerals and organic acidifiers increase apparent mineral retention in trout by reducing inhibitory effect of phytic acid. Moreover, the organic selenium (Se-yeast) has shown higher digestibility and tissue accumulation compared with inorganic sodium selenite. Likewise, amino acid–chelated forms of Zn, Mn and Cu have been reported to enhance oxidative metabolism and antioxidant enzyme activities even at low dietary levels; however, excessive inclusion has been found detrimental and resulted in performance decline. Comparable results were also obtained in European perch and Atlantic salmon larvae, where organic mineral supplementation improved growth, and in rainbow trout larvae, where organic zinc increased mineral deposition. Consequently, improved growth, better FCR and higher mineral retention determined at 0.5% OMM inclusion have given similar results to those reported in the literature. It has been understood that moderate OMM supplementation provides optimum balance between bioavailability and metabolic efficiency; as a result, inclusion of approximately 0.5% OMM in partial replacement of inorganic premix has been found necessary for sustainable growth and mineral utilization in rainbow trout fry.

Organic zinc boosted growth in rainbow trout larvae (Shahpar & Johari, 2019), while mineral complexes enhanced performance in European sea bass and Atlantic salmon (Henry et al., 2020; Kokkali et al., 2023). Organic minerals' higher bioavailability and stability, due to reduced interference from dietary antagonists like phytate and better solubility across pH gradients, drive these benefits (Byrne et al., 2021; Cantwell et al., 2017). Because phytate interference can hinder mineral absorption, Hernández et al. (2012) demonstrated that chelated minerals together with acidifiers improved mineral retention in trout by mitigating phytate interference; therefore, the protective effect was clearly linked to enhanced bioavailability. Supporting this interpretation, element-specific evidence has confirmed that organic selenium

Table 5. Antioxidant enzyme activities of fish after 60 days of feeding

	OMM-0	OMM-50	OMM-75	OMM-100	P-value
SOD	4.20±1.9 ^{b}	8.79±1.9 ^{a}	7.67±1.3 ^{ab}	3.95±1.4 ^{b}	0.02
CAT	97.3±11.9	76.4±14.3	90.4±2.8	86.8±1.4	0.08
GPx	0.10±0.00 ^{b}	0.32±0.11 ^{a}	0.17±0.06 ^{ab}	0.11±0.06 ^{b}	0.01
MDA	3.90±0.94 ^{a}	1.60±0.87 ^{c}	2.83±0.76 ^{b}	3.91±0.84 ^{a}	0.00

Units: SOD, CAT, and GPx activities are expressed as U/mg protein. MDA levels are expressed as nmol/mg protein. The values represent the means and standard error (n=15); different superscripts within a row indicate statistically significant differences (P<0.05).

Table 6. Whole-body mineral concentrations (μg/g dry weight) of rainbow trout fry after 60 days of feeding

Mineral	OMM-0	OMM-50	OMM-75	OMM-100	P-value
Zn	36.03±0.65^{d}	59.35±0.86^{a}	56.51±0.80^{b}	39.08±0.59^{c}	<.001
Cu	3.31±0.07^{d}	6.69±0.10^{a}	5.97±0.07^{b}	3.83±0.07^{c}	<.001
Mn	1.55±0.04^{d}	3.49±0.06^{a}	3.24±0.06^{b}	1.85±0.07^{c}	<.001
Fe	62.19±0.88^{d}	119.80±1.51^{a}	107.35±1.32^{b}	70.76±1.22^{c}	<.001
Se	0.43±0.02^{d}	1.07±0.02^{a}	0.97±0.02^{b}	0.54±0.02^{c}	<.001
Co	0.03±0.00^{d}	0.06±0.00^{a}	0.05±0.00^{b}	0.04±0.00^{c}	<.001

Values represent means±standard error (n=15). Different superscripts within a row indicate significant differences (Tukey's test, P<0.001).

(Se-yeast) provided higher digestibility and whole-body Se deposition compared to inorganic selenite, while zinc retention varied among tissues depending on the mineral form (tissue-specific differences) (Rider et al., 2010). Likewise, amino acid–chelated Zn, Mn, and Cu have been reported to elevate tissue retention and upregulate oxidative metabolism markers even when dietary inclusion was reduced (Apines-Amar et al., 2004). Because low-fishmeal formulations often limit mineral utilization, chelated minerals have maintained growth and enhanced phosphorus efficiency in trout diets, and, consequently, have linked organic supplementation with both performance and sustainability outcomes (Hernández et al., 2012). In contrast, in early-weaned seabream, organic Se/Zn/Mn sources excelled over inorganic and nanoparticulate forms across multiple functional indices (Izquierdo et al., 2017).

Higher growth values were attained in the OMM-50 and OMM-75 groups despite lower feed consumption, suggesting that the nutrients were better utilized due to more effective digestive system function (Hoseinifar et al., 2020). However, the decline in performance at OMM levels above the optimal level is in line with findings from earlier research on shrimp and turbot (Yang et al., 2020; Ramasamy et al., 2021). Even though the current study did not measure the amounts of mineral carriers, the low yield at the highest OMM level suggests osmotic imbalances linked to excessive mineral accumulation (Lee et al., 2016) as well as saturation or competition mechanisms (Lall, 2022; Pan et al., 2008). Likewise, Welker et al. (2018) found that the rainbow trout's physiological performance decreased when mineral levels were higher than ideal; therefore, these results unequivocally show how important it is to carefully optimize the right ratio when adding minerals, and the balance has been found necessary for creation of stable physiological conditions.

The elevated trypsin activity found in the OMM-50 group raises the possibility that moderate OMM supplementation regulates protein metabolism, because trypsin (Jesús-De la Cruz et al., 2020), one of the major serine protease enzymes that cleaves peptide bonds and releases amino acids, needs trace mineral cofactors in order to maintain its catalytic function. The enhanced bioavailability of zinc (Zn) and selenium (Se), which stabilize the enzyme conformation and preserve redox balance, may be cause of this increase in activity (Rungruangsak-Torrissen, 2012; Halliwell & Gutteridge, 2007), while this effect is specific to protease regulation rather than a general digestive adaptation, as evidenced by the lack of change in other digestive enzymes like pepsin, lipase, amylase, and alkaline phosphatase (Lazarević & Janković-Tomanić, 2015). The resultant enhancement of proteolysis likely contributed to the superior growth and feed conversion efficiency observed at 0.5% OMM. By improving amino acid bioavailability, the elevated proteolytic activity is believed to support anabolic processes and, therefore, lead to improved growth and feed conversion efficiency at 0.5% OMM levels (Moyano et al., 1996); moreover, subsequent transcriptomic research could provide more insight into the functions of minerals in the production of trypsin and the control of digestive enzymes.

In tandem with increases in glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, it has been noted that moderate OMM replacement therapy significantly boosts antioxidant enzyme activities, and malondialdehyde (MDA) levels in the OMM-50 and OMM-75 groups decreased concurrently with this increase. This result is consistent with findings from juvenile turbot fed a mixture of organic trace minerals, where the study found that the best organic mineral supplementation, at about 0.075%, reduced MDA levels to the lowest while offering the highest total antioxidant capacity and catalase activity (Yang et al., 2020). This pattern aligns with trout studies showing that selenium form influences digestibility and antioxidant enzyme activity (Rider et al., 2010). These findings corroborate research on trout that indicates the type of selenium has a major impact on MDA levels, digestibility, and catalase activity (Rider et al., 2010); however, the addition of chelated Cu, Zn, and Mn (Mintrex™) to Litopenaeus vannamei boosted growth, tissue mineral accumulation, and SOD activity in comparison to inorganic premixes, according to a study by Katya et al. (2016), and shrimp have shown a similar reaction. All things considered, these results suggest that the chelated trace minerals offer considerable benefit in preserving physiological equilibrium by activating antioxidant enzyme systems; nevertheless, overuse may impair function, and a dose-dependent antioxidant regulation mechanism that is believed to be conserved in aquatic organisms has been revealed by this study. According to the research, consuming enough organic minerals without going overboard improves redox protection and supports element-specific roles of zinc and selenium in SOD and GPx activities (Rider et al., 2010); therefore, under moderate reactive oxygen species (ROS) production, GPx functions as primary detoxification enzyme, as evidenced by constant catalase (CAT) activity across groups. The OMM-0 and OMM-100 groups, on the other hand, showed biochemical patterns suggestive of oxidative stress brought on by mineral excess or deficiency (Lee et al., 2016; Lall, 2022), while the OMM-50 group's concurrent rise in antioxidant enzyme and proteolytic activity suggests that metabolic system responds in a very well-coordinated manner; consequently, this circumstance indicates that in order to sustain cellular functions, stronger redox homeostasis is necessary due to elevated metabolic rate (Martínez-Álvarez et al., 2005).

The results of whole-body mineral composition study unequivocally show that bioavailability is increased by moderate OMM supplementation, and high concentrations of zinc, copper, manganese, iron, selenium, and cobalt in the OMM-50 group suggest efficient mineral absorption that is in line with chelate

stability of mineral proteinates (Byrne et al., 2021). Conversely, even though the OMM-100 group's supplementation rate was higher, their retention efficiency decreased, which suggests that homeostatic excretion or storage mechanisms controlling mineral homeostasis have been triggered; moreover, the downregulation of mineral transporters or binding to metallothioneins may be cause of this circumstance (Prabhu et al., 2020). This reversed retention model points to toxicity-like effects brought on by excessive mineral accumulation or osmotic imbalance when compared to lower survival rate (84.29%), and to verify this theory, histopathological analysis is required (Lee et al., 2016). According to these dose-response relationships, mineral levels need to be carefully managed to prevent saturation effects that might reduce biological efficacy (Meiler & Kumar, 2021).

Increased proteolytic activity, balanced redox homeostasis, tissue mineral accumulation, and physiological regulation were all noted in the OMM-50 group, and it is believed that mutual effects of trace elements, such as synergistic interactions between Se and Zn, are responsible for this coordinated response. Because of increased production of reactive oxygen species (ROS) and accelerated turnover of proteins, increased trypsin activity may have necessitated more robust antioxidant defense; therefore, by boosting enzymatic process efficiency and maintaining structural integrity, optimal mineral assimilation offers dual benefit. These findings suggest that mineral proteinates can be employed as efficient regulating ingredients in precisely formulated aquaculture feeds. Current research suggests that a 0.5% OMM level offers greatest physiological benefit in *O. mykiss* juveniles, preserving systemic homeostasis, even though single "ideal" supplementation level has not yet been precisely identified.

By lowering mineral excretion through feces, increased mineral absorption may lower the risk of eutrophication from aquaculture (Gatlin & Wilson, 1986; El-Sayed et al., 2023). One of the primary limitations of the current study is that this effect needs to be experimentally confirmed in terms of mineral excretion (da Silva Pierri et al., 2021). Future studies should use an integrated approach to address life cycle analysis, mineral flow, and digestive efficiency in order to increase the sustainability of OMM-based feed formulations.

Conclusions

According to the study's findings, rainbow trout (*Oncorhynchus mykiss*) fry grew, metabolized, and retained micronutrients best when the inorganic mineral mixture was replaced by an organic equivalent at a rate of 0.5%. On the other hand, the over-supplementation (1.0%) that has resulted in negative physiological reactions has shown how crucial sensitivity is to preserving micronutrient balance. It has been advised that future factorial experimental designs investigate effects and interactions unique to different types of minerals. Moreover, a more thorough comprehension of mineral synergies and their biological impacts may result from combined assessment of comprehensive systems-level, transcriptomic, and histomorphological analyses. These all-encompassing methods will serve as crucial foundation for creation of aquaculture feeds that are species-specific, nutritionally optimized, and environmentally sustainable.

Ethical Statement

All procedures involving experimental animals were conducted in accordance with the guidelines and regulations of the Republic of Türkiye and were approved by the Animal Ethics Committee of Çanakkale Onsekiz Mart University under the approval number 2021/01-07.

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Author Contribution

T.S.: Conceptualization, Methodology, Resources, Formal Analysis, Investigation, Data Curation, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Supervision. S.E.G.: Investigation, Validation, Writing – Review & Editing. M.G.: Validation, Writing – Review & Editing. S.E.: Conceptualization, Methodology, Resources, Data Curation, Validation, Writing – Review & Editing, Supervision. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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