

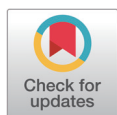
Evaluation of mineral digestibility and physiological status in adult dogs fed an organic mineral-based diet

Seyeon Chang^{1#}, Kangmin Seo^{2#}, Han Tae Bang¹, Kyoung-Min So¹,
Min Young Lee¹, Sang-Yeob Lee¹, Woo-Do Lee¹, Hyun-Woo Cho¹,
Won Yong Jung¹, Ki Hyun Kim^{3*}, Ju Lan Chun^{1*}

¹Animal Welfare Division, National Institute of Animal Science, Rural Development Administration, Wanju, Korea

²Ingredient Examination Division, National Agricultural Products Quality Management Service, Ministry of Agriculture, Food and Rural Affairs, Gimcheon, Korea

³Academic-Industrial Cooperation Organization, Sunchon National University, Suncheon, Korea



Received: Sep 4, 2025

Revised: Dec 3, 2025

Accepted: Dec 5, 2025

#These authors contributed equally to this work.

*Corresponding author

Ki Hyun Kim

E-mail: kihyun@scnu.ac.kr

Ju Lan Chun

E-mail: julanchun@korea.kr

Copyright © 2026 Korean Society of Animal Science and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Seyeon Chang

<https://orcid.org/0000-0002-5238-2982>

Kangmin Seo

<https://orcid.org/0000-0001-6152-8536>

Han Tae Bang

<https://orcid.org/0000-0003-0441-3542>

Kyoung-Min So

<https://orcid.org/0000-0001-9497-3345>

Min Young Lee

<https://orcid.org/0000-0003-4860-6290>

Sang-Yeob Lee

<https://orcid.org/0000-0001-8024-1135>

Woo-Do Lee

<https://orcid.org/0000-0003-4861-4637>

Abstract

The daily requirement for minerals is minor; nevertheless, they are essential for the metabolism, growth, and reproduction of dogs. Therefore, European Pet Food Industry Federation, Nutrient Research Council, and Association of American Feed Control Officials (AAFCO) recommend minimum levels of essential minerals in pet food. This study examined the potential availability and safety of organic minerals in adult dogs. Five-year-old, neutered female beagle dogs were fed an inorganic (IMD) or organic (OMD) mineral-based diet twice daily for two weeks each in a crossover design. The IMD included Ca(IO₃)₂, FeSO₄, MnSO₄, ZnSO₄, and CuSO₄; the OMD included Ca(IO₃)₂, glycine-chelated Fe (Fe-Gly), Mn (Mn-Gly), Zn (Zn-Gly), and Cu (Cu-Gly). The experimental diets were provided in an amount individually estimated by the maintenance energy requirement equation proposed by AAFCO, and water was provided *ad libitum*. No significant differences in food and energy intake, body weight, body condition score, and fecal score were observed between the IMD and OMD groups. The OMD group had significantly higher mineral (K, P, Na, Ca, Fe, Zn, Cu, and Mn) and nutrient (organic matter, dry matter, and nitrogen-free extract) digestibility than the IMD group. All parameters of complete blood count remained within the normal physiological range, despite significant differences in some parameters between these two groups. Therefore, OMD may positively influence nutritional metabolism by improving mineral and nutrient digestibility without negatively affecting body weight, body condition score, and digestive and physiological parameters in adult dogs.

Keywords: Apparent total tract digestibility, Beagle dog, Hematological and biochemical parameters, Nutrition, Organic mineral

INTRODUCTION

Pet food is composed of major nutrients (e.g., protein, fat, and carbohydrates) and micronutrients

Hyun-Woo Cho
<https://orcid.org/0000-0002-3620-9952>
 Won Yong Jung
<https://orcid.org/0000-0002-1751-8418>
 Ki Hyun Kim
<https://orcid.org/0000-0002-9834-2126>
 Ju Lan Chun
<https://orcid.org/0000-0002-4618-586X>

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01560103)" Rural Development Administration, Republic of Korea.

Acknowledgements

This study was supported by the 2026 RDA Fellowship Program of National Institute of Animal Science, Rural Development Administration, Republic of Korea.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Kim KH, Chun JL.
 Data curation: Chang S, Seo K.
 Formal analysis: Lee MY, Lee SY, Lee WD.
 Methodology: Lee MY, Cho HW.
 Software: Lee WD, Cho HW, Jung WY.
 Validation: Bang HT, So KM, Kim KH, Chun JL.
 Investigation: Chang S, Seo K, Lee MY, Lee SY, Lee WD, Cho HW, Jung WY.
 Writing - original draft: Chang S, Seo K, Jung WY, Kim KH, Chun JL.
 Writing - review & editing: Chang S, Seo K, Bang HT, So KM, Lee MY, Lee SY, Lee WD, Cho HW, Jung WY, Kim KH, Chun JL.

Ethics approval and consent to participate

This experiment was approved by the Animal Care and Use Committee National Institute of Animal Science, Wanju, Korea (NIAS2021-0516).

Declaration of generative AI

No AI tools were used in this article.

(e.g., minerals and vitamins). European Pet Food Industry Federation (FEDIAF), Nutrient Research Council (NRC), and Association of American Feed Control Officials (AAFCO) provide recommended levels for a balanced supply of these ingredients [1–3]. In particular, minerals are found in bones, most tissues, and body fluids, and are essential for maintaining the function and structure of living tissues [4]. Although the daily requirement for minerals is minor, they are essential for a dog's metabolism, growth, and reproduction [4]. Therefore, a balanced supply is emphasized [5], and their excess or deficiency should be carefully considered as it can negatively impact animal health [6,7]. Therefore, FEDIAF, NRC, and AAFCO provide minimum and, in some cases, maximum recommended levels for essential minerals (e.g., Ca, P, K, Na, Cl, Mg, Cu, I, Fe, Mn, Se, and Zn) in pet food, and the importance of mineral research in canine nutrition is constantly emphasized [8–11].

Traditionally, mineral supplements for livestock or companion animals have mainly been used as inorganic minerals such as oxides, carbonates, chlorides, and sulphates [12,13]. Inorganic minerals often form insoluble complexes during digestion because of pH changes or interactions with other digestive compounds, leading to their excretion without absorption in the intestine [14,15]. This limited absorption through the small intestine can lead to either mineral deficiency or oversupply in animals, which has raised concerns among researchers. However, organic minerals, which are composed of complexes with organic compounds, such as amino acids, proteins, and carbohydrates, can minimize interactions with dietary components during digestion and can be effectively absorbed through the amino acid or peptide transport pathways [16–18]. These obvious advantages have led to increased usage of various types of organic minerals [15]. Meanwhile, although studies on the bioavailability of specific organic minerals (Zn, P, and Se) in companion dogs have been reported [19,20], those examining the digestibility and physiological safety of a wide range of essential minerals are very limited.

Therefore, this study was conducted to examine the potential availability and safety of organic minerals in the nutrition of adult dogs.

MATERIALS AND METHODS

Animals, management, and housing

This study was conducted on eight healthy beagle dogs (all neutered females, five-year-old) owned by the National Institute of Animal Science (NIAS). All experiments were conducted for four weeks following the methods approved by the Animal Care and Use Committee NIAS, Wanju, Republic of Korea (NIAS2021-0516). All experimental animals were housed in individual indoor spaces (1.7 m × 2.1 m/dog) and maintained under constant temperature (22 °C–24 °C) and consistent lighting cycles (12-h light and 12-h dark). During the experimental period, all dogs were allowed approximately 6 h of outdoor activity each day in an individual outdoor space (2.8 m × 2.5 m/dog) connected to the indoor area. The experimental diets were provided twice daily at an amount individually estimated by the maintenance energy requirement equation (Equation. (1)) proposed by AAFCO [3], and water was provided *ad libitum*.

$$\text{Maintenance energy requirement} = 132 \times \text{Metabolic body weight (mBW, kg; BW}^{0.75}) \quad (1)$$

Preparation of experimental diets

All ingredients for the experimental diets were available in powdered form from commercial sources, except for lard, and no flavoring agents or preservatives were included. The inorganic mineral diet (IMD) included Ca(IO₃)₂, FeSO₄, MnSO₄, ZnSO₄, and CuSO₄; the organic mineral

diet (OMD) included $\text{Ca}(\text{IO}_3)_2$, glycine-chelated Fe (Fe-Gly), Mn (Mn-Gly), Zn (Zn-Gly), and Cu (Cu-Gly). All experimental diets were formulated to meet the nutrient requirements suggested by the AAFCO guidelines [3], and both diets contained equivalent nutrient levels (Tables 1 and 2). Experimental diets were prepared as previously described [21].

Experimental design and sample collection

Each experimental diet (IMD or OMD) was fed for a total of 14 days in a crossover design, including a three-day adaptation period and a four-day fecal sampling period [22]. During the experimental period, food intake and fecal output were daily recorded, and body weight (BW) was weekly measured. For analyzing nutrient digestibility, fecal samples were collected for 4 consecutive days, beginning on day 10 after the initiation of each experimental diet. Fecal samples

Table 1. Analyzed chemical composition of experimental diet

Items	Experiment diets	
	IMD	OMD
Ingredients composition (%)		
Rice flour	29.78	29.61
Chicken breast meal	13.00	13.00
Egg yolk powder	13.00	13.00
Lard	1.63	1.63
Cabbage powder	1.08	1.08
Calcium monophosphate	1.84	1.84
Calcium carbonate	1.63	1.63
Green laver	1.63	1.65
Potassium citrate	0.87	0.87
Choline chloride	0.26	0.24
Vitamin premix ¹⁾	0.04	0.04
Inorganic mineral premix ²⁾	0.13	-
Organic mineral premix ³⁾	-	0.30
Salt	0.11	0.11
Water	35.00	35.00
Chemical composition (DM %) ⁴⁾ (analyzed)		
Crude protein	32.53	32.24
Ether extract	15.08	14.42
Crude fiber	0.51	1.16
Crude ash	7.65	7.30
Nitrogen free extract	44.23	44.88
Organic matter	91.81	91.53
ME (kcal/kg) ⁵⁾ (calculated)	3,968	3,925

¹⁾Vitamin premix was supplied per kilogram of diets at 3,500 IU of vitamin A; 250 IU of vitamin D₃; 25 mg of vitamin E; 0.052 mg of vitamin K; 2.8 mg of vitamin B₁ (thiamine); 2.6 mg of vitamin B₂ (riboflavin); 2 mg of vitamin B₆ (pyridoxine); 0.014 mg of vitamin B₁₂; 6 mg of Cal-d-pantothenate; 30 mg of niacin; 0.4 mg of folic acid; 0.036 mg of biotin; 1,000 mg of taurine.

²⁾Inorganic mineral diet was supplied per kilogram of diets at 44 mg of FeSO₄; 3.8 mg of MnSO₄; 50 mg of ZnSO₄; 7.5 mg of CuSO₄; 0.9 mg of Ca(IO₃)₂.

³⁾Organic mineral diet was supplied per kilogram of diets at 60 mg of Fe-Gly; 10 mg of Mn-Gly; 80 mg of Zn-Gly; 20 mg of Cu-Gly; 1.01 mg of Ca(IO₃)₂.

⁴⁾Values are analyzed value as DM based.

⁵⁾ME (kcal/kg) = [(crude protein × 3.5) + (ether extract × 8.5) + (nitrogen free extract × 3.5)] × 10.

IMD, inorganic mineral diet; OMD, organic mineral diet; DM, dry matter; ME, metabolizable energy.

Table 2. Analyzed mineral composition in experimental diet

Items	Experiment diets	
	IMD	OMD
Macro minerals (g/kg)		
Potassium	6.60	6.30
Magnesium	1.40	1.18
Phosphorus	6.00	6.10
Sodium	1.30	0.92
Calcium	10.10	9.80
Ca:P ratio	1.68	1.61
Micro minerals (mg/kg)		
Iron	96.01	65.30
Zinc	99.00	87.37
Copper	10.75	18.00
Manganese	10.89	11.82

Values are analyzed value as DM based.

DM, dry matter; IMD, inorganic mineral diet; OMD, organic mineral diet.

were collected from each dog and used for analysis. Blood samples were collected from the cephalic vein of the forelimbs at the start and end of intake of each experimental diet, and were immediately divided into EDTA (BD Vacutainer, Becton Dickinson) and serum (BD Vacutainer, Becton Dickinson) vacutainer tubes. Whole blood in EDTA vacutainer tubes was analyzed within 30 min after collection. Whole blood in serum vacutainer tubes was centrifuged (2,000×g, 10 min) to collect serum samples that were stored at -80°C until analysis.

Analysis

Body condition scores (BCS) were weekly assessed according to the 9-point BCS scale [23]. Fecal scores were daily measured using a 5-point fecal score scale (1 = dry to 5 = liquid feces) according to the Waltham feces scoring system [24] and expressed as an average during the intake period of each experimental diet (14 days). The nutritional compositions of the experimental diet and feces were analyzed for moisture (AOAC method 934.01), crude protein (CP; AOAC method 984.13), ether extract (EE; AOAC method 920.39), crude ash (CA; AOAC method 942.05), and crude fiber (CF; AOAC method 978.10) [25]. Nitrogen-free extract (NFE) and metabolizable energy (ME) were calculated as follows:

$$\text{NFE (\%)} = 100 - (\text{Moisture} + \text{CP} + \text{CF} + \text{EE} + \text{CA}) \quad (2)$$

and

$$\text{ME in a diet (kcal/kg)} = [(\text{CP} \times 3.5) + (\text{EE} \times 8.5) + (\text{NFE} \times 3.5)] \times 10 \quad (3)$$

Mineral levels in the experimental diets and feces were analyzed as previously described [26]. Dried feces (1 g) and experimental diet were mixed in 10 mL of 70% nitric acid (Daejung) and acid-decomposed at 190°C for 24 h. Completely decomposed samples were diluted with deionized distilled water to a total volume of 50 mL. Minerals were quantitatively analyzed using inductively coupled plasma-atomic emission spectrometry (ICPS-7510, Shimadzu). The reliability of mineral analysis was evaluated through recovery analysis by spiking the experimental diets with each

standard solution, and the recovery rates for K, Mg, P, Na, Ca, Fe, Zn, Cu, and Mn were 101.8%, 100.9%, 102.7%, 100.7%, 99.8%, 98.9%, 102.3%, 99.3%, and 100.8%, respectively, indicating the accuracy of analysis.

Apparent total tract digestibility (ATTD) of nutrients and minerals was estimated using the total collection method and calculated as follows:

$$\text{ATTD (\%)} = \frac{[(\text{Amount of nutrient intake} - \text{Amount of fecal nutrient excretion}) / \text{Amount of nutrient intake}] \times 100}{(4)}$$

Complete blood count was analyzed using an automatic hematology analyzer (BC-5000, Shenzhen Mindray Bio-medical Electronics), and serum biochemical parameters were analyzed using an automatic biochemical analyzer (Hitachi 7180, Hitachi High-Technologies).

Statistical analysis

All statistical analyses were performed using SPSS v.17.0 (SPSS Statistics). Data are presented as mean $\pm$ standard error. Significant differences between the IMD and OMD groups, excluding BCS and fecal scores, were analyzed using student's *t*-test. The BCS and fecal score between the two groups were compared using the nonparametric test with a Chi-squared test. Differences were considered significant at $p < 0.05$.

RESULTS

Food intake and body parameters

Average daily food and metabolic energy intake for each dog were not changed significantly (Table 3). No significant differences were found in BW, BCS, and fecal score between the IMD and OMD groups.

Table 3. Effects of organic minerals on food intake, body parameters, and fecal score in adult beagle dogs

Items	Experiment diets		<i>p</i> -value ¹⁾
	IMD	OMD	
ADFI (g/day)	288 $\pm$ 12.6	303 $\pm$ 8.6	0.344
MEI (kcal/day)	802 $\pm$ 34.9	833 $\pm$ 23.8	0.466
BW (kg)			
Initial	12.6 $\pm$ 0.7	12.8 $\pm$ 0.5	0.856
Final	12.9 $\pm$ 0.7	12.9 $\pm$ 0.5	0.935
<i>p</i> -value ²⁾	0.831	0.844	
BWG (g)	22.8 $\pm$ 7.1	13.8 $\pm$ 10.5	0.489
BCS			
Initial	5.0 $\pm$ 0.4	5.1 $\pm$ 0.4	0.812
Final	5.0 $\pm$ 0.4	5.1 $\pm$ 0.4	0.812
Fecal score ³⁾	3.0 $\pm$ 0.0	2.9 $\pm$ 0.1	0.590

Eight beagle breed dogs were given an experimental diet containing inorganic minerals for two weeks, followed by a switch to an experimental diet containing organic minerals for another two weeks. Values are expressed as mean $\pm$ SEM.

¹⁾*p*-values for comparisons between inorganic and organic minerals group in a same row.

²⁾*p*-values for comparisons between the initial and final values in a same column.

³⁾Fecal score was expressed as the average during the intake period (14 d) of each experimental diet.

IMD, inorganic mineral diet; OMD, organic mineral diet; ADFI, average daily food intake; MEI, metabolic energy intake; BW, body weight; BWG, body weight gain; BCS, body condition score.

Apparent total tract digestibility of minerals

Tables 4 and 5 show the effects of organic minerals on the average daily macro- (Table 4) and

Table 4. Effects of organic minerals on apparent total tract digestibility of macro minerals in adult beagle dogs

Items	Experiment diets		p-value ¹⁾
	IMD	OMD	
Average daily mineral intake (g/day)			
Potassium	1.33 ± 0.06	1.34 ± 0.04	0.937
Magnesium	0.28 ± 0.01	0.25 ± 0.01	0.046
Phosphorus	1.21 ± 0.05	1.29 ± 0.04	0.213
Sodium	0.26 ± 0.01	0.20 ± 0.01	< 0.010
Calcium	2.04 ± 0.09	2.08 ± 0.06	0.698
Average daily mineral excretion (Fecal) (g/day)			
Potassium	0.08 ± 0.02	0.03 ± 0.00	0.028
Magnesium	0.03 ± 0.00	0.03 ± 0.00	0.243
Phosphorus	0.84 ± 0.05	0.39 ± 0.02	< 0.010
Sodium	0.02 ± 0.00	0.01 ± 0.00	< 0.010
Calcium	1.24 ± 0.07	0.70 ± 0.02	< 0.010
ATTD (%)			
Potassium	93.69 ± 1.40	97.49 ± 0.23	0.032
Magnesium	90.72 ± 0.97	87.72 ± 0.39	0.018
Phosphorus	30.57 ± 3.12	69.69 ± 1.05	< 0.010
Sodium	91.52 ± 0.86	95.76 ± 0.50	< 0.010
Calcium	39.02 ± 2.17	65.98 ± 1.49	< 0.010

Values are expressed as mean ± SEM.

¹⁾p-values for comparisons between inorganic and organic minerals group in a same row.

IMD, inorganic mineral diet; OMD, organic mineral diet; ATTD, apparent total tract digestibility.

Table 5. Effects of organic minerals on apparent total tract digestibility of micro minerals in adult beagle dogs

Items	Experiment diets		p-value ¹⁾
	IMD	OMD	
Average daily mineral intake (mg/day)			
Iron	19.37 ± 0.84	13.86 ± 0.40	< 0.010
Zinc	19.98 ± 0.87	18.55 ± 0.53	0.185
Copper	2.17 ± 0.09	3.82 ± 0.11	< 0.010
Manganese	2.20 ± 0.10	2.51 ± 0.07	0.022
Average daily mineral excretion (Fecal) (mg/day)			
Iron	12.39 ± 1.28	2.25 ± 0.20	< 0.010
Zinc	7.90 ± 0.75	4.02 ± 0.15	< 0.010
Copper	2.07 ± 0.11	2.06 ± 0.07	0.982
Manganese	1.92 ± 0.11	0.80 ± 0.03	< 0.010
ATTD (%)			
Iron	36.81 ± 4.09	83.72 ± 1.51	< 0.010
Zinc	60.89 ± 2.13	78.35 ± 0.55	< 0.010
Copper	4.85 ± 2.33	45.80 ± 1.88	< 0.010
Manganese	12.59 ± 3.17	68.03 ± 0.67	< 0.010

Values are expressed as mean ± SEM.

¹⁾p-values for comparisons between inorganic and organic minerals group in a same row.

IMD, Inorganic mineral diet; OMD, Organic mineral diet; ATTD, apparent total tract digestibility.

micro- (Table 5) mineral intake, excretion (fecal), and ATTD. No significant differences were observed in average daily intake of K, P, Ca, and Zn between the two groups. For Na, Fe, and Mg, significantly lower ($p < 0.05$) intakes were observed in the OMD group than in the IMD group, and for Cu and Mn, significantly higher ($p < 0.05$) intakes were observed in the OMD group than in the IMD group. The average daily excretion of Mg and Cu showed no difference between the two groups, whereas K, P, Na, Ca, Fe, Zn, and Mn excretion were significantly lower ($p < 0.05$) in the OMD group than in the IMD group. The ATTD of macro- and micro-minerals was significantly higher ($p < 0.05$) in the OMD group than in the IMD group for K, P, Na, Ca, Fe, Zn, Cu, and Mn; whereas that of Mg was significantly lower ($p < 0.05$) in the OMD group than in the IMD group.

Apparent total tract digestibility of nutrients

The effects of organic minerals on the nutrient intake, excretion, and digestibility of beagle dogs are shown in Table 6. There was no difference in the average daily intake of organic matter (OM), dry matter (DM), CP, EE, CA, and NFE between the IMD and OMD groups. For CF, the OMD

Table 6. Effects of organic minerals on apparent total tract digestibility (ATTD) of nutrients in adult beagle dogs

Items	Experiment diets		p-value ¹⁾
	IMD	OMD	
Average daily intake (g/day)			
OM	185.25 ± 8.06	194.30 ± 5.54	0.373
DM	201.78 ± 8.78	212.28 ± 6.05	0.344
CP	65.64 ± 2.86	68.44 ± 1.95	0.434
EE	30.43 ± 1.32	30.61 ± 0.87	0.910
CF	1.03 ± 0.04	2.46 ± 0.07	< 0.010
CA	15.44 ± 0.67	15.50 ± 0.44	0.941
NFE	89.18 ± 3.88	95.27 ± 2.72	0.221
Average daily excretion (Fecal) (g/day)			
OM	9.72 ± 0.76	7.63 ± 0.54	0.042
DM	20.88 ± 1.28	19.57 ± 0.83	0.406
CP	4.64 ± 0.33	4.19 ± 0.22	0.278
EE	0.60 ± 0.09	1.26 ± 0.10	< 0.010
CF	0.96 ± 0.18	2.76 ± 0.41	< 0.010
CA	10.20 ± 0.58	9.18 ± 0.35	0.161
NFE	4.48 ± 0.42	2.17 ± 0.39	< 0.010
ATTD (%)			
OM	94.79 ± 0.22	96.09 ± 0.24	< 0.010
DM	89.66 ± 0.39	90.77 ± 0.32	0.045
CP	92.95 ± 0.31	93.86 ± 0.30	0.055
EE	98.07 ± 0.23	95.87 ± 0.35	< 0.010
CF	3.67 ± 18.49	−12.92 ± 17.84	0.529
CA	33.78 ± 2.74	40.62 ± 2.02	0.066
NFE	95.02 ± 0.30	97.76 ± 0.38	< 0.010

Values are expressed as mean ± SEM.

¹⁾p-values for comparisons between inorganic and organic minerals group in a same row.

IMD, Inorganic mineral diet; OMD, Organic mineral diet; ATTD, apparent total tract digestibility; OM, organic matter; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; CA, crude ash; NFE, nitrogen free extract.

group showed a significantly higher ($p < 0.05$) intake than did the IMD group. No differences were observed in the average daily excretion of DM, CP, and CA between the two groups. The excretion of OM and NFE was significantly lower ($p < 0.05$) in the OMD group than in the IMD group, whereas those of EE and CF were significantly higher ($p < 0.05$) in the OMD group than in the IMD group. The ATTD of OM, DM, and NFE was significantly higher ($p < 0.05$) in the OMD group than in the IMD group, whereas EE was significantly lower ($p < 0.05$) in the OMD group than in the IMD group. The ATTD of CP and CA tended to be higher (CP, $p = 0.055$; CA, $p = 0.066$) in the OMD group than in the IMD group.

Hematological and biochemical parameters

The serum biochemical parameters of all groups were within the normal reference range, and no significant differences in the values of these parameters were observed between the IMD and OMD groups (Tables 7 and 8). Meanwhile, the numbers of lymphocytes (LYM) and monocytes (MONO) measured at the end of each experiment were significantly lower ($p < 0.05$) in the OMD group than in the IMD group, whereas the number of basophils (BASO) was significantly higher ($p < 0.05$) in the OMD group than in the IMD group. Nevertheless, all hematological parameters, including LYM, MONO, and BASO, were within the normal reference range during the experimental period.

Table 7. Effects of organic minerals on serum biochemical parameters in adult beagle dogs

Items		Experiment diets		p-value ¹⁾
		IMD	OMD	
GLU (mg/dl) (Ref. range: 70–138)	Initial	101.00 ± 4.57	100.63 ± 2.89	0.946
	Final	97.38 ± 2.18	103.50 ± 3.07	0.126
CREA (mg/dl) (Ref. range: 0.5–1.6)	Initial	0.76 ± 0.03	0.78 ± 0.03	0.587
	Final	0.81 ± 0.04	0.77 ± 0.04	0.513
BUN (mg/dl) (Ref. range: 6.0–31)	Initial	15.65 ± 1.52	14.21 ± 1.17	0.466
	Final	12.73 ± 1.08	14.25 ± 1.05	0.328
T-PRO (g/dl) (Ref. range: 5–7.4)	Initial	6.28 ± 0.14	6.49 ± 0.18	0.368
	Final	6.55 ± 0.25	6.64 ± 0.17	0.777
ALB (g/dl) (Ref. range: 2.7–4.4)	Initial	2.98 ± 0.08	3.03 ± 0.09	0.685
	Final	3.01 ± 0.10	3.09 ± 0.09	0.594
GPT (IU/L) (Ref. range: 12–118)	Initial	40.88 ± 5.18	46.83 ± 5.89	0.460
	Final	42.63 ± 3.71	57.00 ± 11.01	0.236
GGT (U/L) (Ref. range: 0–12)	Initial	5.00 ± 1.18	5.13 ± 1.29	0.944
	Final	5.25 ± 1.29	5.13 ± 1.43	0.949
T-CHO (mg/dl) (Ref. range: 29–291)	Initial	258.63 ± 9.94	269.22 ± 7.81	0.443
	Final	264.14 ± 8.83	286.20 ± 3.83	0.074
TG (mg/dL) (Ref. range: 48–324)	Initial	87.57 ± 10.94	85.00 ± 13.11	0.882
	Final	68.50 ± 10.78	72.75 ± 12.85	0.807
NEFA (mEq/L) (Ref. range: 0.13–1.25)	Initial	0.63 ± 0.05	0.71 ± 0.05	0.303
	Final	0.80 ± 0.14	0.69 ± 0.05	0.475

Values are expressed as mean ± SEM.

¹⁾p-values for comparisons between inorganic and organic minerals group in a same row.

IMD, inorganic mineral diet; OMD, organic mineral diet; GLU, glucose; CREA, creatinine; BUN, blood urea nitrogen; T-PRO, total protein; ALB, albumin; GPT, glutamic pyruvic transaminase; GGT, gamma-glutamyl transferase; T-CHO, total cholesterol; TG, triglycerides; NEFA, non-esterified fatty acids.

Table 8. Effects of organic minerals on complete blood cell counts in adult beagle dogs

Items		Experiment diets		p-value ¹⁾
		IMD	OMD	
WBC ($\times 10^6/\text{mL}$) (Ref. range: 6.00–17.00)	Initial	6.89 $\pm$ 0.55	7.48 $\pm$ 0.55	0.456
	Final	8.73 $\pm$ 0.81	6.83 $\pm$ 0.63	0.085
NEU ($\times 10^3/\mu\text{L}$) (Ref. range: 3.62–12.30)	Initial	4.19 $\pm$ 0.57	4.94 $\pm$ 0.54	0.355
	Final	6.00 $\pm$ 0.80	4.62 $\pm$ 0.58	0.185
LYM ($\times 10^3/\mu\text{L}$) (Ref. range: 0.83–4.91)	Initial	2.16 $\pm$ 0.16	2.08 $\pm$ 0.12	0.716
	Final	2.27 $\pm$ 0.13	1.82 $\pm$ 0.11	0.020
MONO ($\times 10^3/\mu\text{L}$) (Ref. range: 0.14–1.97)	Initial	0.31 $\pm$ 0.03	0.23 $\pm$ 0.02	0.054
	Final	0.24 $\pm$ 0.02	0.16 $\pm$ 0.02	0.021
EOS ($\times 10^3/\mu\text{L}$) (Ref. range: 0.04–1.62)	Initial	0.22 $\pm$ 0.01	0.22 $\pm$ 0.02	0.986
	Final	0.23 $\pm$ 0.03	0.21 $\pm$ 0.03	0.617
BASO ($\times 10^3/\mu\text{L}$) (Ref. range: 0–0.12)	Initial	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.152
	Final	0.00 $\pm$ 0.00	0.01 $\pm$ 0.00	< 0.010
RBC ($\times 10^6/\mu\text{L}$) (Ref. range: 5.10–8.50)	Initial	7.85 $\pm$ 0.19	7.73 $\pm$ 0.20	0.650
	Final	7.80 $\pm$ 0.27	7.53 $\pm$ 0.17	0.415
HGB (g/dL) (Ref. range: 11–19)	Initial	17.74 $\pm$ 0.41	17.60 $\pm$ 0.42	0.813
	Final	17.89 $\pm$ 0.49	17.16 $\pm$ 0.39	0.265
HCT (%) (Ref. range: 33–56)	Initial	49.45 $\pm$ 1.10	48.41 $\pm$ 1.29	0.549
	Final	48.38 $\pm$ 1.67	47.40 $\pm$ 1.17	0.640
MCV (fL) (Ref. range: 60–76)	Initial	63.04 $\pm$ 0.90	62.72 $\pm$ 1.04	0.821
	Final	62.06 $\pm$ 0.95	63.06 $\pm$ 1.34	0.552
MCH (pg) (Ref. range: 20–27)	Initial	22.64 $\pm$ 0.38	22.80 $\pm$ 0.31	0.739
	Final	22.99 $\pm$ 0.27	22.79 $\pm$ 0.40	0.687
MCHC (g/dL) (Ref. range: 30–38)	Initial	35.89 $\pm$ 0.21	36.38 $\pm$ 0.20	0.114
	Final	37.08 $\pm$ 0.46	36.19 $\pm$ 0.23	0.104
RDW-CV (%) (Ref. range: 12.5–17.2)	Initial	13.50 $\pm$ 0.28	13.56 $\pm$ 0.33	0.894
	Final	13.51 $\pm$ 0.32	13.66 $\pm$ 0.39	0.771
RDW-SD (fL) (Ref. range: 33.2–46.3)	Initial	33.64 $\pm$ 0.70	33.74 $\pm$ 0.68	0.920
	Final	33.54 $\pm$ 0.70	34.04 $\pm$ 0.67	0.614
PLT ($10^3/\mu\text{L}$) (Ref. range: 117–490)	Initial	313.75 $\pm$ 29.23	324.17 $\pm$ 31.34	0.812
	Final	340.25 $\pm$ 24.74	318.50 $\pm$ 47.98	0.693
MPV (fL) (Ref. range: 8–14.1)	Initial	10.36 $\pm$ 0.30	9.93 $\pm$ 0.31	0.330
	Final	9.69 $\pm$ 0.30	9.74 $\pm$ 0.41	0.924
PCT (mL/L) (Ref. range: 0.9–5.8)	Initial	3.21 $\pm$ 0.24	3.15 $\pm$ 0.25	0.863
	Final	3.27 $\pm$ 0.18	2.97 $\pm$ 0.40	0.518

Values are expressed as mean $\pm$ SEM.

¹⁾p-values for comparisons between inorganic and organic minerals group in a same row.

IMD, inorganic mineral diet; OMD, organic mineral diet; WBC, white blood cell; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, Red blood cell distribution width-coefficient of variation; RDW-SD, Red blood cell distribution width-standard deviation; PLT, platelet; MPV, Mean platelet volume; PCT, plateletcrit.

DISCUSSION

Body parameters and feeding

This study indicated that feeding a diet containing four organic minerals (Fe-Gly, Mn-Gly, Zn-Gly, and Cu-Gly) did not affect food and energy intake, BW, and BCS in beagle dogs. Studies

evaluating the effects of organic minerals chelated with glycine on companion dogs are minimal. However, those evaluating the effects of some organic minerals chelated with organic substances (e.g., protein, organic acid, and organic compound) have reported that their supply does not affect changes in food intake, BW, or BCS [19,27–30]. Supplementation of organic minerals chelated with glycine exerts a positive effect on feed intake or BW increase in livestock such as broiler, pig, and mink [31–34]. These differential effects may result from differences in the physiological status of animals, such as age or growth. Variations in nutritional study methods could also contribute to these effects. For example, livestock studies provide unlimited diets, whereas studies on companion dogs typically limit diets to maintain an ideal BCS [35,36]. Nevertheless, the common result that feed intake and BW did not decrease by OMD suggests that feeding of organic minerals does not negatively affect palatability and nutrient metabolism of animal diet.

Apparent total tract digestibility of nutrients

In the present study, supplementation of Fe-Gly, Mn-Gly, Zn-Gly, and Cu-Gly showed positive effects on improving both macro and micro mineral digestibility compared to that by inorganic minerals. These effects may be owing to the contribution of organic minerals that are chemically stable during digestion. Organic minerals, when complexed with organic compounds, such as amino acids, proteins, and carbohydrates, may minimize interactions with dietary components during digestion and are highly effectively absorbed via amino acid or peptide transport pathways [16–18]. In particular, minerals chelated with glycine may minimize gastrointestinal interference, exhibiting higher ATTD than does the sulfate form [12]. Consistent with our findings, replacing inorganic minerals with organic minerals in weaned pigs or broiler breeders results in decreased excretion of Zn, Fe, and Mn [37,38]. This is probably because organic minerals can improve the bioavailability compared to that by inorganic minerals, thereby increasing the amount of absorbed minerals and their concentration in the circulatory system or tissues, thereby decreasing excretion [39,40]. Interestingly, in this study, although only Fe-Gly, Mn-Gly, Zn-Gly, and Cu-Gly were supplied, the digestibility of K, Na, and Ca increased, while that of Mg decreased. Organic minerals have high absorption rates, which can reduce the concentration of residual mineral ions in the intestines, thereby alleviating competition for absorption of other minerals such as K, Na, and Ca [41,42]. In particular, organic minerals can improve Ca digestibility by regulating intestinal pH and increasing the activity of the calcium-binding protein transient receptor potential cation channel subfamily V member 6 [43,44]. Additionally, Fe-Gly shows higher ameliorating effect than FeSO₄ on intestinal mucosal damage, thereby preserving the absorption surface area of the small intestine and contributing to improving the digestibility of K, Na, and Ca [45]. However, Mg is mainly absorbed in the large intestine, and its absorption is inhibited when that of Ca or Zn increases [46,47]. The high absorption rate of organic minerals may change the intestinal environment (pH or short-chain fatty acids), which reduces the absorption rate of Mg. Although a slight difference in CF content existed between the two treatments (0.51% vs. 1.16%), both levels were below 1.5%, which is far lower than the physiological threshold (above 5–10%) known to affect mineral absorption [48]. In addition, the fiber in this study mainly originated from insoluble plant-based ingredients, which are known to have a much lower mineral-binding capacity compared with soluble or fermentable fibers [15,49]. Both experimental diets were formulated with identical ingredients and nutrient levels, and the minor variation in CF is considered to be within the normal analytical variation of proximate analysis [50,51]. Therefore, the improvement in mineral digestibility observed in this study is interpreted as resulting from the chemical form of the mineral sources rather than from differences in fiber content.

In this study, organic minerals increased mineral digestibility, as well as OM, DM, and NFE

digestibility. Organic minerals likely improve digestibility by protecting the intestinal mucosa, increasing nutrient absorption surface area, and balancing intestinal microbiota, thereby optimizing the digestive environment [52,53]. Organic minerals reduce the concentrations of residual ions in the intestines compared to inorganic minerals [19]. This reduction minimizes competition for absorption among minerals and reduces binding between nutrients [54]. Consequently, enzyme activity and energy metabolism efficiency increase, thereby enhancing the decomposition and absorption of various nutrients [19]. This combined action suggests that the structure of organic minerals facilitates their absorption in the body, contributing to improved nutrient utilization in dogs.

Safety and blood parameters

In this study, all biochemical parameters were within the normal range after OMD and IMD supplementation, and no negative effects on changes in feces form were observed. This suggests that both forms of the mineral can maintain physiological stability in dogs. Minerals are found in bones, most tissues, and body fluids, and are essential for maintaining the function and structure of living tissues [4]. However, excessive supply of minerals causes cell damage through mechanisms similar to those of heavy metal toxicity, and minerals exceeding the recommended dietary amount may cause chronic toxicity owing to long-term accumulation [55,56]. Among blood biochemical parameters, normal maintenance of glutamic pyruvic transaminase, gamma-glutamyl transferase, albumin, and total protein, which are related to liver function, suggests that organic minerals do not cause liver toxicity compared to inorganic minerals. Although certain minerals, such as Cu, can cause liver damage when accumulated in excess [10,57], the copper content used in this study (10.75–18 mg/kg DM) is within both AAFCO and FEDIAF guidelines (minimum 7.3–8.3 mg/kg, maximum 28 mg/kg DM) and is considered safe for use in dogs [2,3]. Creatinine and blood urea nitrogen are key indicators for evaluating kidney function, and both indicators remained within the normal range, suggesting that the mineral form did not negatively affect kidney filtration [58]. Total cholesterol (T-CHO), triglycerides (TG), and non-esterified fatty acids (NEFA) in blood are parameters, which comprehensively reflect the lipid metabolic status, inflammation level, and endocrine function of dogs [59–61]. In this study, the maintenance of normal ranges of T-CHO, TG, and NEFA indicated that organic or inorganic minerals did not interfere with the lipid metabolic pathways. Zn is involved in regulating lipid metabolism [62]; however, in this study, no significant difference in lipid metabolism was observed between the OMD and IMD groups.

All hematological parameters were within the normal reference range during the experimental period; however, compared to those of the IMD group, LYM and MONO counts decreased in the OMD group, whereas BASO count increased. The exact mechanism by which organic minerals alter hematological parameters in dogs has not been elucidated. However, since minerals can influence key immune cell functions, including differentiation, activation, and cytokine production [63], these changes may be due to differences in immune cell differentiation and functional regulation mechanisms depending on the mineral form. Zn-proteinate in dietary form promotes T cell differentiation in beagle dogs, and the proportion of CD4⁺ T cells, the core component of adaptive immunity, increases compared to that by feeding ZnSO₄ [30]. Considering that all hematological values remained within the normal reference range, the slight decreases observed in LYM and MONO counts may be a related phenomenon associated with T cell proliferation rather than representing physiologically significant changes [64–66]. Given the crossover design employed in this study, further studies are warranted to elucidate the long-term effects and underlying mechanisms of organic mineral supplementation on immune response in dogs. The crossover design allowed for within-subject comparison, thereby reducing individual variability, but potential carryover effects and the limited treatment duration should be considered when interpreting the

results [67]. However, the finding that organic mineral supplementation did not exert any adverse effects on hematological or metabolic parameters, and that all values remained within normal ranges, highlights the significance of this study in demonstrating the safety of applying organic minerals in pet foods.

CONCLUSION

Results of this study showed that OMD did not have a negative effect on energy intake, BW, BCS, and fecal score in adult dogs. The OMD showed higher mineral (K, P, Na, Ca, Fe, Zn, Cu, and Mn) and nutrient (OM, DM, NFE, CP, and CA) digestibility than IMD. Serum biochemical parameters and hematological parameters were all within normal ranges and showed no negative effects of OMD feeding. In conclusion, OMD feeding can positively affect nutritional metabolism in adult dogs by improving mineral and nutrient digestibility without causing adverse effects from physiological, biochemical, or food nutritional perspectives. However, the additional physiological safety of OMD needs to be evaluated through long-term feeding tests.

REFERENCES

1. NRC (National Research Council). Nutrient requirements of dogs and cats.: The National Academies Press; 2006.
2. FEDIAF (The European Pet Food Industry). Nutritional guidelines: for complete and complementary pet food for cats and dogs. FEDIAF; 2021.
3. AAFCO (Association of American Feed Control Officials). Official publication. AAFCO; 2021.
4. McDowell LR. General introduction. In: McDowell LR, editor. Minerals in animal and human nutrition. 2nd ed. Elsevier; 2003. p. 1-32.
5. Davies M. Veterinary clinical nutrition: success stories: an overview. *Proc Nutr Soc.* 2016;75:392-7. <https://doi.org/10.1017/S002966511600029X>
6. McDowell LR. Minerals in animal and human nutrition. Academic Press Incorporated; 1992.
7. Tal M, Parr JM, MacKenzie S, Verbrugge A. Dietary imbalances in a large breed puppy, leading to compression fractures, vitamin D deficiency, and suspected nutritional secondary hyperparathyroidism. *Can Vet J.* 2018;59:36-42.
8. Davies M, Alborough R, Jones L, Davis C, Williams C, Gardner DS. Mineral analysis of complete dog and cat foods in the UK and compliance with European guidelines. *Sci Rep.* 2017;7:17107. <https://doi.org/10.1038/s41598-017-17159-7>
9. Cargo-Froom C. Mineral nutrition of adult canines: whole ingredients as a primary source of minerals instead of supplemental minerals [Ph.D. dissertation]. University of Guelph; 2018.
10. Pereira AM, Pinto E, Matos E, Castanheira F, Almeida AA, Baptista CS, et al. Mineral composition of dry dog foods: impact on nutrition and potential toxicity. *J Agric Food Chem.* 2018;66:7822-30. <https://doi.org/10.1021/acs.jafc.8b02552>
11. Kazimierska K, Biel W, Witkiewicz R. Mineral composition of cereal and cereal-free dry dog foods versus nutritional guidelines. *Molecules.* 2020;25:5173. <https://doi.org/10.3390/molecules25215173>
12. Liu Y, Ma YL, Zhao JM, Vazquez-Añón M, Stein HH. Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. *J Anim Sci.* 2014;92:3407-15. <https://doi.org/10.2527/jas.2013-7080>
13. Goi A, Simoni M, Righi F, Visentin G, de Marchi M. Application of a handheld near-infrared

- spectrometer to predict gelatinized starch, fiber fractions, and mineral content of ground and intact extruded dry dog food. *Animals*. 2020;10:1660. <https://doi.org/10.3390/ani10091660>
14. Powell JJ, Whitehead MW, Ainley CC, Kendall MD, Nicholson JK, Thompson RPH. Dietary minerals in the gastrointestinal tract: hydroxypolymerisation of aluminium is regulated by luminal mucins. *J Inorg Biochem*. 1999;75:167-80. [https://doi.org/10.1016/S0162-0134\(99\)00094-X](https://doi.org/10.1016/S0162-0134(99)00094-X)
 15. Byrne L, Murphy RA. Relative bioavailability of trace minerals in production animal nutrition: a review. *Animals*. 2022;12:1981. <https://doi.org/10.3390/ani12151981>
 16. Du Z, Hemken RW, Harmon RJ. Copper metabolism of holstein and jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *J Dairy Sci*. 1996;79:1873-80. [https://doi.org/10.3168/jds.S0022-0302\(96\)76555-4](https://doi.org/10.3168/jds.S0022-0302(96)76555-4)
 17. Aldridge BE, Saddoris KL, Radcliffe JS. Copper can be absorbed as a Cu-peptide chelate through the PepT1 transporter in the jejunum of weanling pigs. *J Dairy Sci*. 2007;90:154-5.
 18. Liu S, Lu L, Li S, Xie J, Zhang L, Wang R, et al. Copper in organic proteinate or inorganic sulfate form is equally bioavailable for broiler chicks fed a conventional corn-soybean meal diet. *Biol Trace Elem Res*. 2012;147:142-8. <https://doi.org/10.1007/s12011-012-9329-5>
 19. Trevizan L, Fischer MM, Rodenbusch CR, Labres RV, de Mello Kessler A. Effects of diets containing organic and inorganic zinc sources on hair characteristics, zinc concentration in blood and hair, and the immune response of dogs. *Acta Sci Vet*. 2013;41:1154.
 20. Dobenecker B, Reese S, Herbst S. Effects of dietary phosphates from organic and inorganic sources on parameters of phosphorus homeostasis in healthy adult dogs. *PLOS ONE*. 2021;16:e0246950. <https://doi.org/10.1371/journal.pone.0246950>
 21. Seo K, Cho HW, Chun J, Jeon J, Kim C, Kim M, et al. Evaluation of fermented oat and black soldier fly larva as food ingredients in senior dog diets. *Animals*. 2021;11:3509. <https://doi.org/10.3390/ani11123509>
 22. Seo K, Cho HW, Lee MY, Kim CH, Kim KH, Chun JL. Prediction of apparent total tract digestion of crude protein in adult dogs. *J Anim Sci Technol*. 2024;66:374-86. <https://doi.org/10.5187/jast.2024.e20>
 23. Laflamme D. Developmental and validation of a body condition score system for dogs. *Canine Pract*. 1997;22:10-5.
 24. Moxham G. Waltham feces scoring system—a tool for veterinarians and pet owners: how does your pet rate. *Waltham Focus*. 2001;11:24-5.
 25. AOAC (Association of Official Analytical Chemists International). Official methods of analysis of AOAC International. 18th ed. AOAC International; 2006.
 26. Kim KH, Ishizaki N, Iguchi E, Funaba M, Matsui T. Effect of magnesium deficiency on various mineral concentrations in rat liver. *Biol Trace Elem Res*. 2011;144:865-71. <https://doi.org/10.1007/s12011-011-9042-9>
 27. Wedekind KJ, Lowry SR. Are organic zinc sources efficacious in puppies? *J Nutr*. 1998;128:S2593-5. <https://doi.org/10.1093/jn/128.12.2593S>
 28. Guo R, Henry PR, Holwerda RA, Cao J, Littell RC, Miles RD, et al. Chemical characteristics and relative bioavailability of supplemental organic copper sources for poultry. *J Anim Sci*. 2001;79:1132-41. <https://doi.org/10.2527/2001.7951132x>
 29. van Heugten E, Spears JW, Kegley EB, Ward JD, Qureshi MA. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. *J Anim Sci*. 2003;81:2063-71. <https://doi.org/10.2527/2003.8182063x>
 30. Pereira AM, Guedes M, Matos E, Pinto E, Almeida AA, Segundo MA, et al. Effect of zinc source and exogenous enzymes supplementation on zinc status in dogs fed high phytate diets. *Animals*. 2020;10:400. <https://doi.org/10.3390/ani10030400>

31. Wang Y, Tang JW, Ma WQ, Feng J, Feng J. Dietary zinc glycine chelate on growth performance, tissue mineral concentrations, and serum enzyme activity in weanling piglets. *Biol Trace Elem Res*. 2010;133:325-34. <https://doi.org/10.1007/s12011-009-8437-3>
32. Cui H, Zhang TT, Nie H, Wang ZC, Zhang XL, Shi B, et al. Effects of sources and concentrations of zinc on growth performance, nutrient digestibility, and fur quality of growing-furring female mink (*Mustela vison*). *J Anim Sci*. 2017;95:5420-9. <https://doi.org/10.2527/jas2017.1810>
33. Jarosz Ł, Marek A, Grądzki Z, Kwiecień M. Effects of dietary supplementation of iron as sulphates or glycine chelates on the productive performance and concentrations of acute-phase proteins and iron in the serum and liver tissues of broiler chickens. *Ann Anim Sci*. 2021;21:267-90. <https://doi.org/10.2478/aoas-2020-0069>
34. Lei H, Du Q, Lu N, Jiang X, Li M, Xia D, et al. Comparison of the microbiome-metabolome response to copper sulfate and copper glycinate in growing pigs. *Animals*. 2023;13:345. <https://doi.org/10.3390/ani13030345>
35. Bray EE, Zheng Z, Tolbert MK, McCoy BM, Dog Aging Project Consortium, Kaeblerlein M, et al. Once-daily feeding is associated with better health in companion dogs: results from the Dog Aging Project. *GeroScience*. 2022;44:1779-90. <https://doi.org/10.1007/s11357-022-00575-7>
36. Nam J, Kim JN, Kim HB, Cho JH, Kim Y, Ahn J, et al. Effects of dietary aluminosilicate on growth performance, frequency of diarrhea, and blood profiles of weaned pigs. *J Anim Sci Technol*. 2025;67:375-82. <https://doi.org/10.5187/jast.2024.e21>
37. Wang G, Liu L, Wang Z, Pei X, Tao W, Xiao Z, et al. Comparison of inorganic and organically bound trace minerals on tissue mineral deposition and fecal excretion in broiler breeders. *Biol Trace Elem Res*. 2019;189:224-32. <https://doi.org/10.1007/s12011-018-1460-5>
38. Zhang WF, Tian M, Song JS, Chen F, Lin G, Zhang SH, et al. Effect of replacing inorganic trace minerals at lower organic levels on growth performance, blood parameters, antioxidant status, immune indexes, and fecal mineral excretion in weaned piglets. *Trop Anim Health Prod*. 2021;53:121. <https://doi.org/10.1007/s11250-021-02561-1>
39. Burkett JL, Stalder KJ, Powers WJ, Bregendahl K, Pierce JL, Baas TJ, et al. Effect of inorganic and organic trace mineral supplementation on the performance, carcass characteristics, and fecal mineral excretion of phase-fed, grow-finish swine. *Asian-Australas J Anim Sci*. 2009;22:1279-87. <https://doi.org/10.5713/ajas.2009.70091>
40. Yenice E, Mızrak C, Gültekin M, Atik Z, Tunca M. Effects of organic and inorganic forms of manganese, zinc, copper, and chromium on bioavailability of these minerals and calcium in late-phase laying hens. *Biol Trace Elem Res*. 2015;167:300-7. <https://doi.org/10.1007/s12011-015-0313-8>
41. Ghasemi HA, Hajkhodadadi I, Hafizi M, Taherpour K, Nazaran MH. Effect of advanced chelate technology based trace minerals on growth performance, mineral digestibility, tibia characteristics, and antioxidant status in broiler chickens. *Nutr Metab*. 2020;17:94. <https://doi.org/10.1186/s12986-020-00520-5>
42. Zhang R, Wei M, Zhou J, Yang Z, Xiao M, Du L, et al. Effects of organic trace minerals chelated with oligosaccharides on growth performance, blood parameters, slaughter performance and meat quality in sheep. *Front Vet Sci*. 2024;11:1366314. <https://doi.org/10.3389/fvets.2024.1366314>
43. Meena AS, Shukla PK, Bell B, Giorgianni F, Caires R, Fernández-Peña C, et al. TRPV6 channel mediates alcohol-induced gut barrier dysfunction and systemic response. *Cell Rep*. 2022;39:110937. <https://doi.org/10.1016/j.celrep.2022.110937>
44. Khattar V, Wang L, Peng JB. Calcium selective channel TRPV6: structure, function, and

- implications in health and disease. *Gene*. 2022;817:146192. <https://doi.org/10.1016/j.gene.2022.146192>
45. Gao Q, Zhang Y, Wu Y, Gu D, Chen J, Yin C, et al. Dietary Fe-Gly supplementation attenuates enterotoxigenic *Escherichia coli* (ETEC)-induced inflammation response and intestinal barrier dysfunction in piglets. *Front Vet Sci*. 2025;12:1537604. <https://doi.org/10.3389/fvets.2025.1537604>
 46. Cargo-Froom CL, Fan MZ, Pfeuti G, Pendlebury C, Shoveller AK. Apparent and true digestibility of macro and micro nutrients in adult maintenance dog foods containing either a majority of animal or vegetable proteins. *J Anim Sci*. 2019;97:1010-9. <https://doi.org/10.1093/jas/skz001>
 47. Stojiljković S, Stojiljković S, Riess SP. The importance of minerals in the health of dogs. *EC Nutr*. 2023;18:1-10.
 48. Montserrat-Malagarriga M, Castillejos L, Salas-Mani A, Torre C, Martín-Orúe SM. The impact of fiber source on digestive function, fecal microbiota, and immune response in adult dogs. *Animals*. 2024;14:196. <https://doi.org/10.3390/ani14020196>
 49. Pinna C, Vecchiato CG, Bolduan C, Grandi M, Stefanelli C, Windisch W, et al. Influence of dietary protein and fructooligosaccharides on fecal fermentative end-products, fecal bacterial populations and apparent total tract digestibility in dogs. *BMC Vet Res*. 2018;14:106. <https://doi.org/10.1186/s12917-018-1436-x>
 50. AOAC (Association of Official Analytical Chemists International). Guidelines for standard method performance requirements. AOAC International; 2019.
 51. Hill RC, Choate CJ, Scott KC, Molenberghs G. Comparison of the guaranteed analysis with the measured nutrient composition of commercial pet foods. *J Am Vet Med Assoc*. 2009;234:347-51. <https://doi.org/10.2460/javma.234.3.347>
 52. Barroso C, Fonseca AJM, Cabrita ARJ. Vitamins, minerals and phytonutrients as modulators of canine immune function: a literature review. *Vet Sci*. 2024;11:655. <https://doi.org/10.3390/vetsci11120655>
 53. Xu W, Zhou M, Yang Z, Zheng M, Chen Q. Organic trace elements enhance growth performance, antioxidant capacity, and gut microbiota in finishing pigs. *Front Vet Sci*. 2024;11:1517976. <https://doi.org/10.3389/fvets.2024.1517976>
 54. Pereira AM, Maia MRG, Pinna C, Biagi G, Matos E, Segundo MA, et al. Effects of zinc source and enzyme addition on the fecal microbiota of dogs. *Front Microbiol*. 2021;12:688392. <https://doi.org/10.3389/fmicb.2021.688392>
 55. Zafalon RVA, Perini MP, Vendramini THA, Pedrinelli V, Rentas MF, Morilha IB, et al. Vitamin-mineral supplements do not guarantee the minimum recommendations and may imply risks of mercury poisoning in dogs and cats. *PLOS ONE*. 2021;16:e0250738. <https://doi.org/10.1371/journal.pone.0250738>
 56. Kępińska-Pacelik J, Biel W, Witkiewicz R, Podsiadło C. Mineral and heavy metal content in dry dog foods with different main animal components. *Sci Rep*. 2023;13:6082. <https://doi.org/10.1038/s41598-023-33224-w>
 57. Amundson LA, Kirn BN, Swensson EJ, Millican AA, Fahey GC. Copper metabolism and its implications for canine nutrition. *Transl Anim Sci*. 2024;8:txad147. <https://doi.org/10.1093/tas/txad147>
 58. Fielder SE. Serum biochemical analysis reference ranges. MSD veterinary manual. Rahway, NJ: Merck and Company Incorporated; 2024.
 59. Chen X, Zhang W, Tian Y, Wang L, Chen C, Qiu C. Liraglutide suppresses non-esterified free fatty acids and soluble vascular cell adhesion molecule-1 compared with metformin in patients

- with recent-onset type 2 diabetes. *Cardiovasc Diabetol*. 2018;17:53. <https://doi.org/10.1186/s12933-018-0701-4>
60. Sieber-Ruckstuhl NS, Tham WK, Baumgartner F, Selva JJ, Wenk MR, Burla B, et al. Serum lipidome signatures of dogs with different endocrinopathies associated with hyperlipidemia. *Metabolites*. 2022;12:306. <https://doi.org/10.3390/metabo12040306>
 61. Kim TW, Kang MH, Park HM. Lipid metabolism alterations in hyperlipidemic dogs with biliary tract or endocrine diseases. *Animals*. 2025;15:256. <https://doi.org/10.3390/ani15020256>
 62. Zohal M, Jam-Ashkezari S, Namiranian N, Moosavi A, Ghadiri-Anari A. Association between selected trace elements and body mass index and waist circumference: a cross sectional study. *Diabetes Metab Syndr Clin Res Rev*. 2019;13:1293-7. <https://doi.org/10.1016/j.dsx.2019.01.019>
 63. Alghamdi M, Gutierrez J, Komarnytsky S. Essential minerals and metabolic adaptation of immune cells. *Nutrients*. 2022;15:123. <https://doi.org/10.3390/nu15010123>
 64. Karamitros D, Kotantaki P, Lygerou Z, Kioussis D, Taraviras S. T cell proliferation and homeostasis: an emerging role for the cell cycle inhibitor geminin. *Crit Rev Immunol*. 2011;31:209-31. <https://doi.org/10.1615/critrevimmunol.v31.i3.30>
 65. Wang YS, Chi KH, Liao KW, Liu CC, Cheng CL, Lin YC, et al. Characterization of canine monocyte-derived dendritic cells with phenotypic and functional differentiation. *Can J Vet Res*. 2007;71:165-74.
 66. Ricklin Gutzwiller ME, Moulin HR, Zurbriggen A, Roosje P, Summerfield A. Comparative analysis of canine monocyte- and bone-marrow-derived dendritic cells. *Vet Res*. 2010;41:40. <https://doi.org/10.1051/vetres/2010012>
 67. Lim CY, In J. Considerations for crossover design in clinical study. *Korean J Anesthesiol*. 2021;74:293-9. <https://doi.org/10.4097/kja.21165>