Comparative Effects of Zinc Methionylglycinate and Zinc Sulfate on Hair Coat Characteristics and Zinc Concentration in Plasma, Hair, and Stool of Dogs

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Abstract

There are two form of Zn supplement used in commercial dog foods, organic and inorganic forms. These forms can influence Zn absorption and utilization of the animals. The goal of the current study was to evaluate the effects of zinc methionylglycinate (ZnMG) compared to zinc sulfate ($ZnSO_4$) supplementations in commercial dog foods on haircoat characteristics, and Zn concentration in plasma, hair, and stool of the dogs. Eight mature female beagles were randomly divided into two groups of four dogs each. A Cross-over design was used for this study. The treatments composed of 120 ppm Zn supplement of either ZnMG or $ZnSO_4$. A commercial dry dog food formulated with no Zn supplementation (only from raw materials) was used as the basal diet. Each experimental period lasted 5 wk with the first 2 wk as adaptation period and the last 3 wk as time of Zn supplement. Blood samples were collected for the measurement of serum ALP activity and plasma Zn concentration. Hair was shaved and used to analyze for Zn deposition. Haircoat characteristics were determined under electron microscope. The dogs supplemented with ZnMG had greater hair growth rate, level of Zn deposition in hair, serum ALP activity, amount of Zn absorption ($p<0.05$), and plasma Zn concentration ($p<0.10$). The hair of the dogs received ZnMG supplement appeared to be smoother and lesser fragmented than the dogs received $ZnSO_4$ supplement. In conclusion, the organic Zn as ZnMG was found to be the form that could enhance the haircoat characteristics and suitable for supplementation into the commercial dry dog foods.

บทคัดย่อ

การเปรียบเทียบผลของสังกะสีเมทไธโอนิลไกลซีเนทและสังกะสีซัลเฟต ต่อลักษณะขน และ ปริมาณสังกะสีในพลาสมา ขนและมูลของสุนัข

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สังกะสีที่ใช้เติมในอาหารสำเร็จรูปสำหรับสุนัขมีอยู่สองรูปแบบ คือ สังกะสีอินทรีย์และสังกะสีอนินทรีย์ รูปแบบของแร่ธาตุ
นี้สามารถมีผลต่อการดูดซึมและการนำไปใช้ประโยชน์ของตัวสัตว์ การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมสังกะสี
เมทไธโอนิลไกลซีเนทเปรียบเทียบกับสังกะสีซัลเฟต ในอาหารสำเร็จรูปสำหรับสุนัข ต่อลักษณะขน และปริมาณสังกะสีในพลาสมา
ขน และมูลของสุนัข กลุ่มทดสอบมี 2 กลุ่ม ประกอบด้วย กลุ่มที่ได้รับการเสริมสังกะสีเมทไธโอนิลไกลซีเนทเปรียบเทียบและกลุ่ม
ที่ได้รับการเสริมสังกะสีซัลเฟต ที่ระดับ 120 พีพีเอ็ม ในสุนัขพันธุ์บีเกิ้ล เพศเมีย ที่โตเต็มที่ จำนวน 8 ตัว ทำการสุ่มแบ่งสุนัขออก
เป็น 2 กลุ่มๆ ละ 4 ตัว วางแผนการทดลองเป็นแบบเปลี่ยนสลับ อาหารสำเร็จรูปชนิดแห้งสำหรับสุนัขที่ผลิตขึ้นโดยมีเพียงสังกะสี
ที่เป็นองค์ประกอบของวัตถุดิบที่ใช้ในการผลิตอาหารเท่านั้นเป็นอาหารพื้นฐาน ระยะเวลาทดลองในแต่ละช่วงนาน 5 สัปดาห์ โดย
2 สัปดาห์แรกเป็นระยะปรับตัวที่สุนัขได้รับเพียงอาหารพื้นฐานเท่านั้น ส่วนใน 3 สัปดาห์หลัง สุนัขจะได้รับอาหารพื้นฐานพร้อม
การเสริมสังกะสีแบบใดแบบหนึ่ง ทำการเก็บตัวอย่างเลือดเพื่อตรวจวัดประสิทธิภาพการทำงานของเอนไซม์อัลคาไลน์ฟอสฟาเตส
และปริมาณสังกะสี ทำการเก็บตัวอย่างขน โดยการโกนเพื่อตรวจวัดปริมาณของสังกะสีที่สะสมและตรวจลักษณะที่ปรากฏภายใต้
กล้องจุลทรรศน์อิเลคตรอน สุนัขกลุ่มที่ได้รับการเสริมด้วยสังกะสีเมทไธโอนิลไกลซีเนทมีอัตราการเจริญของขน ปริมาณสังกะสีที่
สะสมในขน ประสิทธิภาพการทำงานของเอนไซม์อัลคาไลน์ฟอสฟาเตส การดูดซึมสังกะสี ($p < 0.05$) และปริมาณสังกะสีในพลาสมา
($p < 0.10$) สูงกว่า และมีสภาพเส้นขนที่มีเกล็ดขนเรียงตัวราบเรียบดีกว่าและแตกน้อยกว่าสุนัขกลุ่มที่ได้รับการเสริมด้วยสังกะสี
ซัลเฟต สรุปได้ว่า สังกะสีอินทรีย์ในรูปแบบของสังกะสีเมทไธโอนิลไกลซีเนทเป็นรูปแบบที่เหมาะจะใช้เสริมในอาหารสำเร็จรูป
สำหรับสุนัข เนื่องจากการเสริมสังกะสีเมทไธโอนิลไกลซีเนทสามารถช่วยปรับปรุงสภาพเส้นขน โดยทำให้สุนัขมีอัตราการเจริญ
ของขนและปริมาณสังกะสีในพลาสมาและขนที่สูงกว่า และมีสภาพเส้นขนที่ดีกว่าการเสริมด้วยสังกะสีซัลเฟต

คำสำคัญ : สุนัข เส้นขน พลาสมา มูล สังกะสี

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Introduction

Zinc, a micromineral, presents in the body and the diet at level less than 100 ppm (Hellman and Carlson, 2003). It†is widely distributed in many tissues of the body (McDonald, 1995). Zinc is important for many metabolic function, necessary for the maintenance and cofactor of metalloenzymes in all six classes (Case et al., 2000; Kidd et al., 1996). Zinc deficiency occur in the dogs from several causes such as genetic defect that results in diminished intestinal Zn absorption, rapidly growing puppies fed Zn-deficient diet or diets containing substances which prevents the absorption and utilization of Zn, dog food that produced from raw materials which have low amount Zn and varies, and raw materials containing an antinutrition factor such as phytic acids. The first clinical signs of Zn deficiency have been described as alopecia, dull, coarse hair coat, and focal erythemia encircle the eyes, ears, nose, mouth and pressure points (Case et al., 2000; Colombini, 1999). Therefore, Zn is added to most of commercial dog foods to meet the animal requirement. Assessment of Zn status in animal is considered complicate. Concentration of Zn in plasma has been denigrated as a measure of zinc status because it responds to metabolic conditions unrelated to zinc status and because it is insensitive to changes in dietary zinc (King, 1990).

There are two form of Zn supplement used in the commercial dog foods, organic and inorganic forms. These forms can have influence on the absorption and utilization of Zn. At present, inorganic Zn such as $ZnSO_4$ or ZnO are the popular forms commonly used in most commercial dog foods. However, these forms can dissociate to Zn^{2+} in the gastrointestinal tract (GIT) and interact with other substances (e.g., phytic acid) resulting in the formation of strong and insoluble complexes that animal cannot absorb (Wilaison, 2002). Moreover, divalent cations (e.g., Ca^{2+}, Cu^{2+} and Fe^{2+}) can inhibited Zn absorption possibly due to these cations compete one another for binding ligands in the intestinal lumen or within the cell as well as for receptor sites on the brush border of the enterocytes (Gropper et al., 2005). Only organic mineral in chelated form is stable in a wide pH range encountered within the different segments of the digestive tract and so it does not dissociate before reaching the absorption site (Vandergrift, 1994). It can be absorbed as an intact molecule (Ashmead, 1992). An amino acid which is bound to mineral and acts as a carrier is used to transport that mineral across the intestinal enterocyte and into the circulation.

Nowadays, most animal feed industries are interesting in organic Zn including dog foods industries. However, a few research studies on organic Zn supplementation for dog foods are limited. The hypothesis of the present study is that organic Zn could be utilized more efficiently than inorganic Zn regarding it absorbability. Therefore, the dogs supplemented with ZnMG should have better haircoat characteristics, greater Zn concentration in plasma and hair, greater Zn absorption, but lower Zn excretion in stool than the dogs supplemented with $ZnSO_4$. The objectives of this experiment was to evaluate the effects of ZnMG supplement compared to $ZnSO_4$ supplement on hair coat characteristics and Zn concentration in plasma, hair, and stool of dogs.

Materials and Methods

Animals: Eight mature female beagles with body weight of 8.7±0.4 kg were used in the study. The Cross-over design was used as an experimental design. All dogs were housed individually in the metal cages (1.0 x 1.2-m) with a plastic slat floor at temperature between 24.9 and 31.4°C. Each cage was cleaned twice daily. In the pretest period, all dogs were fed a basal diet for 2 wk. The purpose of this period was to reduce the variability of Zn stores of the dogs. At the end of this pretest period, four dogs were randomly allotted to one of two treatments. However, dogs receiving the basal diet would be removed from the test if sign of Zn deficiency were severe. The dogs were fed the basal diets with Zn supplement in the form of solution for 3 wk (test period). Fresh water was available ad libitum throughout the experiment.

Feed and feeding: The commercial extruded dog diet formulated with no Zn supplementation in accordance with the AAFCO (2000) nutrient guide for adult dog was used as the basal diet. The calculated values and chemical analysis of nutrient composition in basal diet are presented in Table 1. This basal diet contained Zn at 58.50 ppm DM as composition of raw materials. Treatments composed of Zn supplementation to meet the minimum requirement for maintenance (120 ppm DM) according to AAFCO (2000) either in the form of ZnMG (20% Zn; 2 mol Met-Gly: 1 mol Zn) or $ZnSO_4$ (35% Zn; $ZnSO_4 \cdot H_2O$). Each form of Zn was prepared as solution by dissolving ZnMG or $ZnSO_4$ with deionized water. The amount of food was calculated by using standard equations to determine energy requirements of active adult dogs (Case et al., 2000) and adjusted every 2 wk. Everyday food was weighed and divided into two equal portions and fed to the dogs at 0700 and 1530 h in stainless steel bowls. Zinc solution was served at 15 min after offering each meal.

Data collection:

Data collection of both experiment 1 and 2 were similar. Throughout of the experiment, BW of dogs was recorded twice weekly for adjusting the amount of food. Food intake was measured daily.

Table 1 Calculated values and chemical analysis of nutrient composition in the basal diet (DM basis).

Item	Calculation	Analysis
Metabolizable energy (kcal/g)	3.63	3.67[*]
Crude protein (%)	29.64	25.79
Ether extract (%)	9.04	7.64
Crude fiber (%)	1.12	0.81
Ash (%)	8.21	5.54
Calcium (%)	1.23	0.95
Phosphorus (%)	1.05	0.80
Zinc (ppm)	31.33	58.50

[*]Calculated by use of equation from NRC (2006): ME, kcal/g = [(3.5 x CP) + (8.5 x EE) + (3.5 x NFE)]/100

Sample collection and determination:

Sample collection and determination were as follow:

Food: Throughout the study, the food sample was collected daily and pooled into plastic bags and stored at -20ºC until nutrient content analysis. Food sample was ground through a 1-mm screen mill (cyclotec 1093 sample mill). It was analyzed in duplicate for DM, CP, EE, CF, ash, Ca and P using AOAC (1990) procedures. Concentrations of Zn were determined by atomic absorption spectrophotometer (AAS; Model spectr AA - 300) using Sullivan and Carpenter (1993) method.

Blood: At d 14 and d 35, 6 ml of blood was collected from cephalic vein. These collections were performed between 3 and 4 h after offering the meal at 0700 h. Blood samples 5 ml were collected into heparinized polypropylene (PP) tubes and placed on ice then centrifuged at 2000g for 15 mins at 6ºC (modified from Brinkhaus et al., 1998). Another 1 ml of blood samples were collected into nonheparinized PP tubes then placed on ice until it was analyzed for serum ALP activity. The separated plasma was stored at -20ºC in 5 ml PP tubes until plasma Zn analysis was performed. Plasma was prepared for analysis of Zn concentration by diluting plasma with deionized water (modified from Wedekind et al., 1994) using AAS. Serum ALP activity was determined by automated analyzer (BT2000Plus, Biotecnica Instrument S.p.A.) and test kit Cat. No. AD711AP and AD701AP (Audit diagnostics).

Hair: A patch of similar colored hair was shaved from the dogís neck using a 20 cm^2 template on the day before the beginning of the test period (d 14). Thereafter the same patch was shaved on d 35. The hair was handled, collected in plastic bag, weighed and stored at room temperature until Zn analysis was performed. At d 35, chest hair was shaved using a 10 cm^2 template and collected in plastic bag for analysis of hair condition. Neck hair samples were weighed for determination of hair growth rate (Lowe and Wiseman, 1998) and analyzed for Zn concentrations by AAS using Van den Broek (1988) method. The Zn deposition in hair values were calculated by equation: Zn deposition in hair (μg/21d · 20cm^2)=[Zn concentration in neck hair sample (μg/g)] x [weight of neck hair sample (μg/21d·20cm^2)]. Chest hair samples were screened and photographed under scanning electron microscopy photographic (JSM-5800LV, JEOL) using Kuhlman and Rompala (1998) method.

Stool: All glassware used for Zn analysis was soaked in 10% nitric acid and rinsed (3 times) with deionized water.

From d 25 to d 32, the dogs were dosed orally twice a day, before each feeding, with a gelatin capsule containing 250 mg of chromic oxide, in order to use as an indigestible marker. On the first day of fecal collection (d 29), all stool before 0700 h were removed and discarded from the cages. Fecal output of individual dog was collected and placed into labeled plastic bags from this

point until d 32. Fecal samples of each dog were stored at -20ºC and dried at 60ºC in a forced-air oven. After drying, the samples were ground through a 1-mm screen mill and collected in labeled plastic bottles at room temperature until further analysis. Fecal samples were determined for Zn and Cr concentrations by AAS using Sullivan and Carpenter (1993) and Williams et al. (1962) procedures, respectively.

Statistical analysis: All data was expressed as mean±SE. Data were analyzed as a Cross-over using the GLM. Each dog represented an experimental unit. The model included period, dog, and treatment, and the error was residual error mean square. The mean differences between treatments were tested by LSD using procedure of SAS (1988). Differences were considered significant when $p<0.05$ and were regarded as trends if $0.05 \leq p < 0.10$.

Results

No observation of any abnormal behavior and appearance throughout the experiment and also no sign of severe Zn deficiency.

Effects of either ZnMG or ZnSO$_4$ supplementation on hair coat characteristics and Zn deposition in hair

The hair growth rate and Zn deposition in hair was greater ($p<0.05$) for the dog supplemented with ZnMG

when compared to the dog supplemented with ZnSO$_4$ (Table 2). Scanning electron microscopy revealed differences between treatments in hair condition with the hair taken from the ZnMG supplemented dogs apparently smoother and less fragmented than ZnSO$_4$ supplemented dogs (Fig.1).
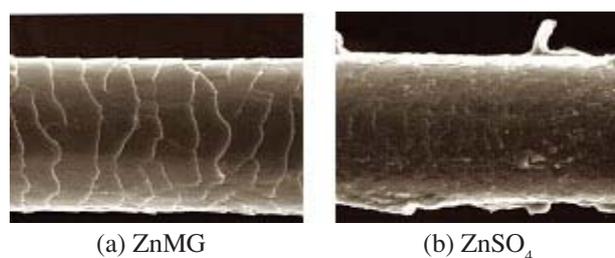

(a) ZnMG (b) ZnSO$_4$

Figure 1 Comparison of scanning electron microscopy photographs of a strand of hair from dog supplemented with ZnMG versus ZnSO$_4$ (Mag. x 1,500).

Effects of either ZnMG or ZnSO$_4$ supplementation on Zn concentration in plasma and serum ALP activity

At the end of the pretest period, plasma Zn concentration and serum ALP activity of all dogs were 8.4±0.26 µmol/l and 145.5±15.62 U/L, respectively. Levels of Zn concentration in plasma and serum ALP after receiving either ZnMG or ZnSO$_4$ supplementation are shown in Table 2. The dogs fed ZnMG treatment tended to have plasma Zn concentration greater ($p<0.10$) than the dogs fed ZnSO$_4$ treatment. The value of serum

Table 2 Hair growth rate and Zn deposition in hair, levels of Zn concentration in plasma and serum ALP activity, amounts of fecal Zn excretion and Zn absorption after receiving either ZnMG or ZnSO$_4$ supplementation[1].

Item	Treatment		
	ZnMG	**ZnSO$_4$**	**P-value**
Hair growth rate (mg/d . 20cm^2)	6.2±0.34[a]	3.8±0.38[b]	0.02
Zn deposition in hair (µg/21d 20cm^2)	26.5±1.39[a]	19.2±1.79[b]	0.03
Plasma Zn (µmol/l)	9.0±0.09	8.6±0.07	0.06
Serum ALP activity (U/L)	193.9±4.77[a]	164.4±7.29[b]	0.04
Fecal Zn (mg/d)	11.1±0.16[a]	12.4±0.24[b]	0.02
Fecal Zn (%)	63.0±0.89[a]	70.2±1.36[b]	0.02
Zn absorption (mg/d)	6.5±0.16[a]	5.2±0.25[b]	0.02
Zn absorption (%)	37.0±0.88[a]	29.8±1.34[b]	0.02

[1]Mean±SE
[a,b]Mean in the same row with different superscripts differed significantly ($p<0.05$)

ALP activity when the dogs fed ZnMG was greater ($p<0.05$) than the dogs fed $ZnSO_4$.

Effects of either ZnMG or $ZnSO_4$ supplementation on fecal Zn excretion and Zn Absorption

Table 2 shows the mean value of the fecal Zn excretion and Zn absorption. The dogs supplemented with ZnMG had lower ($p<0.05$) fecal Zn excretion but greater ($p<0.05$) Zn absorption, calculated by subtracted the amount of Zn intake with the amount of fecal Zn excretion, than the dogs supplemented with $ZnSO_4$.

Discussion

Hair coat characteristics and Zn deposition in hair

ZnMG supplementation resulted in greater ($p<0.05$) hair growth rate and more ($p<0.05$) Zn deposition than $ZnSO_4$ supplementation. These result were similar to Lowe et al. (1994b) studied. They reported that the hair growth rate and Zn deposition in hair were greater ($p<0.05$) in dogs fed diet containing zinc amino acid chelate (ZnAAC) than dogs fed ZnO diet. Similarly, França et al. (2005) shown that cats supplemented with Zn proteinate resulted in the greater ($p<0.05$) level of Zn deposition in hair than cats supplemented with ZnO and $ZnSO_4$. Results from these reports demonstrated that animals could utilize Zn in the chelate form better than the inorganic form.

The taken hair of the dogs supplemented with ZnMG showed apparently smoother and less fragmented than the taken hair of the dogs supplemented with $ZnSO_4$. Similar the result was reported by Kuhlman and Rompala (1998) that partial replacement of inorganic Zn, Mn, and Cu with proteinated forms of Zn, Mn, and Cu in diet gave the better hair condition than diet containing inorganic forms of Zn, Mn, and Cu only.

ZnMG supplementation had greater amount of Zn absorption and utilization when compare to $ZnSO_4$ supplementation. ZnMG is absorbed then moved directly into the plasma as an intact molecule. This intact molecule will be metabolized at the target tissue (Albion, 2004). Borges and Silva (n.d.) suggested that the use of minerals chelated to amino acids related to the specific needs of certain tissues and chelated Zn promotes Zn deposition in hair. When the amino acids (chelated to the mineral) are transported to specific tissues, they carry with them the mineral that they are chelated to, ensuring the absorption

and deposition of the mineral on the tissue. The target tissue of the current study is hair. Hair has such great requirement of both Zn and sulfur-containing amino acids for proper hair growth rate. ZnMG that used in the present study composed of Zn and sulfur-containing amino acid (methionine). Zn is known to be associated with three key functions in the keratinization process as follow: 1) catalytic roles; 2) structural roles; and 3) regulatory roles (Tomlinson et al., 2004). The availability of sulfur-containing amino acids would affect to the rate of synthesis of high-sulfur hair matrix proteins (Tscharner and Halliwell, 1990). Consequently, if the ZnMG was absorbed readily, proper hair growth and condition would be achieved because optimal both levels of Zn and methionine were provided to the hair follicle.

Zn concentration in plasma and ALP activity

Van den Broek (1988) reported that serum Zn concentrations of normal adult dogs ranged 4.3-16 μmol/l. While the previous report of the normal range values of plasma Zn concentration in dogs were not found. Kirk and Bonagura (1992) reported that serum ALP activity of adult dogs ranged 35-280 U/L. In the present study, plasma Zn concentrations and level of serum ALP activity, the biochemical liver function test of serum, at the end of pretest and the end of test periods were in normal range for both experiments. Serum ALP activity usually was reported in various wide reference ranges. In addition, plasma Zn concentrations and level of serum ALP activity at the end of test period seem to be greater than at the end of pretest period for both experiments. These probably due to all dogs were fed only basal diets in order to reduce the Zn storage in the body.

The plasma Zn concentration tended to be different ($p<0.10$) between ZnMG and $ZnSO_4$ treatments. These results were similar to the reported of Lowe et al. (1994a). At the same time of blood collection (3 to 4 h after meal), they found that dogs fed ZnAAC diet tended to have greater plasma Zn concentration than the dogs fed ZnO diet. Moreover, they found that feeding dogs with ZnAAC diet had greater ($p<0.05$) plasma Zn concentration than ZnO diet. But the significant differences were observed at the different time of peak value between ZnAAC and ZnO. The ZnAAC was observed at 4.5 h while ZnO was observed at 2.25 h. Thus,

the difference between forms of Zn supplementation had affected to plasma Zn determination. According to Valberg et al. (1985), Zn form influenced the Zn transportation from the intestinal lumen to either blood circulation or cell incorporation such as hair. Van den Broek (1993) found that normal beagles had the peak of plasma Zn concentration at 2 h after supplemented with $ZnSO_4$. In Exp.2, plasma Zn concentration increased ($p<0.05$) with increasing the amount of Zn supplementation. These results were in agreement with the report by Van den Broek (1993) who found that dogs fed $ZnSO_4$ increased from 0.50, 0.75, and 1.00 mg Zn/kg BW resulted in increased ($p<0.05$) plasma Zn concentration. On the other hand, ALP is a Zn-containing enzyme and Zn is essential to maintain its activity (Gropper et al., 2005). In Exp.1, the dogs supplemented with $ZnSO_4$ had lower ($p<0.05$) level of serum ALP activity than the dogs supplemented with ZnMG which could cause by the form of Zn. Since $ZnSO_4$ possibly provided the exact amount of Zn for ALP less than ZnMG. Although both ZnMG and $ZnSO_4$ gave the values of serum ALP activity in the normal range.

Fecal Zn excretion and Zn absorption

The dogs supplemented with ZnMG had lower ($p<0.05$) amount of fecal Zn excretion but greater ($p<0.05$) Zn absorption than the dogs supplemented with $ZnSO_4$. These results were similar to the study of Lowe et al. (1994b) that Zn excretion was greater ($p<0.05$) in the dogs fed ZnO diet than the dogs fed ZnAAC diet. In cats, Borges and Oliveira (2003) and França et al. (2005) reported that Zn proteinate supplementation resulted in greater ($p<0.05$) Zn absorption and retention than $ZnSO_4$ supplementation.

In fact, soybean meal was used in the most commercial dog foods. In the present study, soybean meal was used as a raw material in basal diet. Furthermore, these basal diets might contain antagonistic substance from soybean meal such as phytic acid. Edwards and Baker (2000) reported that phytic acid from some raw materials had affected on Zn utilization when $ZnSO_4$ supplemented to soybean meal diet. Because $ZnSO_4$ can dissociate to Zn^{2+} in GIT and could interact with phytic acid to form strong and insoluble complexes that inhibit Zn absorption by animal

(Wilaison, 2002; Gropper et al., 2005). Whereas ZnMG is stable in wide pH range encountered within the different segments of the GIT so it would neither dissociate nor interact with other substances (Vandergrift, 1994). It is transported across the intestinal enterocyte and into the circulation as an intact molecule (Ashmead, 1992).

In conclusion, the organic Zn as ZnMG was found to be the form that could enhance the haircoat characteristics and suitable for supplementation into the commercial dry dog foods.

Acknowledgement

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