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Effects of Diets Containing Organic and Inorganic Zinc Sources on Hair Characteristics, Zinc Concentration in Blood and Hair, and the Immune Response of Dogs

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ABSTRACT

***Background*:** There are two commercial forms of zinc, organic and inorganic, and its form may influence absorption and utilization by the animals. The inorganic form dissociates to Zn^{2+} in the gastrointestinal tract and can interact with other substances that the animal cannot absorb. The interest in using organic minerals has increased because of the reported potential of higher bioavailability compared to inorganic sources. In dogs, little research has been done concerning the bioavailability of organic and inorganic mineral sources. This study compared the effects of diets containing organic and inorganic zinc on hair characteristics, zinc concentration in blood and hair, and the influence of organic minerals on the immune response of dogs.

***Materials, Methods & Results*:** Eighteen healthy adult dogs of different breeds (Labrador, German Shepherd, Malinois Shepherd, and Rottweiler) were separated into two groups of nine animals per treatment and fed diets for 30 days. Two diets were used: an inorganic mineral source diet (IMSD) and an organic mineral source diet (OMSD). Four parameters were evaluated (days -14, 0, 10, 20, 30) to determine the hair characteristics: brightness, texture, looseness, and greasiness. On days 0 and 30, two blood samples were taken and two hair samples were collected to measure zinc concentrations. On the 10th day of the trial period, the animals received an injection of 4 mL of a 10% solution of sheep red blood cells (SRBC) subcutaneously as a stimulus to assess the humoral response. Blood samples were taken prior to injection as well as on days 10 and 20 post-injection for antibody titer. All dogs consumed adequate amount of the diets and body weight did not change during the experiment. The daily dry matter, energy, and zinc consumption did not differ between groups. The zinc concentration in the blood remained stable throughout the experiment in animals fed the OMSD but decreased significantly in the animals fed the IMSD ($P = 0.0145$). The zinc concentration in hair increased ($P = 0.0075$) in dogs fed the OMSD, while the dogs fed the IMSD had no difference. The consumption of the OMSD resulted in higher brightness in the hair of the face, muzzle, armpit, back, abdomen, and tail compared with the IMSD. The animals fed the chelated mineral-supplemented diet also showed better texture in hair of the abdomen ($P = 0.0327$), chest ($P = 0.0335$), and tail ($P = 0.0291$). The parameters looseness and greasiness showed no significant differences. No differences were observed in the production of antibodies against SRBC between groups; however, the antibody concentration was maintained throughout the experiment in the animals supplemented with OMSD, in contrast to the animals supplemented with IMSD, which reduced their antibody concentration.

***Discussion*:** There is controversy in the literature regarding the benefits of organic over inorganic zinc source. This may be due to two factors: the time of zinc supplementation, and the levels of zinc included in the diets, which varies in each experiment. In the present study, both criteria were adequate for the responses evaluated and the results suggest that organic zinc is suitable for supplementation into the commercial dog foods and could enhance the hair characteristics of adult dogs. Moreover, the improvement in immune status, although subtle, must be taken into account. It should be noted that the diets were formulated with high levels of minerals, especially calcium, which increases the possibility of interactions and competition in the absorptive process, increasing the challenge and impairing absorption of divalent minerals.

Keywords: bioavailability, organic zinc source, inorganic zinc source, immune response, hair characteristics.

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INTRODUCTION

Organic trace minerals are forms in which the mineral is complexed or chelated to an organic ligand, such as an amino acid, polysaccharide, or organic acid [18]. It is known that certain minerals such as selenium, chromium, and iron presented as an organic form are better utilized than inorganic forms [9]. For other minerals such as zinc, there is controversy concerning the degree of availability of organic vs. inorganic forms. In most commercial dog foods, inorganic Zn form - such as $ZnSO_4$ or ZnO - are the most commonly used, but these forms dissociate more into Zn^{2+} in the gastrointestinal tract and interact with other substances, which reduces Zn absorption by the animal [17,18]. Furthermore, the competition between other divalent cations (e.g., Ca^{2+}, Cu^{2+}, and Fe^{2+}) can inhibit zinc absorption in the intestinal lumen [3]. Conversely, the organic mineral in chelated form is stable in the wide range of pH encountered in different segments of the digestive tract, so it does not dissociate before reaching the absorption site [14].

The effects of zinc on the immune system have been studied in several experiments in various species and there is controversy regarding the results. There is a lack of information regarding the effect of zinc on immunity of dogs. The objectives of this study were to compare the bioavailability of organic and inorganic mineral forms in dogs by evaluating hair characteristics, Zn concentrations in blood and hair, and the immune system response.

MATERIALS AND METHODS

Animals and diets

Eighteen adult dogs of different breeds (Labrador, German Shepherd, Malinois Shepherd, and Rottweiler), intact males and females, aged between 2 and 6 years, and body weight (BW) 33.7±1.27 kg were used in the experiment. The dogs belonged to the State Army Kennel, and were trained to rescue people and to detect drugs and explosives. During the trial they were maintained performing the same activities they were trained for. Health of the dogs was assessed before the start of the study and at the end by clinical and hematologic examination. Serum biochemical profiles, including urea, creatinine, alkaline phosphatase, alanine aminotransferase, total plasma protein, and albumin were also determined. All dogs were considered healthy. The metabolizable energy (ME) of the diets was estimated from their chemical composition, and the quantity of diet provided was initially calculated using standard equations to determine proper energy requirements for dog maintenance (ME, kcal = 140 x kg $BW^{0.75}$) in accordance with the NRC [10] guidelines. Dogs were weighed every 10 days, and food supply was individually adjusted to achieve constant BW during the experiment. Water was available *ad libitum*. The experiment was conducted with two groups of nine dogs each (six males and three females) assigned to two diets in a randomized assay. Two diets were used (Table 1): an inorganic mineral source diet (IMSD) and an organic mineral source diet (OMSD). Diets were formulated in accordance with the AAFCO [1] nutrient guide for adult dogs to be balanced and to meet maintenance requirements. Except for four minerals (Zn, Se, Cu, and Mn) the diets were formulated containing the same ingredients. The IMSD was made by adding inorganic mineral premix, while the OMSD was made by replacing part of the mineral requirements by organic minerals. The organic source (Mintrex®) contributed with 40, 40, 0,3 and 20 mg of Zn, Mn, Se, Cu per kg of diet, respectively, and the same amount was supplied in the IMSD by inorganic sources (zinc oxide, manganese sulfate, sodium selenite, copper sulfate). The replacement of minerals was made by adding 1kg/t Mintrex®, which also contains 2-hydroxy 4-(methylthio) butanoic acid (HMTBa). The HMTBa is a precursor of methionine, and the IMSD was supplied with same inclusion of HMTBa (Table 1). After manufacture, the diets were analyzed by the analytic laboratory of the manufacturer and found to be within expected analytic variance of these target values. All dogs received the IMSD 14 days prior to the onset of the feeding trial (adaptation period). During the adaptation, the diet acceptance was tested by measuring the consumption of energy and variation in the BW. The experimental period was begun when dogs were weighted and half of them were maintained on the same diet and the others were fed with the OMSD for 30 days.

Table 1. Ingredient and nutrient composition of inorganic mineral source diet (IMSD) and organic mineral source diet (OMSD) for adult dogs.

Ingredient (%)	IMSD	OMSD
Brewers rice	34.20	34.20
Poultry byproduct meal 60%	21.00	21.00
Corn grain	15.00	15.00
Meat and bone meal 45%	12.00	12.00
Wheat bran	10.00	10.00
Poultry fat	3.00	3.00
Flavor coating	1.90	1.90
Soybean oil	1.50	1.50
Sodium cloride	0.50	0.50
Linseed	0.40	0.40
Yeast	0.10	0.10
Mineral premix	0.10[1]	0.10[2]
Mintrex®[3]	0.00	0.10
HMTBa 50%[4]	0.10	0.00
Yucca extract	0.04	0.04
Vitamin premix[5]	0.03	0.03
Condroitine	0.02	0.02
Glicosamine	0.01	0.01
Artificial dye	0.01	0.01
Composition Analysed		
Dry Matter (%)	93.90	92.20
Crude Protein (%)	24.06	24.79
Ether Extract (Acid hidrolysys) (%)	10.87	10.25
Crude Fiber (%)	1.91	1.58
Ash (%)	9.07	8.95
Calcium (%)	2.02	2.05
Phosphorus (%)	1.45	1.42
ME (kcal/g)[6]	3.67	3.61
Zinc (mg/kg)[7]	138.78	147.20
Manganese (mg/kg)[7]	62.07	59.15
Copper (mg/kg)[7]	38.47	38.84
Selenium (mg/kg)[8]	0.41	0.41
HMTBa (%)[4]	0.032	0.064

[1]Mineral premix content added per kg of diet: ferrous sulfate 267 mg; calcium iodate 2.42 mg; zinc oxide 158 mg; copper sulfate 78 mg; sodium selenite 0.91 mg; manganese sulfate 75 mg. [2]Mineral premix content added per kg of diet: ferrous sulfate 267 mg; calcium iodate 2.42 mg; zinc oxide 105 mg; copper sulfate 21 mg; sodium selenite 0.24 mg; manganese sulfate 8.3 mg. [3]Mintrex® PSe containing organic mineral: zinc HMTBa 40 mg of Zn; manganese HMTBa 40 mg of Mn; Se Yeast 0,3 mg of Se; Copper HMTBa 20 mg of Cu, added per kg of diet. [4]2-Hydroxy-4-(Methylthio) Butanoic Acid. [5]Vitamin premix content added per kg of diet: vitamin A 9,000 UI; vitamin D_3 800 UI; vitamin E 50 mg; vitamin K_3 4 mg; thiamine 7 mg; riboflavin 5 mg; pyridoxine 5 mg; vitamin B_{12} 25 mcg; pantothenate 10 mg; nicotinic acid 50 mg; folic acid 1 mg; biotin 70 mcg; [6]Metabolizable energy estimated according to NRC [10]. [7]Value obtained by Atomic absorption method. [8]Calculated value.

Zinc concentration in whole blood

On the first day of the experimental period and 30 days after feeding, blood was drawn directly from the saphenous vein into a 6 mL Vaccutainer tube containing EDTA. The concentration of zinc in the total blood was measured by inductively coupled plasma mass spectrometry after nitric acid digestion in a pressurized tube warmed by microwaves (ICP -AES) (IRIS-AP - Thermo Jarrell Ash). Values are expressed in µg of Zn per µg of whole blood.

Zinc concentration in hair

The thorax ventral-lateral and cranial-caudal hair was shaved (5x10 cm), 14 days prior to the start of feeding experimental diets to keep 20 mm in length of the hair in the area. On day 0, hair was shaved to

3 mm in length in the area and the sample was collected in paper bags for analysis. After 30 days, the local area was totally shaved and the hair was collected again and both samples were sent to the lab to measure Zn concentration. The Zn concentration was measured after washing hair in Triton X, rinsing with acetone and then deionized water. Nitric acid digestion of the hair was performed, followed by mass spectrometry analysis (IRIS-AP - Thermo Jarrell Ash).

In vivo antibody production

On the 10th day, dogs were injected subcutaneously with 1mL of 10% suspension of sheep red blood cells (SRBC) in four different points: each side of the lateral thorax and each side of the flank, totaling 4 mL. Blood samples were taken prior to the injections as well as on days 20 and 30, and serum was separated by centrifugation and frozen at -20ºC until analysis. Serum antibody titers to SRBC were determined according to the procedure of Nunes [11]. Data were expressed as log base 2 of the reciprocal of the titer dilution.

Hair evaluation

The hair was evaluated 14 days previous to the start of feeding, and on days 0, 10, 20, and 30 of the experimental assay. Two veterinarian evaluators were blinded to the diets consumed by the groups to perform the subjective evaluations. The characteristics (brightness, texture, looseness, and greasiness) were assessed by the observers, who scored the hair of dogs from 1 to 3, where 1 = worst hair characteristics, and 3 = best hair characteristics. The dog's body was divided into regions to perform the evaluations: muzzle, chest, face and ears, ventral abdomen, armpits, shoulders, thoracic members, pelvic members, back, croup, and tail.

Statistical analyses

Values are reported as mean ± SEM. All data, except for the antibody titers against SRBC (anti-SRBC), were normally distributed as determined by the use of the Shapiro-Wilk test [12]. The anti-SRBC values were transformed by arc sin. The skin and hair evaluations were averaged between evaluators to perform the analysis. All data were submitted to ANOVA to compare diets. For all variables, the Tukey multiple comparison of means was performed. For all analyses, values of $P < 0.05$ were considered significant.

RESULTS

All dogs consumed adequate amount of the diets and BW did not change during the experiment. The daily dry matter (DM), energy, and zinc consumption did not differ between groups as shown in Table 2.

Table 2. Dry matter and energy consumption, zinc intake of dogs fed inorganic mineral source diet (IMSD) and organic mineral source diet (OMSD).

Intake, x kg $(BW^{0.75})^{-1}$ d^{-1}	IMSD	OMSD	SEM	P-value
Dry matter (g)	38.06	35.30	2.45	0.4386
Energy (kcal)	144	134	9.30	0.4326
Zinc (mg)	5.62	5.63	0.38	0.9836

Zinc concentration values in whole blood and hair are shown in Figure 1. The zinc concentration in the blood remained stable throughout the experiment in animals fed the OMSD but decreased significantly in the animals fed the IMSD (P = 0.0145). The zinc concentration in hair increased (P = 0.0075) from day 0 to 30 in the animals fed the OMSD, while the animals fed the IMSD had no difference.

The animals fed the OMSD showed a higher value of brightness in the hair of the face, muzzle, armpit, back, abdomen, and tail (Figure 2) compared with the IMSD. The animals fed the chelated mineral-supplemented diet also showed a better texture in the abdomen (P = 0.0327), chest (P = 0.0335), and tail hair (P = 0.0291). The parameters looseness and greasiness showed no significant differences.

As shown in Figure 3, after injection with sheep red blood cells, the antibody production was similar between groups, however, from day 20 to 30 the concentration decreased in dogs fed the IMSD (P = 0.0183).

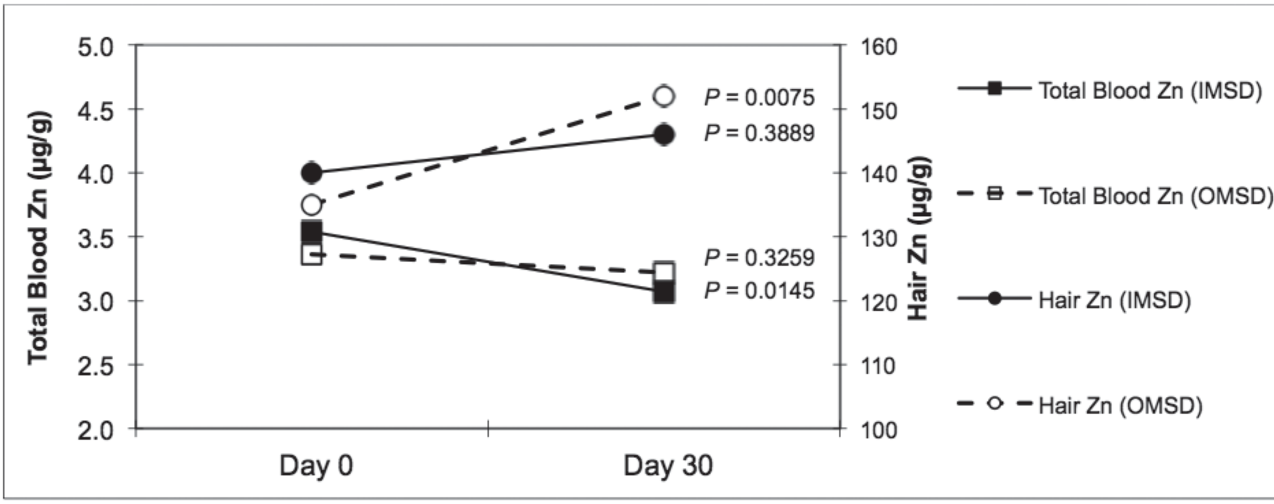

Figure 1. Zinc concentration in whole blood (µg/g) and hair (µg/g) on days 0 and 30 of dogs fed organic mineral source diet (OMSD) and inorganic mineral source diet (IMSD).

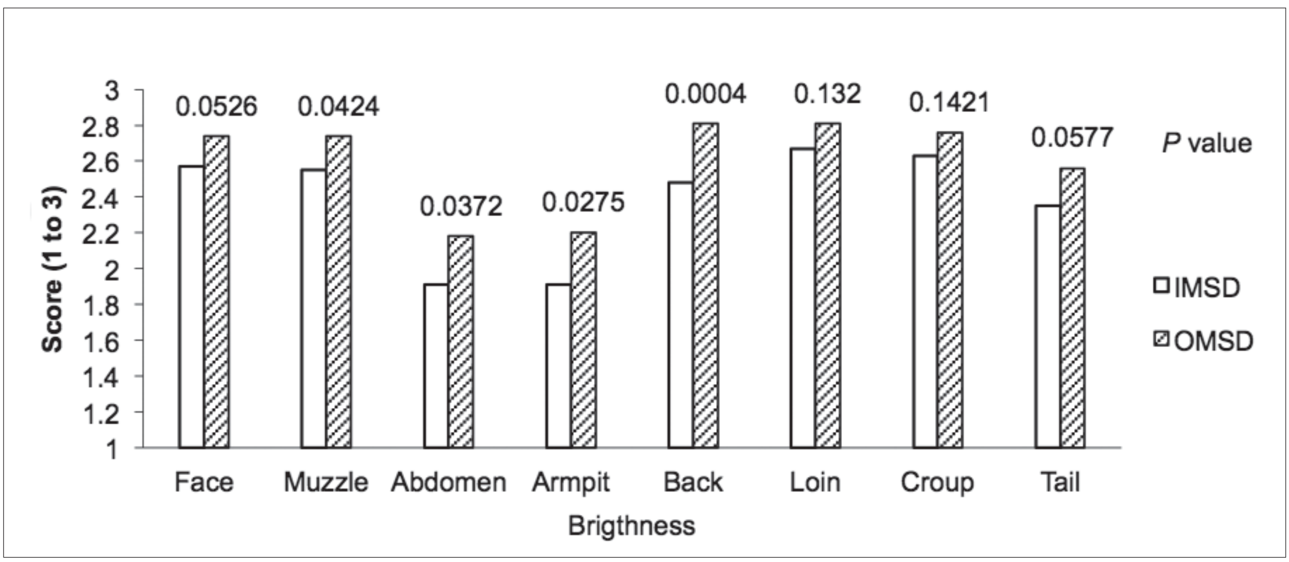

Figure 2. Brightness of face, muzzle, abdomen, armpit, back, loin, croup, and tail of dogs fed organic mineral source diet (OMSD) and inorganic mineral source diet (IMSD).

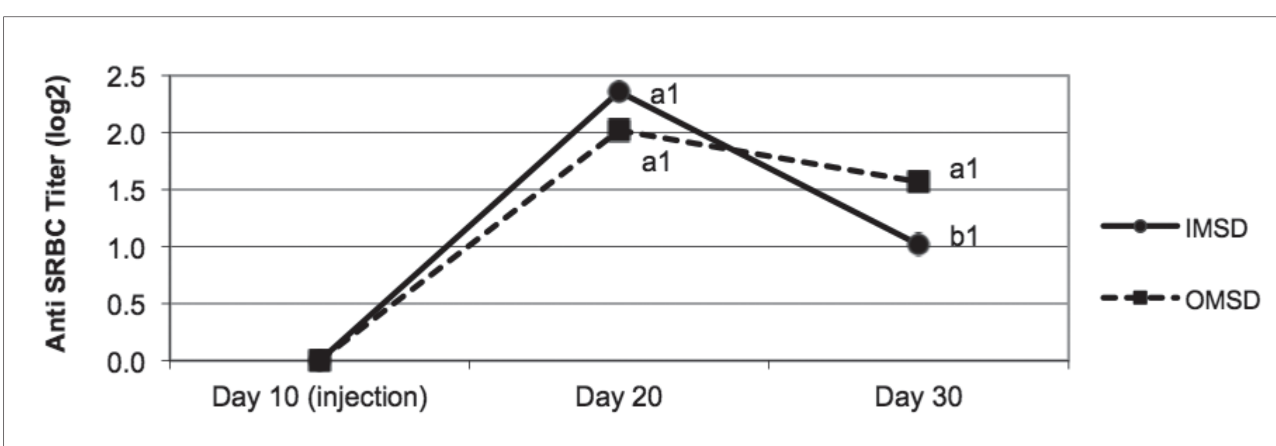

Figure 3. Titer of anti-SRBC on days 10 (injection), 20, and 30 of dogs fed organic mineral source diet (OMSD) and inorganic mineral source diet (IMSD). Different letters show the difference between days in each diet. Different numbers show the difference in each day between diets.

DISCUSSION

The zinc concentration in the blood of dogs fed OMSD remained stable throughout the experiment, while the concentration in the blood of dogs fed IMSD decreased significantly. Other studies in dogs have been conducted comparing Zn bioavailability between organic and inorganic Zn sources, however the most recent publication was in 2008 [4]. Lowe *et al*. [6] demonstrated that when chelated Zn was fed to dogs, it was absorbed better and retained in the body longer than the inorganic form of this mineral. Thus, minor inclusions of mineral organic forms are necessary to meet essential requirements, which reduce the interactions between minerals in the gut and improve the absorptive process. These authors reported that feeding dogs with a zinc amino acid chelate diet had greater plasma Zn concentration than dogs fed a zinc oxide diet. Other authors have found that the bioavailability of organic zinc (Zn propionate) is 60-80% greater than inorganic zinc (ZnO) in puppies [16]. It should be noted that, in this study, diets were formulated with high levels of minerals (considering minerals present in the ingredients + mineral premix + supplementation), which increases the possibility of interactions in the absorptive mechanism, increasing the challenge and impairing absorption, mainly in the IMSD. It is important to emphasize that the levels of calcium used in the diets are in accordance with practical diets. If diets had been formulated with reduced levels of minerals, especially calcium, perhaps the zinc concentration in the blood of dogs fed IMSD would have been improved.

Regarding the zinc deposition in hair, no differences were observed between groups. However, organic zinc supplementation resulted in a significant increase of zinc in hair while the inorganic zinc diet did not alter the zinc concentration in hair during the study. Some authors found greater Zn deposition in hair with organic zinc compared to inorganic zinc. Jamikorn & Preedapattarapong [4] reported that ZnMG (zincmethionylglycinate, organic source) supplementation resulted in greater ($P < 0.05$) Zn deposition than $ZnSO_4$ in dogs (zinc sulfate, inorganic source). Lowe *et al*. [7] reported similar results; they found that the Zn deposition in hair was greater ($P < 0.05$) in dogs fed diets containing zinc amino acid chelate than dogs fed a ZnO diet. These results seem to indicate that animals can use organic zinc better than inorganic zinc. Conversely, no differences were observed in hair samples taken from bitches fed control or chelated mineral-supplemented diets [5]. On the other hand, they reported differences between treatments in hair follicle condition when they evaluated the hair taken from the dogs supplemented with chelated mineral, which appeared smoother and less fragmented than hair from dogs fed an inorganic diet. This is in agreement with the present study, which observed that the brightness and the texture was higher and better, respectively, in some parts of the body of dogs fed the OMSD compared to the IMSD. No studies comparing brightness were found, perhaps because brightness and smoothness parameters are related. In a more recent study, the hair of dogs that received ZnMG supplement appeared to be smoother and less fragmented than the hair of dogs that received $ZnSO_4$ supplement [4].

The antibody production against SRBC was similar between groups, however, from day 20 to 30 the concentration decreased in dogs fed the IMSD, demonstrating that the OMSD has an effect on the persistence of antibodies, thus improving the immune status. The effect of zinc on the immune system has been studied in several experiments in various species. In cattle, researchers found a better immune response when Zn propionate was added to the diet versus Zn sulfate [8]. In a study with guinea pigs', the authors reported that supplementation of 20 ppm of zinc in the diet significantly improved the immune response, and the impact was more prominent when an organic source was used compared to an inorganic source [13]. However, all the effects reported in this study may not be addressed only to the organic zinc added to the diet once organic copper, manganese and selenium were also included. Also, the time of supplementation in each experiment as well as the inclusion levels of zinc may cause controversial results. Researchers found in their studies that the antibody response to sheep red blood cells in pigs was not affected by supplementation of Zn at different levels (80-160 ppm) in a basal diet, regardless of whether zinc was supplied as an organic or inorganic source [2,15]. In this study, no differences were observed in the production of antibodies against SRBC between groups supplemented with 40 ppm OMSD. However, the antibody concentration was maintained throughout the experiment in the animals supplemented with OMSD, in contrast to the animals supplemented with IMSD, which had reduced antibody concentrations.

CONCLUSION

Organic zinc supplementation showed some benefits in adult dogs. The results of this study suggest that organic Zn is suitable for supplementation into commercial dog foods and may enhance the haircoat characteristics and improve the immune status of adult dogs.

SOURCE AND MANUFACTURER

[1]Mintrex®, Novus International Inc., 20 Research Park Drive, St. Charles, MO 63304, USA.

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Ethical approval. All procedures involving animals were in accordance with Brazilian guidelines and approved by the Ethics Committee of the Federal University of Rio Grande do Sul.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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