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The effect of inorganic and organic mineral premix in broiler diets on growth performance, and fecal mineral excretion

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육계 사료내 무기태 또는 유기태 미네랄 프리믹스 첨가가 생산성 및 분변내 미네랄 배설에 미치는 영향

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Abstract This study examined the effect of diets supplemented with organic and inorganic mineral premixes on growth performance and mineral excretion. Seven hundred and fifty one-day-old ROSS 308 (initial BW= 42.32±0.32 g) were assigned randomly to one of three dietary treatments, repeated five times: mash-type basal diet supplemented with an inorganic mineral premix (1.0 g/kg) and a basal diet supplemented with an organic mineral premix (1.8 g/kg). In the starter period (0 to 14 days), grower period (15 to 28 days), and overall periods (0 to 28 day), no significant differences were observed between any of the performance parameters measured. On the other hand, the mortality rate was lower ($p < 0.05$) in the inorganic and organic mineral premix groups than in the basal diet group. The concentrations of iron, copper, zinc, manganese, and magnesium in the fecal were different ($p < 0.05$) among the treatment groups, with the highest values reported in the inorganic mineral premix group. The concentrations of iron, copper, zinc, manganese, and magnesium in the spleen and liver were different ($p < 0.05$) among the treatment groups, with the highest values reported in the organic mineral premix group. In conclusion, replacing inorganic mineral premix with organic mineral premix does not adversely affect the productivity of broilers and can reduce the concentration of minerals in feces that cause environmental pollution.

요 약 본 연구는 육계사료에 무기태 또는 유기태 형태의 미네랄 프리믹스 첨가가 생산성 및 분변내 미네랄 배설에 미치는 영향을 구명하고자 수행하였다. 총 750수의 1일령 ROSS 308 (Initial BW = 42.32±0.32g)을 공시하여 3처리 5반복으로 반복당 50수씩 완전 임의 배치하여 자유 섭식케 하였으며 28일간 전기(0~14일), 후기(15~28일)로 나누어 실시하였다. 처리구들은 대조구(프리믹스 무첨가), 무기태 미네랄(대조구 +무기태 미네랄 프리믹스 1.0 g/kg), 유기태 미네랄(대조구 + 유기태 미네랄 프리믹스 1.8 g/kg) 총 3처리구 였다. 전기(0~14일) 및 후기(15~28일) 및 전기간(0~28일) 생산성에는 처리구간에 유의적 차이는 나타나지 않았지만, 폐사율은 무기태 및 유기태 미네랄 처리구가 대조구와 비교하여 유의적으로 ($p<0.05$) 낮았다. 분변 내 철, 구리, 아연, 망간, 마그네슘 함량은 처리구간에 차이가 유의적으로 나타났으며 ($p<0.05$), 무기태 처리 그룹에서 가장 높은 함량을 보여주었다. 비장과 간에서 철, 구리, 아연, 망간, 마그네슘의 함량은 유기태 처리그룹에서 유의적으로 ($p<0.05$) 가장 높은 함량을 보여주었다. 결론적으로 유기태 미네랄 프리믹스의 첨가는 육계의 생산성에 영향을 미치지 않으며, 환경오염을 유발하는 분변 내 미네랄 함량을 낮출 수 있는 것으로 확인되었다.

Keywords : Broiler, Organic, Inorganic, Fecal Mineral Excretion, Mortality

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1. Introduction

Trace minerals such as copper, iron, zinc, manganese and magnesium are essential nutrients for sustaining animal life and growth. These factors are directly or indiectly involved in the biochemical processes and conditions of the animals's body[1]. Inorganic minerals (sulfate or oxide) have traditionally met the trace element requirements of animals, but the environmental pollution caused by livestock manure is a very serious situation worldwide[2]. In Korea, as the livestock industry is scaled up and commercialized, manure excreted from livestock is becoming a source of water and soil pollution[3]. As a result, the government, recognizing the environmental pollution caused by livestock waste water, strengthened environmental laws and regulated the level of mineral additions in feed. However, not only nutrient requirements but also the mineral requirements are increasing due to the increase in the capacity of livestock, and the current situation is that active research is being conducted on organic minerals[4]. Iron (Fe) is an essential trace mineral for all living organisms, playing important roles in oxygen and electron transport as well as in DNA synthesis[5]. Copper (Cu) is indispensable trace element for poultry maintenance, growth, health, and survival. As co-factor for several metalloenzymes, Cu is involved in a plethora of biological processes, which include mitochondrial reparation, erythropoiesis, connective tissue maturation, free redical scavenging, hormone secretion pathways and immune system defenses, among others[6,7]. Zinc (Zn) is essential since it serves as a cofactor in more than 240 enzymes and helps to metabolize nutrients, such as carbohydrates and proteins, thus helping to increase growth and reproductive performance[8,9]. Manganese (Mn) plays an important role in bone formation and in many biochemical processes by activating enzymes, such as pyruvate carboxylase, superoxide, dismutase and glycosyl transferase[10]. Magnesium (Mg) was indispensable for animal growth and survival. It is fourth most abundant cation in living organisms[11]. Most mineral additives currently used in livestock production come from inorganic compounds, such as oxides, sulfates, carbonate, and phosphates[12]. Chelated or complex trace elements may improve the bioavailability of minerals for pigs and poultry[13-17]. Some studies have reported that organic mineral materials have a reduction mineral excretion compared to inorganic mineral material[17]; however, most mineral research has focused on single-supplementation effect. In general, organic minerals are in a form that can be best absorbed by body tissues, and organically chealted minerals, such as amino acids and small molecule peptides, can be affectively absorbed and utilized by the body, reducing the amount of minerals excreted and thus reducing fecal pollution[18]. However, since methionine, an amino acids widely used as a chelating agent for mineral amino acid chelation, is expensive, this study used soybean meal as a chealting agent to prepare organic minerals to reduce manufacturing costs[4]. In this study, we evaluated the effect of diets supplemented with multiple organic and inorganic trace minerals, including iron, copper, zinc, manganese, and magnesium on the growth performance and mineral excretion in broilers.

2. Materials and Methods

2.1 Preparation of mineral proteinate

Trace mineral (Fe, Cu, Zn, Mn, and Mg) soy proteinate was developed in the animal nutrition laboratory at Chung-Ang University[19]. Soybean meal digest prepared by hydrolysis of soybean meal mixed with an enzyme (Alkalase 2.4 L; Novozymes®, Bagsvaerd, Denmark) under an aqueous condition of pH 8 and at a temperature of 60°C for 8 h. Then, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot H_2O$, $ZnSO_4 \cdot H_2O$, $MnSO_4 \cdot H_2O$, and MgO were allowed to react with the soybean meal digest at a 1:1 weight ratio at pH values of 8.8, 8.4, 8.8, 8.3, and 8.7, respectively. The precipitates were separated and dried. The final products contained 191 g/kg Fe, 174 g/kg Cu, 180 g/kg Zn, 168 g/kg manganese, and 90 g/kg Mg, respectively.

2.2 Bird and experimental design

A total of 600 one-day-old broiler chicks (initial BW = 42.32±0.32 g) of a commercial broiler strain (Ross × Ross 308; Yang-Ji Hatchery, Pyeong-Taek, South Korea) were randomly allotted to 1 of 2 dietary treatments. The experiment was performed as a completely randomized design with 6 replicate pens consisting of 50 birds [pen size: 2.0m(width) × 2.4m (length)]. A 2-phase feeding program with a starter diet from 0 to 14 d and a grower diet from 15 to 28d was used in this experiment (Table 1). The basal diet was a commercial-type formulated to exceed the nutrient recommendations of the National Research Coulcil (NRC)[20] for broilers, with Fe, Cu, Zn, Mn, and Mg concentrations of 80 mg/kg, 8 mg/kg, 40 mg/kg, 60 mg/kg, and 600 mg/kg, respectively. The other 2 diets were prepared by adding an inorganic mineral premix (sulfate and oxide) or the organic mineral premix (soy-proteinate) to the basal diet. The analyzed concentrations of Fe, Cu, Zn, Mn, and Mg in the inorganic and organic diet were 83.3 or 84.3 mg/kg, 7.9 or 8.1 mg/kg. 45.3 or 46.1 mg/kg, 59.4 or 59.9 mg/kg, 624 or 618 mg/kg, respectively. All feeds were analyzed for mineral content. Mineral analyses in feed was determined by Inductively Coupled Plasma Mass Spectrometry(ICP-MS; Optima 5300 DV) after wet-ash digestion with nitric/perchloric acid [21]. During the experimental period of 28 d, feed and water ad libitum and the light were exposed to a 23L:1D schedule. Brooder temperature was maintained at 32°C, and barn temperature was maintained at 32°C during the first week of the experiment and gradually decreased 26°C at the end of the experiment.

2.3 Growth performance

Body weight gain (BW gain) and feed intake were recorded at the 14 d and 28 d. The feed conversion ratio (F:G) was calculated as BWG divided by FI after adjusting for mortality.

2.4 Collection of fecal, spleen, liver and blood sample

At the end of the experiment period, the blood samples were collected, using EDTA treated BD Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA), from the wing vein of 10 birds randomly selected from each treatment. The tubes were placed on ice, and the whole-blood samples were immediately evaluated. The white and red blood cell, heterophil, and lymphocyte

Table 1. Cmposition and nutrient content of experimental diets.

Ingredients, g/kg	0 to 14d	15 to 28d
Corn	520.3	547.7
Soybean meal	281.0	230.0
Wheat meal	50.0	100.0
Corn gluten	38.4	19.9
Fish meal	40.0	35.0
Tallow	35.0	35.0
Dicalcium phosphate	18.6	15.9
Limstone	10.0	10.0
Sodium chloride	2.2	2.5
Choline-50%	0.6	0.4
Methionine-99%	1.1	1.1
Lysine-78%	1.4	1.1
Vitamin premix	1.4	1.4
Total	1,000	1,000
Calculated composition 3		
ME, kcal/kg	3,100	3,150
Crude protein, g/kg	220	190
Calcium, %	10.0	9.2
Available phosphate, %	5.1	4.5
Lysine, %	12.0	10.2
Methionine + Cystein, %	8.7	7.5
Analysed composition		
Iron, g/kg	75.2	79.2
Copper, g/kg	13.5	14.1
Zinc, g/kg	14.4	14.8
Manganese, g/kg	50.6	49.8
Magnesium, g/kg	1,125	1,139

[1] Provided per kilogram of the complete diet: vitamin A (vitamin A acetate), 12,500 IU; vitamin D_3, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_1, 2mg; vitamin B2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 18 μg; calcium pantotenate, 8 mg; folic acid, 1 mg; biotin 50 μg; niacin, 24 mg.

counts and hemoglobin and hematocrit levels were analyzed using the Hemavet Multispcies Hematology System (Drew Scientific Inc., Oxford, CT, USA). The H/L ratio was calculated by dividing lymphocytes in heterophils. At the end of the experiment, fecal samples were taken for mineral analysis. 10 birds per pen were randomly selected and placed in cages, where lighting was reduced for approximately 15 min, until sufficient droppings were produced (minimum of 50 g per 10 birds). Fecal samples from each pen were homogenously mixed and analyzed for Fe, Cu, Zn, Mn, and Mg contents, to determine the level of mineral excretion. 10 birds per treatment were sacrificed by cervical dislocation for spleen and liver sampling. The spleen and livers were washed extensively under running water and immediately stored in individual sample bags at -20°C. The samples were dried for 4 days at 50°C. The dried samples were cooled to room temperature (20°C), pulverized bagged, and stored in a refrigerator until chemical analysis of Fe, Cu, Zn, Mn, and Mg levels. The concentrations of Fe, Cu, Zn, Mn, and Mg in the fecal, spleen, and liver were determined by ICP-MS (Optima 5300 DV) after wet-ash digestion with nitric/perchloric acid[21].

2.5 Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) with a fully randomized design using the Proc Mixed procedure from SAS (SAS Inst., Inc., Cary, NC). Outlier data were identified using the UNIVARIATE procedure in SAS and no outliers were found. Differences among the least squares means were evaluated using the PDIFF option with Tukey's adjustment. In this process, the output values were converted to letter groups using a macro program[22]. The LSMEANS procedure was used to calculate the means of either basal diet vs. mineral premix or inorganic mineral premix vs. organic mineral premix. The significance and trend of statistical tests were set at p-values < 0.05 and $0.05 \leq p \leq 0.10$, respectively.

3. Results and discussion

3.1 Growth performance

In the starter period (0 to 14 d), grower period (15 to 28 d), and overall periods (0 to 28 d) no significant differences were observed between any of the performance parameters measured (Table 2). However, mortality rate was lower ($p <$ 0.05) in the inorganic and organic mineral premix groups than in the basal diet group (starter period, grower period, and overall periods).

Table 2. Productive performance of broilers fed diets formulated with inorganic or organic mineral premix[1)]

Items	Treatments[2)]			SEM	p-value	
	Basal	Inorganic	Organic		B vs. M	I vs. O
Starter periods (0 to 14 d)						
[3)]BW gain, g	332	333	333	4.41	0.571	0.201
Feed intake, g	418	415	407	4.40	0.320	0.301
[4)]FCR	1.26	1.25	1.22	0.012	0.358	0.405
Motality, %	4.29^a	0.81^b	0.61^b	0.968	0.015	0.500
Grower periods (5 to 28 d)						
BW gain, g	1,068	1,063	1,0798	19.6	0.427	0.861
Feed intake, g	1,129	1,121	1,116	11.7	0.328	0.242
FCR	1.06	1.05	1.03	0.023	0.399	0.425
Motality, %	2.99^a	0.80^b	0.80^b	0.232	0.023	0.359
Overall periods (0 to 28 d)						
BW gain, g	1,400	1,396	1,412	21.8	0.762	0.772
Feed intake, g	1,547	1,536	1,523	12.3	0.145	0.345
FCR	1.11	1.10	1.08	0.018	0.715	0.662
Motality, %	7.28^a	1.61^b	1.41^b	1.647	0.032	0.425

$^{a-b}$Means with different letters in the same line are significantly different ($p<0.05$).
[1)]Data are least squares means of 6 replicates per treatment.
[2)]Basal = Basal diet(no mineral premix); Inorganic = Basal diet + 1.0 g/kg inorganic mineral premix; Organic = Basal diet + 1.8 g/kg organic mineral premix.
[3)]BW gain = body weight gain. [4)]FCR = feed conversion ratio.

It is well established that organic forms are environment-friendly because of their lower excretion rate and it remains long time in the gut consequently improves the growth performance[23]. Organic minerals are highly bioavailable because they have higher retention rate in the body compared with inorganic minerals[16]. Pacheco et al.[24] reported that organic Zn and inorganic Zn has no significant effect on the body weight which was also found by several researches[25,26]. In another study[27], broiler birds fed organic mineral supplemented diet has better effect on body weight compared with inorganic mineral supplemented in diet. Our result was coincided with the result of another study[23], were it was shown that although organic form facilitated grater bioavailability, they did not significantly affect body weight gain of birds. Feed intake data revealed that birds of all treatments consumed more or less similar amounts of feed up to 28 d of age and, so the differences in feed intake did not differ significantly. Organic minerals are chelating agents that help in better feed absorption and utilization in the body tissue and decline mineral excretion from the body. Therefore, organic mineral enhanced feed intake, Baloch et al.[28] reported that organic mineral supplementation did not significantly affect feed intake. Sunder et al.[29] also found that feed intake was not significantly affected by feeding organic mineral and inorganic mineral. In this study, FCR was not affected by feeding organic and inorganic mineral in diets. Since the values were close to each other in different dietary treatments, there was no statistical differences. Baloch et al.[28] and Zhao et al.[30] observed that organic and inorganic mineral, when used in the diet, had no significant effect on FCR, which agreed with our result. Although birds fed the basal diet numerically showed a higher mortality rate compared with those fed supplemental treatments, the difference was not affected. It may indicate that when zinc is inadequate to maintain growth or cellular metabolism, reduced feed intake may be a protective mechanism to allow survival[31]. Thus, zinc deficiency may not result in definite higher mortality.

3.2 Hematological analysis

Leukocyte (WBC, HE, LY, MO, EO, and BA) and erythrocyte (RBC, Hb, HCT, MCV, MCH, MCHC), no significant difference between inorganic and organic mineral premix in the diets (Table 3). Livestock blood status is a good indicator that reflects physiological and nutritional changes that indicate the animal's physical condition[32]. The leukocyte count has been used a measure of immune function and stress index[33].

Table 3. Blood parameter (leukocyte and erythrocyte) of broilers fed diets formulated with inorganic or organic mineral premix1)

Items	Treatments2)			SEM	p-value	
	Basal	Inorganic	Organic		B vs. M	I vs. O
		Leukocyte3)				
WBC, K/uL	31.3	28.2	28.6	1.48	0.482	0.131
HE, K/uL	10.6	8.2	8.9	0.43	0.285	0.262
LY, K/uL	15.4	15.3	15.2	0.87	0.325	0.162
SI, HE/LY	0.69	0.54	0.59	0.230	0.398	0.363
MO, K/uL	3.1	2.6	2.9	0.05	0.421	0.283
EO, K/uL	1.7	1.7	2.0	0.08	0.410	0.222
Erythrocyte4)						
RBC, m/uL	3.1	3.2	3.1	0.325	0.325	0.864
Hb, g/dL	8.2	9.3	8.7	0.369	0.369	0.842
HCT, %	30.8	32.3	30.6	0.591	0.591	0.849
MCV, f/L	100.5	100.5	99.9	0.142	0.142	0.363

1)Data are least squares means of 10 replicates per treatment.
2)Basal = Basal diet(no mineral premix); Inorganic = Basal diet + 1.0 g/kg inorganic mineral premix; Organic = Basal diet + 1.8 g/kg organic mineral premix.
3)Leukocyte: WBC = white blood cell, HE = heterophil, LY = lymphocyte, MO = monocyte, EO = Eosinophil, BA = Basophil.
4)Erythrocyte: RBC = red blood cell, Hb = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume.

Hosienpour et al.[34] and Kim et al.[16] reported that the supplementation of organic minerals did not affect hematological parameters of lamb and laying hens. In contrast, previous research reported that supplementation of organic trace minerals increased RBV parameters and hematological response in animals[35,36]. However, further studies are needed to determine the effects of various forms of mineral premix in the diet on poultry hematological parameters.

3.3 Mineral concentrations in fecal, spleen, and liver

We found that the concentrations of Fe, Cu, Zn, Mn, and Mg in the fecal were different ($p < 0.05$) among the treatment groups, with the highest values reported in the inorganic mineral premix group. The concentrations of Fe, Cu, Zn, Mn, and Mg in the spleen and liver were different ($p < 0.05$) among the treatment groups, with the highest values reported in the organic mineral premix group (Table 4).

Table 4. Effect of dietary supplementation with different mineral premix on mineral concentrations in fecal, spleen, and liver[1)]

Items	Treatments[2)]			SEM	p-value	
	Basal	Inorganic	Organic		B vs. M	I vs. O
Fecal (DM-basis, mg/kg)						
Fe	236.2^{b}	278.9^{a}	248.5^{b}	8.92	0.762	0.732
Cu	35.7^{b}	44.3^{a}	37.6^{b}	31.52	0.432	0.042
Zn	87.2^{b}	184.0^{a}	95.2^{b}	36.49	0.025	0.042
Mn	41.1^{b}	102.8^{a}	82.3^{b}	12.65	0.035	0.025
Mg	866.3^{ab}	903.1^{a}	619.8^{b}	97.76	0.432	0.038
Spleen (DM-basis, mg/kg)						
Fe	415.7^{b}	408.0^{b}	467.9^{a}	57.36	0.022	0.032
Cu	3.2^{b}	3.1^{b}	3.6^{a}	0.28	0.018	0.028
Zn	42.5^{b}	41.7^{b}	44.7^{a}	2.18	0.028	0.035
Mn	4.5^{b}	4.4^{b}	6.7^{a}	1.15	0.019	0.042
Mg	425.3^{b}	433.0^{b}	450.3^{a}	44.4	0.011	0.020
Liver (DM-basis, mg/kg)						
Fe	367.9^{b}	366.1^{b}	385.4^{a}	75.57	0.013	0.035
Cu	7.5^{b}	8.4^{b}	9.8^{a}	0.62	0.027	0.024
Zn	143.9^{b}	143.2^{b}	180.4^{a}	11.59	0.032	0.012
Mn	18.2^{b}	17.8^{b}	19.9^{a}	1.14	0.017	0.048
Mg	700.5^{b}	655.5^{b}	864.3^{a}	35.16	0.031	0.025

$^{a-b}$Means with different letters in the same line are significantly different ($p<0.05$).
[1)]Data are least squares means of 10 replicates per treatment.
[2)]Basal = Basal diet(no mineral premix); Inorganic = Basal diet + 1.0 g/kg inorganic mineral premix; Organic = Basal diet + 1.8 g/kg organic mineral premix.

Inorganic trace minerals are widely used in livestock production due to their easy accessibility and low cost, but their bioavailability is low. Thereby, in commercial production, producers generally supplement feed stuffs with a large safety margin in order to obtain higher economic benefits, thus causing a greater burden on the environment. It might improve bioavailability when trace minerals are chelated to a ligand, because the likelihood of interaction with other elements or combining with gastrointestinal antagonistic molecules in reduced [37]. Studies on replacing inorganic trace mineralswith organic forms partially or entirely in animal feed have shown that organic trace minerals have higher bioavailability and reduced excretion in feces. An earlier study indicated that supplemented inorganic trace mineral with segmental organic modality could enhance the absorption coefficient of these trace minerals[38]. Nollet et al. [17] reported that lower levels of organic trace minerals can be added to broiler diet to replace inorganic forms without any negative effects on broiler performance, even increasing feed conversion rate. In previous study, the supplementaion of organic and inoreganic minerals to broiler diets significantly reduced fecal concentrations of Cu, Fe, Zn, and Mn by 43.4%, 26.5%, 36.5% and 55.3%, respecively, based on the supplementation of organic minerals [17]. Further, replacement with organic trace minerals resulted in significantly reduced concentrations of minerals in manure. As environmental protection issues are urgently addressed, organic trace minerals can be used in poultry feed, reducing the risk of environmental contamination from fecal minerals without affecting performance and metabolic status of animals. Yang et al.[39] reported that the higher bioavailability of chelates is probably based on their ring structure, which protects trace minerals from chemical reactions in the gastrointestinal tract and maintains stability, even at low pH. Additionally, chelates are negatively charged and therefore pass more effectively through the intestinal wall by various mechanisms (mainly by amino acid transport) than inorganic compounds. The rate of passive diffusion is higher because of the lower extent of interaction between the mineral and other nutrients. This alternative absorption pathway reduces the excretion. The bioavailability of mineral is defined as degree to which an ingested mineral in a particular source is absorbed in a form that can be utilized in metabolic process for animals.

4. Conclusion

In conclusion, it has been confirmed that replacing inorganic mineral premix with organic mineral premix does not adversely affect the productivity if broilers and can reduce the concentration of minerals in feces that cause environmental pollution.

References

[1] C. D. Espinosa, H. H. Stein, "Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: a review", *Journal of Animal Science Biotechnology*, Vol. 13, pp.12-13, 2021.
DOI: https://doi.org/10.1186/s40104-020-00533-3

[2] G. M. Pesti, R. Bakalli, "Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens", Poultry Science, Vol. 75, pp.1086-1091, 1996.
DOI: https://doi.org/10.3382/ps.0751086

[3] Y. H. Kim, J. S. Yoo, J. C. Park, H. J. Jung, J. H. Cho, Y. J. Chen, H. J. Kim, I. C. Kim, S. J. Lee, I. H. Kim, "Effects of copper and zinc sources on growth performance, nutrient digestibility, carcass traits and meat characteristics in finishing pigs", *Food Science of Animal Resources*, Vol. 28, pp.27-31, 2008.
DOI: https://doi.org/10.5851/kosfa.2008.28.1.27

[4] C. H. Kim, J. Y. Jung, S. N. Cheon, G. Z. Yoo, J. H. Jeon, "Effects of organic copper and zinc supplementation on the growth performance, blood parameter, nutrient digestibility and fecal microflora of weaning pigs" *Jounral of Korea Academic Cooperation Society*, Vol. 22, pp.345-351, 2021.
DOI: https://doi.org/10.5762/KAIS.2021.22.8.345

[5] D. A. Von, J. W. Adamson, "Iron metabolism in man", *Journal of Parenteral and Enteral Nutrition*, Vol. 37. pp.599-606, 2013.
DOI: https://doi.org/10.1177/0148607112459648

[6] A. V. Jegede, O. O. Oduguwa, A. M. Bamgbose, A. O. Fanimo, L. Nollet, "Growth response, blood characteristics and copper accumulation in organs of broilers fed on diets supplemented with organic and inorganic dietary copper sources", *British Poultry Science*, Vol. 52, pp.133-139,2011.
DOI: https://doi.org/10.1080/00071668.2010544714

[7] L. M. Gaetke, H. S. Chow-Johnson, C. K. Chow, "Copper: toxicological relevance and mechanisms", *Archives of Toxicology*, Vol. 88, pp.1929-1938, 2014.
DOI: https://doi.org/10.1007/s00204-014-1355-y

[8] O. Kucuk, N. Sahin, K. Sahin, "Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens", *Biological Trace Element Research*, Vol. 94, pp. 225-235, 2003.
DOI: https://doi.org/10.1385/BTER:94:3-225

[9] K. Sahinm O. Kucuk, "Zinc supplementation alleviates heat stress in laying Japanese quail", *Journal of Nutrition*, Vol. 133, pp.2008-2011, 2003.
DOI: https://doi.org/10.1093/jn/133.9.2808

[10] O. Olgun, "Manganese in poultry nutrition and its effect on performance and eggshell quality", *World's Poultry Science Journal*, Vol. 73, pp.45-56, 2017.
DOI: https://doi.org/10.1017/S0043933916000891

[11] F. I. Wolf, A. Cittadini, "Chemistry and biochemistry of magnesium", *Molecular Aspects of Medicine*, Vol. 24, pp.3-9, 2003.
DOI: https://doi.org/10.1016/50098-2997(02)00087-0

[12] C. Stefanello, T. C. Santos, A. E. Murakami, E. N. Martins, T. C. Carneiro, "Productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals", *Poultry Science*, Vol. 93, pp.104.113, 2014.
DOI: https://doi.org/10.3382/ps.2013-03190

[13] K. J. Wdedekind, A. E. Horthin, D. H. Baker, "Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-mtehionine, zinc sulfate, and zinc oxide", *Journal of Animal Science*, Vol. 70, pp.178-187, 1992.
DOI: https://doi.org/10.2527/1992.701178x

[14] S. Aoyagi, D. H. Baker, "Nutritional evaluation of a copper-methionine complex for chicks", *Poultry Science*, Vol. 72, pp.2309-2315, 1993.
DOI: https://doi.org/10.3382/ps.0722309

[15] G. B. Kim, Y. M. Seo, K. S. Shin, A. R. Rhee, J. Han, I. K. Paik, "Effects of supplemental coppr-soy proteinate on the performance, blood parameters, liver mineral content, and intestinal microflora of broiler chickens", *Journal of Applied Poultry Research*, Vol. 20, pp.21-32.
DOI: https://doi.org/10.3382/japr.2010-00177

[16] C. H. Kim, S. H. Jeong, S. J. Lim, S. N. Cheon, K. H. Kim, J. L. Chun, J. H. Jeon, "Effect of organic or inorganic mineral premix in the diet on laying performance of aged laying hens and eggshell quality", *Animals*, Vol. 12. pp.2378, 2022.
DOI: https://doi.org/10.3390/ani12182378

[17] L. Nollet, J. D. Van der Kils, M. Lensing, P. Spring, "The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion", *Jounral of Applied Poultry Research*, Vol. 16, pp.592-597, 2007.
DOI: https://doi.org/10.3382/japr.2006-00115

[18] J.W. Spears., E.B. Kegley. "Effect of zinc source(zinc oxide vs. zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers", *Journal of Animal*

Science. Vol. 80, pp.2747-2753, 2002.
DOI: https://doi.org/10.2527/2002.80102747x

[19] I. K. Paik, H. K. Lee, S. W. Park, "ffects of organic iron supplementation on the performance and iron content in the egg yolk of laying hens", *Journal of Poultry Science*, Vol. 46, pp.198-202, 2009.
DOI: https://doi.org/10.2141/jpsa.46.198

[20] NRC. Nutrient Requirements of Poultry. 9th ed. National Academic. Press, Washington, DC. USA, 1994.

[21] AOAC. 2007. Official methods of analysis (18th ed.). Association of Official Agricultural Chemists. Washington, DC, USA.

[22] A. M. Saxton, "A macro for converting means separation output to letter groupings in Proc Mixed", In Proceedings of the 23nd SAS Users Group International, Nashville, TN, USA, 22-25 March, pp. 1243-1246, 1998.

[23] S. Leeson, L. Caston, "Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure", *Animal Feed Science Technology*, Vol. 142, pp.339-347. 2008.
DOI:https://doi.org/10.1016/j.anifeedsci.2007.08.004

[24] B. H. C. I. Pacheco, V. S. I. Nakagi, E. H. Kobashigawa, D. E. I. Faria, "Dietary levels of zinc and manganese on the performance of broilers between 1 to 42 days", *Brazail Journal of Poultry Science*, Vol. 19, pp.171-178, 2017.
DOI:https://doi.org/10.1590/1806-9061-2016-0323

[25] S. D. Bun, Y, M, Guo, F. C. Guo, F. J. Ji, H. Cao, "Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella", *Poultry Science*, Vol. 90, pp.1220-1226, 2011.
DOI:https://doi.org/10.3382/ps.2010-01308

[26] H.M. Salim, H.R. Lee, C. Jo, S.K. Lee, B.D. Lee, "Effect of sex and dietary organic zinc on growth performance, carcass traits, tissue mineral content, and blood parameters of broiler chickens", *Biological Trace Element Research*, Vol. 147, pp.120-129. 2012.
DOI:https://doi.org/10.1007/s12011-011-9282-8

[27] Y. M. Bao, M. Choct, P. A. Iji, K. Bruerton, "Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues", *Journal of Applied Poultry Research*, Vol. 16, pp.448-455, 2007.
DOI: https://doi.org/10.1093/japr/16.3.448

[28] Z. Baloch, N. Yasmeen, T.N. Pasha, "Effect of replacing inorganic with organic trace minerals on growth performance, carcass characteristics and chemical composition of broiler thigh meat", *African Journal of Agriculture Research*, Vol. 12, pp.1570-1575, 2017.
DOI: https://doi.org/10.5897/AJAR2016.12.104

[29] G. S. Sunder, C. V. Kumer, A. K. Panda, "Effect of supplemental organic Zn and Mn on broiler performance, bone measures, tissue mineral uptake and immune response at 35 days of age", *Current Research in Poultry Science*, Vol. 3, pp.1-11, 2013.
DOI: https://doi.org/10.3923/crpsaj.2013.1.11

[30] J. Jhao, B. Robert, J. J. Shirley, J. Dibner, "Superior growth performance in broiler chicks fed chelated compared to inorganic zinc in presence of elevated dietary copper", *Journal of Animal Science biotechnology*, Vol. 7, pp.13-21, 2016.
DOI: https://doi.org/10.1186/s40104-016-0072-1

[31] R. S. McDonald, "The role of zinc in growth and cell proliferation", *Jounral of Nutrition*, Vol. 130, pp.1500S-1508, 2000.
DOI: https://doi.org/10.1093/jn/130.5.1500S

[32] A. Masoudi., M. Chagi, M. Bojaropour, K. Mirzadeh. "Effects of diiferent levels of date pits on performance, carcass characteristics and blood parameters of broiler chickens" *Journal of Applied Animal Research*, Vol. 39, pp.399-405, 2011.
DOI: https://doi.org/10.1080/09712119.2011.621790

[33] T. S. Jhonson, M. Zuk, "Parasites, morphology, and blood characters in male red jungle fowl during development", *Condor,* Vol. 100, pp.749-752, 1998.
DOI: https://doi.org/10.2307/1369760

[34] N. Hosienpour, M. A. Norouzian, A. Afzalzadeh, A. A. Khadem, A. Adadi Alamouti, "Source copper may have regressive effects on serum cholesterol and urea nitrogen among male fattening lambs", *Biological Trace Element Research*, Vol. 159, pp.147-151, 2014.
DOI: https://doi.org/10.1007/s12011-014-0004-x.

[35] M. H. Bami, M. Mohri, H. A. Seifi, A. A. A. Tavatabaee, "Effects of parenteral supply of iron and copper on hematology, weight gain and health in neonatal dietary calves", *Vet Res Commun*, Vol. 32, pp.553-561, 2008.
DOI: https://doi.org/10.1007/s11259-008-9058-6

[36] M. A. Noriuzian, M. Malaki, A. A. Khadem, "Effect of parenteral administration of cobalt, copper and iron in late pregnancy on ewe hematology and lamb vigour", *Iran Journal of Applied Animal Science*, Vol. 4, pp. 285-289, 2014.

[37] M. K. Managi, M. Vazquer-Anon, J. D. Richard, S. Carter, R. E. Buresh, K. D. Christensen, "Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace minerals on broiler performance, yield, footpad health, and litter mineral concentration", *Journal of Applied Poultry Research*, Vol. 21, pp.881-890, 2012.
DOI: https://doi.org/10.3382/japr.2012-00531.

[38] S. Mondal, S. Haldar, S, P. Saha, T. K. Ghosh, "Metabolism and tissue distribution of trace elements in broiler chickens' FED diets containing deficient and plethoric levels of copper, manganese, and zinc", *Biological Trace Element Research*, Vol. 137, pp.190-205, 2010.
DOI: https://doi.org/10.1007/s12011-009-8570-z

[39] K. Yang, S. Hu, Ri, Y. Qing, L. Xie, L. Zhou, K. M.

Ajuwin, R. Fang, "Effects of different patterns and sources of trace elements on laying performance, tissue mineral deposition, and fecal excretion in laying hens", *Animals*, Vol. 11, pp.1164, 2021.
DOI: https://doi.org/10.3390/ani11041164

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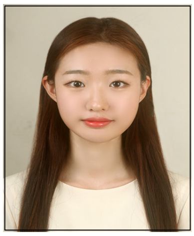

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