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Effect of Adding Dietary Organic Trace Minerals (Zn, Cr, And Mn) and Their Mixtures on Physiological Traits Laying Hens

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ABSTRACT

This study was conducted to investigate the effect of adding dietary organic trace minerals (zinc, chromium, and manganese) and their mixtures on the levels of zinc, manganese, chromium, calcium, and phosphorus on some physiological traits. The experiment included the following treatments: Treatment (T1) served as control group without any addition. Treatment (T2) involved the addition of zinc (40 mg/kg diet). treatment (T3) included the addition of chromium (0.4 mg/kg diet). treatment (T4) consisted of the addition of manganese at a concentration of 40 mg/kg diet. Treatment (T5) involved a combination of zinc (40 mg), manganese (40 mg), and chromium (0.4 mg) per kg diet. The results demonstrated significant improvements in most of the studied traits. In terms of blood serum, there was a significant ($p < 0.05$) increase in the levels of zinc, manganese, and calcium in all treatments containing mineral additives and their mixture compared to control group, which showed a noticeable decline. However, non. significant differences were observed in the serum levels of chromium and phosphorus across the treatments. Regarding bone mineral levels, there was a significant increase ($p < 0.05$) in zinc, chromium, manganese, phosphorus, and calcium in all supplemented treatments compared to the control group. These findings suggest that the addition of organic trace minerals, either individually or as a mixture, enhances the physiological levels of these minerals in both blood serum and bones, thereby improving mineral bioavailability.

Key word: blood, bones,layer chickens, Lohmann, minerals.

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INTRODUCTION

Poultry meat production accounts for approximately 36% of global meat production, with chicken meat representing about 89% of total poultry production (Fakharzadeh, et al. 2020). In 2020, global poultry meat production exceeded 136.5 million tons, marking a 2.4% increase compared to 2019 (Duncan's, 1955, Elgeddawy, et al. 2020). To ensure proper nutrition and achieve targeted goals, scientific studies in animal and poultry nutrition reveal that producers increasingly focus on developing and using feed supplements. These are natural or synthetic substances added to feed to enhance its nutritional value, thereby improving animal performance, particularly in terms of growth (Amata, 2013). These supplements include nutrients such as minerals (Haq, et al. 2016), amino acids (FAO. 2020.), vitamins (Ahmad, et al. 2019), fatty acids, enzymes, herbal extracts, antioxidants, and others (Chen, et al. 2020). They are common components of poultry diets, either individually or as mixtures (Gálvez, et al. 2020). Among the essential trace minerals, zinc plays a pivotal role in growth, development, and immune enhancement in poultry (Horváth, and Babinszky, 2018.). Zinc is involved in the repair of damaged tissues, contributing to faster recovery from diseases (Kong, et al. 2022). Similarly, manganese is a key mineral that participates in various vital processes in poultry (Khatun, et al. 2019). Manganese plays a vital role and works

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synergistically with calcium and phosphorus to support skeletal development (Azad et al., 2017). Furthermore, this trace mineral enhances meat quality, assists in blood glucose regulation, and helps maintain optimal cholesterol levels (Burezq, 2021).Another indispensable trace element is chromium. Despite being required in minuscule quantities, it significantly accelerates growth rates by improving amino acid absorption (Alagawany et al., 2021). Chromium also participates in glucose metabolism to help stabilize blood sugar levels in poultry (Idowu et al., 2011) while mitigating the negative impacts of environmental stress (Ford, 2009). the aim of this study to balance avian health with environmental sustainability specifically by minimizing mineral excretion in poultry manure—this investigation evaluates how dietary supplementation of zinc, chromium, manganese, and their combination influences blood serum and bone mineral profiles in Lohmann layer chickens.

MATERIALS AND METHODS

Study Methodology: This 16-week study was conducted at the Poultry Research Station, Department of Animal Wealth, Agricultural Research Directorate under the Ministry of Agriculture in Abu Ghraib, Iraq, spanning from 12th , 2022, to 1st , 2022.. The trial comprised four production cycles, starting when the birds reached 20 weeks of age.

Experimental Design and Housing

Animals: A total of 100 Lohmann Brown laying hens were randomly allocated into 15 cages, establishing replicates of five chickens per cage.

Enclosure Specifications: Each cage measured 60 cm × 60 cm and was fitted with three overhead nipple drinkers to provide continuous water. Feed was manually distributed using longitudinal feeders designed specifically for cage systems.

Environmental Controls: Photoperiod: A automated timer maintained a strict lighting schedule of 16 hours of light and 8 hours of darkness daily.

Climate: During winter, electric heaters maintained target temperatures, while ventilation and temperature control were managed via exhaust fans positioned opposite evaporative cooling windows.

Serum Analysis: Serum mineral concentrations were measured using commercial mineral-specific kits. All analytical procedures strictly adhered to the protocols and instructions provided by the respective kit manufacturers.

Sample Collection and Laboratory Analysis

At 35 weeks of age, blood samples were gathered from the brachial vein of all birds across the treatment groups. Approximately 5 mL of blood was collected per bird using a syringe and transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to preserve the samples. To prepare the samples for biochemical analysis, the blood was centrifuged (Gemmy Industrial Corp, Taiwan) at 3000 rpm for 15 minutes. The separated plasma or serum was immediately collected and stored at -20°C for subsequent biochemical testing. Additionally, tibia bone ash was prepared and analyzed to quantify bone mineral concentrations, following established protocols described by Lilburn and McIntyre (2024) and Malik et al. (2011).

Statistical Analysis: Data were analyzed using the Statistical Analysis System (SAS) to evaluate the impact of dietary treatments on the measured physiological and biochemical parameters (Khatun et al., 2019).

Experimental Design: A Completely Randomized Design (CRD) was employed for the study.

Mean Comparison: Treatment means were compared and separated using Duncan's multiple range test (Belloir et al., 2019), with statistical significance assessed against the specified thresholds.

Table 1. composition and calculated chemical analysis of the production diet for Lohmann brown layers (105 g/bird/day)

Material	Mount for 100kg
Corn	55
Wheat	7.85
Soybean	24.6
Apply sunflower oil	0.5
Calcium diphosphate	0.4
Limestone	8.9
Salt	0.25
Premix	2.5
chemical composition	
C.P	17.59
M.E	2732
Lysin	0.87
Meth	0.46
M+C	0.73
Phosphate	3.91

RESULTS AND DISCUSSION

Physiological Traits

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Serum Concentrations of Minerals: Table (2) revealed the effect of adding different levels of organic zinc (Zn), chromium (Cr), manganese (Mn), and their mixture (ZMC) to the diet of layer chickens on the concentrations of calcium, zinc, manganese, and phosphorus in blood serum. The results revealed a significant increase ($p < 0.05$) in mineral levels of the supplemented groups with organic minerals compared to the control group (T1). For calcium, zinc, and manganese, the mixture treatment (T5) showed the highest significant levels at 0.023, 0.168, and 0.98 (mg/dl), respectively (Table 1). Other supplemented groups (T2, T3, T4) exhibited slight variations in the mineral concentrations, while the control group (T1) showed significantly lower concentration of calcium, chromium and phosphorus among the treatments (Table 2). These findings highlight the positive effects of organic trace mineral supplementation, particularly in the (0.66) zin, and manganese (0.086). However, the serum complex (ZMC) was observed to improve serum mineral concentrations, which may contribute to improved physiological performance in laying hen

Table 2. Effect of adding different levels of dietary organic zinc, chromium, manganese, and their mixture to the diet on blood serum mineral concentrations in layer chickens (mean ± s e)

	Organic minerals(mg/dl)				
treatment	**Ca**	**Zn**	**Cr mg/dl**	**Mn**	**P**
T1	0.016 ± 0.0044[c]	0.66 ± 0.011[c]	0.15 ± 0.006	0.086 ± 0.0008[c]	4.16 ± 0.024
T2	0.019 ± 0.0037[b]	0.82 ± 0.005[b]	0.18 ± 0.005	0.094 ± 0.0009[c]	4.16 ± 0.008
T3	0.018 ± 0.0049[b]	0.71 ± 0.008[c]	0.24 ± 0.005	0.091 ± 0.0004[c]	4.20 ± 0.008
T4	0.020 ± 0.0081[b]	0.73 ± 0.006[c]	0.16 ± 0.003	0.120 ± 0.0014[b]	4.19 ± 0.024
T5	0.023 ± 0.0051[a]	0.98 ± 0.003[a]	0.29 ± 0.005	0.168 ± 0.0020[a]	4.17 ± 0.005
Significance Level	<0.05	<0.05	NS	<0.05	NS

***Different superscript letters within the same column indicate significant differences (*p* < 0.05) among the treatment. Means**
NS no significant differences.
T1 (control, no mineral supplementation), T2 (addition of 40 mg zinc/kg of feed), T3 (addition of 0.4 mg chromium/kg of feed), T4 (addition of 40 mg manganese/kg of feed), and T5 (a mixture of 40 mg zinc, 0.4 mg chromium, and 40 mg manganese/kg of feed).

Minerals Content in Bone

Table (3) demonstrates the effect of adding different levels of organic zinc (Zn), chromium (Cr), manganese (Mn), and their mixture (ZMC) of layer hens on the concentrations of calcium, zinc, chromium, manganese, and phosphorus in bones. The results show a significant increase ($p < 0.05$) in the mineral levels for the supplemented group receiving the mineral mixture (T5), which recorded the highest values of 20.143, 1.626, 23.140, 2.350, and 2.166 (mg/dl) for calcium, zinc, manganese, chromium, and phosphorus, respectively. This highlights the enhanced effect of mineral mixtures on bone mineralization. While treatments T2, T3, and T4 also showed slight increases in mineral levels, the control group (T1) exhibited notably lower values of 19.076, 1.350, 22.093, 0.943, and 1.043 (mg/dl) for calcium, zinc, manganese, chromium, and phosphorus, respectively (Table 3). The improvements observed can be attributed to the high bioavailability of organic minerals. The supplementation of zinc, manganese, chromium, and their combination in the diet positively impacted bone structure, emphasizing the importance of these minerals in enhancing skeletal health in layer chickens.

Table 3. Effect of adding different levels of dietary organic zinc, chromium, manganese, and their mixture to the diet on bone mineral content in layer chickens (mean ± s e)

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treatment	Ca(%)		Zn(%)		Cr(%)		Mn(%)		P(%)	
T1	22.093	± 0.029[c]	1.043	± 0.02333[c]	1.350	± 0.0152[c]	0.943	± 0.023[c]	19.076	± 0.017[c]
T2	22.336	± 0.021[b]	1.460	± 0.01528[b]	1.496	± 0.0120[b]	1.130	± 0.011[b]	19.240	± 0.020[b]
T3	22.920	± 0.011[b]	2.027	± 0.01453[b]	2.140	± 0.0057[b]	1.500	± 0.011[b]	19.890	± 0.015[b]
T4	22.683	± 0.023[b]	1.840	± 0.02082[b]	1.923	± 0.0145[b]	1.260	± 0.011[b]	19.593	± 0.035[b]
T5	23.140	± 0.005[a]	2.166	± 0.00882[a]	2.350	± 0.0152[a]	1.626	± 0.014[a]	20.143	± 0.018[a]
Significance Level	<0.05		<0.05		<0.05		<0.05		<0.05	

Different superscript letters within the same column indicate significant differences (*p* < 0.05) between the treatment means
NS: no significant differences.
included T1 (control, no mineral supplementation), T2 (addition of 40 mg zinc/kg of feed), T3 (addition of 0.4 mg chromium/kg of feed), T4 (addition of 40 mg manganese/kg of feed), and T5 (a mixture of 40 mg zinc, 0.4 mg chromium, and 40 mg manganese/kg of feed).

Results of physiological measurements in blood serum studied in this experiment indicate that the physiological conditions of the birds, especially during the different production phases, were not adversely affected in most treatments. The addition of minerals and their mixtures to the diets contributed to stabilizing and optimizing the birds' condition under production circumstances and various stress factors encountered during rearing stages. The addition of zinc, higher mineral deposition in bones and liver. This effect might be attributed to zinc's role in stimulating the secretion of reproductive hormones, such as estrogen, which promotes oviduct growth and increases blood calcium concentrations. manganese, iron, and copper to the diet did not negatively impact bird growth. Furthermore, organic mineral supplements not only enhanced broiler productivity but also had a positive effect on bone structure and blood parameters. Our findings with those of (Amata, 2013), who reported that diets supplemented with organic zinc (Zn-methionine) led to the elevated blood zinc levels, which are associated with increased absorption in the digestive system. Similarly, Abdallah, et al. (2009) and Sheoran, (2017) observed that birds fed a diet supplemented with organic zinc and manganese exhibited These results are consistent with the findings of (Lilburn, & McIntyre, 2024, Sirri, et al. 2016), who noted a rise in calcium and phosphorus levels in bones due to zinc supplementation. Although minor differences in supplementation rates exist, (Sunder, et al. 2008) highlighted that organic zinc (50 mg Zn-Gly) improved bone quality and mineral content, specifically calcium and phosphorus. In contrast, (Ghoreyshi, et al. 2019, Xu, et al. 2006) found non. significant effect of organic zinc on calcium and phosphorus in bones, indicating variability in responses to supplementation. (Karam, et al. 2007, Turek, et al. 2019) further reported that rare organic trace minerals are stored at higher concentrations in tissues and organs, such as blood, liver, and bones. (Al-Hassani, 2022) stated that blood calcium levels were not significantly affected by chromium supplementation, though phosphorus levels increased due to chromium addition. They suggested that higher chromium levels enhance phosphorus concentrations in the blood without impacting calcium levels. Phosphorus (P) is an essential mineral for poultry, vital for physiological functions and skeletal development. It also plays a critical role in carbohydrates, protein, and fat metabolism. Additionally, the acidic environment and digestive functions are necessary for calcium and phosphorus breakdown to enhance their absorption (Niknia, et al. 2023, Yenice, et al. 2015).

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The authors declare that they have not received a fund.

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AUTHOR/S DECLARATION:

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

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تأثير اضافة المعادن العضوية النادرة (زنك، كروم، منغنيز) ومخاليطها الى العليقة في الصفات الفسلجية للدجاج البياض

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المستخلص

أجـــريت هذه الدراســـة بهدف بيان "تأثير اضافة المعادن العضوية النادرة (الزنك والكروم والمنغنيز) ومخالطيها الى العليقة في قياس نسبة المعادن الزنك والمنغنيز والكروم والكالسيوم والفسفور بمصل الدم وفي العظام لدى دجاج البياض اذ شـــملت معاملات التجربة الآتي: المعاملة (T1) : معاملة السيطرة بدون اضافة .المعاملة (T2) : اضافة الزنك بمقدار 40 ملغم / كغم علف، المعاملة (T3) : اضافة الكروم بمقدار 0.4 ملغم /كغم علف ، المعاملة (T4) : اضافة المنغنيز بمقدار 40 ملغم / كغم علف والمعاملة (T5) : تقديم علف مضاف له خليط الزنك والكروم والمنغنيز بمقدار40 ملغم زنك + 40 ملغم منغنيز +0.4 ملغم كروم/ كغم علف. اظهرت الدراسة وجود تفوق في اغلب الصفات المدروسة ففي قياس مستويات الزنك والمنغنيز والكالسيوم بمصل الدم حصل ارتفاع ملحوظ معنويا ($p < 0.05$) لمعاملات المضافة لها المعادن وخليطها مع انخفاض ملحوظ في معاملة السيطرة الخالية من الاضافة السيطرة ولم تسجل النتائج وجود فروق معنوية بالنسبة الى الكروم والفسفور بالمصل الدم. اما فيما يخص مستويات الزنك والكروم والمنغنيز والفسفور والكالسيوم بالعظام لوحظ ارتفاع معنوي ($p < 0.05$) لجميع معاملات الاضافة مقارنة بالسيطرة. هذه النتائج بينت ان اضافة المعادن العضوية النادرة سواء بشكل مفرد او خليط قد ادت الى الحث على زيادة مستوياتها في كل من مصل الدم والعظام مما يؤدي الى توافرها الحيوي.

الكلمات المفتاحية: الدم، العظام، دجاج بياض، اللوهمان، المعادن.