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## 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 $Ca(IO_3)_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, and $CuSO_4$; the OMD included $Ca(IO_3)_2$, 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, nitrogen-free extract, crude protein, and crude ash) 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 (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, 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 (22–24℃) 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 diets twice daily at an amount individually estimated by the maintenance energy requirement equation (Eq. 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 $Ca(IO_3)_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, and $CuSO_4$; the organic mineral diet (OMD) included $Ca(IO_3)_2$, 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, 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 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, NJ, USA) 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 (2000 ×g, 10 min) to collect serum samples that were stored at -80℃ 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:

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

and

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

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, Siheung, Republic of Korea) and acid-decomposed at 190℃ 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, Kyoto, Japan). 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:

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

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

**Statistical analysis**

All statistical analyses were performed using SPSS v.17.0 (SPSS Statistics, IL, USA). Data are presented as mean ± 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.

## ATTD of minerals

Tables 4 and 5 show the effects of organic minerals on the average daily macro- (Table 4) and micro- (Table 5) mineral intake, excretion (fecal), and ATTD. No significant differences were observed in average daily intake of K, Mg, P, Ca, and Zn between the two groups. For Na and Fe, 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, Mg, 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.



## ATTD 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 group showed a significantly higher ($p < 0.05$) digestibility 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.

## 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.

### ATTD 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 $FeSO_4$ 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 $ZnSO_4$ [30]. Considering that all hematological values remained within the normal reference range, the slight decreases observed in LYM and MONO counts may be a possible 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.

# Competing Interests

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

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## Tables

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[4)], DM % (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 $D_3$; 25 mg of vitamin E; 0.052 mg of vitamin K; 2.8 mg of vitamin $B_1$ (thiamine); 2.6 mg of vitamin $B_2$ (riboflavin); 2 mg of vitamin $B_6$ (pyridoxine); 0.014 mg of vitamin $B_{12}$; 6 mg of Cal-d-pantothenate; 30 mg of niacin; 0.4 mg of folic acid; 0.036 mg of biotin; 1000 mg of taurine. [2)]Inorganic mineral diet was supplied per kilogram of diets at 44 mg of $FeSO_4$; 3.8 mg of $MnSO_4$; 50 mg of $ZnSO_4$; 7.5 mg of $CuSO_4$; 0.9 mg of $Ca(IO_3)_2$. [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 $Ca(IO_3)_2$. [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.

Abbreviations: 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 (K) | 6.60 | 6.30 |
| Magnesium (Mg) | 1.40 | 1.18 |
| Phosphorus (P) | 6.00 | 6.10 |
| Sodium (Na) | 1.30 | 0.92 |
| Calcium (Ca) | 10.10 | 9.80 |
| Ca:P ratio | 1.68 | 1.61 |
| **Micro minerals, mg/kg** | | |
| Iron (Fe) | 96.01 | 65.30 |
| Zinc (Zn) | 99.00 | 87.37 |
| Copper (Cu) | 10.75 | 18.00 |
| Manganese (Mn) | 10.89 | 11.82 |

Values are analyzed value as DM based.
Abbreviations: IMD, Inorganic mineral diet; OMD, Organic mineral diet;

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

| Items | Experiment diets | | *p*-value[1] |
|---|---|---|---|
| | IMD | OMD | |
| ADFI, g/day | 288 ± 12.6 | 303 ± 8.6 | 0.344 |
| MEI, kcal/day | 802 ± 34.9 | 833 ± 23.8 | 0.466 |
| BW, kg | | | |
| Initial | 12.6 ± 0.7 | 12.8 ± 0.5 | 0.856 |
| Final | 12.9 ± 0.7 | 12.9 ± 0.5 | 0.935 |
| *p* value[2] | 0.831 | 0.844 | |
| BWG, g | 22.8 ± 7.1 | 13.8 ± 10.5 | 0.489 |
| BCS | | | |
| Initial | 5.0 ± 0.4 | 5.1 ± 0.4 | 0.812 |
| Final | 5.0 ± 0.4 | 5.1 ± 0.4 | 0.812 |
| Fecal score[3] | 3.0 ± 0.0 | 2.9 ± 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 ± standard error of the mean (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 days) of each experimental diet.

Abbreviations: 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.

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

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

Values are expressed as mean ± standard error of the mean (SEM).
*p*-values for comparisons between inorganic and organic minerals group in a same row.
Abbreviations: IMD, Inorganic mineral diet; OMD, Organic mineral diet; ATTD, apparent total tract digestibility.

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

| Items | Experiment diets | | *p*-value[1] |
|---|---|---|---|
| | IMD | OMD | |
| **Average daily mineral intake, mg/day** | | | |
| Iron (Fe) | 19.37 ± 0.84 | 13.86 ± 0.40 | < 0.01 |
| Zinc (Zn) | 19.98 ± 0.87 | 18.55 ± 0.53 | 0.185 |
| Copper (Cu) | 2.17 ± 0.09 | 3.82 ± 0.11 | < 0.01 |
| Manganese (Mn) | 2.20 ± 0.10 | 2.51 ± 0.07 | 0.022 |
| **Average daily mineral excretion (Fecal), mg/day** | | | |
| Iron (Fe) | 12.39 ± 1.28 | 2.25 ± 0.20 | < 0.01 |
| Zinc (Zn) | 7.90 ± 0.75 | 4.02 ± 0.15 | < 0.01 |
| Copper (Cu) | 2.07 ± 0.11 | 2.06 ± 0.07 | 0.982 |
| Manganese (Mn) | 1.92 ± 0.11 | 0.80 ± 0.03 | < 0.01 |
| **ATTD, %** | | | |
| Iron (Fe) | 36.81 ± 4.09 | 83.72 ± 1.51 | < 0.01 |
| Zinc (Zn) | 60.89 ± 2.13 | 78.35 ± 0.55 | < 0.01 |
| Copper (Cu) | 4.85 ± 2.33 | 45.80 ± 1.88 | < 0.01 |
| Manganese (Mn) | 12.59 ± 3.17 | 68.03 ± 0.67 | < 0.01 |

Values are expressed as mean ± standard error of the mean (SEM).
*p*-values for comparisons between inorganic and organic minerals group in a same row.
Abbreviations: IMD, Inorganic mineral diet; OMD, Organic mineral diet; ATTD, apparent total tract digestibility.

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

| Items | Experiment diets IMD | | | Experiment diets OMD | | | *p*-value |
|---|---|---|---|---|---|---|---|
| **Average daily intake, g/day** | | | | | | | |
| Organic matter (OM) | 185.25 | ± | 8.06 | 194.30 | ± | 5.54 | 0.373 |
| Dry matter (DM) | 201.78 | ± | 8.78 | 212.28 | ± | 6.05 | 0.344 |
| Crude protein (CP) | 65.64 | ± | 2.86 | 68.44 | ± | 1.95 | 0.434 |
| Ether extract (EE) | 30.43 | ± | 1.32 | 30.61 | ± | 0.87 | 0.910 |
| Crude fiber (CF) | 1.03 | ± | 0.04 | 2.46 | ± | 0.07 | < 0.01 |
| Crude ash (CA) | 15.44 | ± | 0.67 | 15.50 | ± | 0.44 | 0.941 |
| Nitrogen free extract (NFE) | 89.18 | ± | 3.88 | 95.27 | ± | 2.72 | 0.221 |
| **Average daily excretion (Fecal), g/day** | | | | | | | |
| Organic matter (OM) | 9.72 | ± | 0.76 | 7.63 | ± | 0.54 | 0.042 |
| Dry matter (DM) | 20.88 | ± | 1.28 | 19.57 | ± | 0.83 | 0.406 |
| Crude protein (CP) | 4.64 | ± | 0.33 | 4.19 | ± | 0.22 | 0.278 |
| Ether extract (EE) | 0.60 | ± | 0.09 | 1.26 | ± | 0.10 | < 0.01 |
| Crude fiber (CF) | 0.96 | ± | 0.18 | 2.76 | ± | 0.41 | < 0.01 |
| Crude ash (CA) | 10.20 | ± | 0.58 | 9.18 | ± | 0.35 | 0.161 |
| Nitrogen free extract (NFE) | 4.48 | ± | 0.42 | 2.17 | ± | 0.39 | < 0.01 |
| **ATTD, %** | | | | | | | |
| Organic matter (OM) | 94.79 | ± | 0.22 | 96.09 | ± | 0.24 | < 0.01 |
| Dry matter (DM) | 89.66 | ± | 0.39 | 90.77 | ± | 0.32 | 0.045 |
| Crude protein (CP) | 92.95 | ± | 0.31 | 93.86 | ± | 0.30 | 0.055 |
| Ether extract (EE) | 98.07 | ± | 0.23 | 95.87 | ± | 0.35 | < 0.01 |
| Crude fiber (CF) | 3.67 | ± | 18.49 | -12.92 | ± | 17.84 | 0.529 |
| Crude ash (CA) | 33.78 | ± | 2.74 | 40.62 | ± | 2.02 | 0.066 |
| Nitrogen free extract (NFE) | 95.02 | ± | 0.30 | 97.76 | ± | 0.38 | < 0.01 |

Values are expressed as mean ± standard error of the mean (SEM).
*p*-values for comparisons between inorganic and organic minerals group in a same row.
Abbreviations: IMD, Inorganic mineral diet; OMD, Organic mineral diet; ATTD, apparent total tract digestibility.

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

| Items | | Experiment diets | | *p*-value |
|---|---|---|---|---|
| | | 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, IU/L (Ref. range: 23 – 102) | 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 ± standard error of the mean (SEM).
*p*-values for comparisons between inorganic and organic minerals group in a same row.
Abbreviations: 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 |
|---|---|---|---|---|
| | | IMD | OMD | |
| WBC, $\times 10^6$/mL (Ref. range: 6.00–17.00) | Initial | 6.89 ± 0.55 | 7.48 ± 0.55 | 0.456 |
| | Final | 8.73 ± 0.81 | 6.83 ± 0.63 | 0.085 |
| NEU, $\times 10^3$/uL (Ref. range: 3.62–12.30) | Initial | 4.19 ± 0.57 | 4.94 ± 0.54 | 0.355 |
| | Final | 6.00 ± 0.80 | 4.62 ± 0.58 | 0.185 |
| LYM, $\times 10^3$/uL (Ref. range: 0.83–4.91) | Initial | 2.16 ± 0.16 | 2.08 ± 0.12 | 0.716 |
| | Final | 2.27 ± 0.13 | 1.82 ± 0.11 | 0.020 |
| MONO, $\times 10^3$/uL (Ref. range: 0.14–1.97) | Initial | 0.31 ± 0.03 | 0.23 ± 0.02 | 0.054 |
| | Final | 0.24 ± 0.02 | 0.16 ± 0.02 | 0.021 |
| EOS, $\times 10^3$/uL (Ref. range: 0.04–1.62) | Initial | 0.22 ± 0.01 | 0.22 ± 0.02 | 0.986 |
| | Final | 0.23 ± 0.03 | 0.21 ± 0.03 | 0.617 |
| BASO, $\times 10^3$/uL (Ref. range: 0–0.12) | Initial | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.152 |
| | Final | 0.00 ± 0.00 | 0.01 ± 0.00 | $< 0.01$ |
| RBC, $\times 10^6$/uL (Ref. range: 5.10–8.50) | Initial | 7.85 ± 0.19 | 7.73 ± 0.20 | 0.650 |
| | Final | 7.80 ± 0.27 | 7.53 ± 0.17 | 0.415 |
| HGB, g/dL (Ref. range: 11–19) | Initial | 17.74 ± 0.41 | 17.60 ± 0.42 | 0.813 |
| | Final | 17.89 ± 0.49 | 17.16 ± 0.39 | 0.265 |
| HCT, % (Ref. range: 33–56) | Initial | 49.45 ± 1.10 | 48.41 ± 1.29 | 0.549 |
| | Final | 48.38 ± 1.67 | 47.40 ± 1.17 | 0.640 |
| MCV, fL (Ref. range: 60–76) | Initial | 63.04 ± 0.90 | 62.72 ± 1.04 | 0.821 |
| | Final | 62.06 ± 0.95 | 63.06 ± 1.34 | 0.552 |
| MCH, pg (Ref. range: 20–27) | Initial | 22.64 ± 0.38 | 22.80 ± 0.31 | 0.739 |
| | Final | 22.99 ± 0.27 | 22.79 ± 0.40 | 0.687 |
| MCHC, g/dL (Ref. range: 30–38) | Initial | 35.89 ± 0.21 | 36.38 ± 0.20 | 0.114 |
| | Final | 37.08 ± 0.46 | 36.19 ± 0.23 | 0.104 |
| RDW-CV, % (Ref. range: 12.5–17.2) | Initial | 13.50 ± 0.28 | 13.56 ± 0.33 | 0.894 |
| | Final | 13.51 ± 0.32 | 13.66 ± 0.39 | 0.771 |
| RDW-SD, fL (Ref. range: 33.2–46.3) | Initial | 33.64 ± 0.70 | 33.74 ± 0.68 | 0.920 |
| | Final | 33.54 ± 0.70 | 34.04 ± 0.67 | 0.614 |
| PLT, $10^3$/uL (Ref. range: 117–490) | Initial | 313.75 ± 29.23 | 324.17 ± 31.34 | 0.812 |
| | Final | 340.25 ± 24.74 | 318.50 ± 47.98 | 0.693 |
| MPV, fL (Ref. range: 8–14.1) | Initial | 10.36 ± 0.30 | 9.93 ± 0.31 | 0.330 |
| | Final | 9.69 ± 0.30 | 9.74 ± 0.41 | 0.924 |
| PCT, mL/L (Ref. range: 0.9–5.8) | Initial | 3.21 ± 0.24 | 3.15 ± 0.25 | 0.863 |
| | Final | 3.27 ± 0.18 | 2.97 ± 0.40 | 0.518 |

Values are expressed as mean ± standard error of the mean (SEM).
*p*-values for comparisons between inorganic and organic minerals group in a same row.
Abbreviations: 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.