CHAPTER V

DISCUSSION

Hair Coat Characteristics and Zinc Deposition in Hair

In experiment 1, 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 ZnAAC than dogs fed ZnO diet (added 50 ppm Zn to basal diet containing 56 ppm Zn). Similarly, França et al. (2005) showed 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 $ZnSO_4$ (added 30 mg Zn to commercial diet containing 184 mg Zn). Results from these reports demonstrated that animals could utilize Zn in the chelate form better than the inorganic form. In experiment 2, as the amount of ZnMG supplementation increased the hair growth rate and the amount of Zn deposition in hair increased ($P < 0.05$). These results were similar to the reported of Lowe and Wiseman (1998) who fed diet (56 ppm Zn) supplementing ZnAAC at 50, 75, and 100 ppm Zn. They found that an increased amount of Zn supplementation in the diet would increase both hair growth rate and the amount of Zn deposition in hair. In addition, the results of experiment 2 demonstrated that ZnMG supplementation at 160 ppm had the greatest rate of hair growth and amount of Zn deposition in hair.

The results of experiment 1 showed that 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 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. In experiment 2, the taken

hair of the 120 and 160 ppm ZnMG supplemented dogs showed apparently smoother and less fragmented than 80 ppm. Supplementation of 80 ppm Zn resulted in the lowest level of Zn deposition in hair. These could indicate the Zn supplemented at 80 ppm was inadequate for improving hair condition.

From the experiment 1, ZnMG supplementation had greater amount of Zn absorption and utilization when compare to $ZnSO_4$ supplementation. Zinc methionylglycinate 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 present study is hair. Hair has a great requirement of both Zn and sulfur-containing amino acids for proper hair growth rate. Zinc methionylglycinate that used in the present study composed of Zn and sulfur-containing amino acid (methionine). Zinc 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.

Zinc Concentration in Plasma and Alkaline Phosphatase Activity

Van den Broek (1988) reported that serum Zn concentrations of normal adult dogs ranged between 4.3 and 16 µmol/l. While the previous report of the normal range values of plasma Zn concentration in dogs were not found. Although plasma and serum give similar results, analysis of plasma Zn is less subject to contamination by release of Zn from platelets during clotting and from red blood cells by

haemolysis (Thoday, 1989). Kaneko (1989) and Kirk and Bonagura (1992) reported that serum ALP activity of adult dogs ranged between 39 and 222 U/L and 35 and 280 U/L, respectively. In the present study, plasma Zn concentrations and level of serum ALP activity at the initiation of the experiment, the end of pretest period, and the end of test period were in normal range for both experiments. Serum ALP activity usually were reported in various wide reference range. 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.

In experiment 1, 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. Van den Broek (1993) found that normal beagles had the peak of plasma Zn concentration at 2 h after supplemented with $ZnSO_4$. Further investigation shall use an appropriate indicator (e.g., plasma metallothionein) that can demonstrate the better value of Zn availability than plasma Zn when supplement in different form of Zn. In experiment 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 experiment 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 be caused 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. In experiment 2, no difference ($P > 0.05$) was observed in the level of serum ALP activity between treatments but the dogs received the 160 ppm Zn treatment tended to have greater ($P < 0.10$) level of serum ALP activity than the dogs received the 80 ppm Zn treatment. Similarly, Puls (1990) reported that serum ALP activity increased with increased Zn intake. In pigs, Revy et al. (2002) reported that plasma ALP activity increased ($P < 0.001$) with increasing dietary Zn methionine complex (a molar ratio of 2 methionines for 1 Zn) from 10, 20, and 30 ppm Zn to basal diet (28 ppm Zn). These results contradicted to the reported of Lowe and Wiseman (1998) who found that the forms and the amount of Zn supplementation did not affect the plasma ALP activity.

Fecal Zinc Excretion and Zinc Absorption

The results of experiment 1 showed that 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 (Gropper et al., 2005; Wilaison, 2002). 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 experiment 2, percentage and daily Zn absorption and daily fecal Zn excretion increased with increasing the amount of Zn supplementation. Previous research done by Carlson et al. (2004) with pigs demonstrated that percentage and daily Zn absorption and daily fecal Zn excretion increased ($P < 0.05$) when pigs fed increase dietary Zn proteinate (added 0, 200, and 400 ppm Zn to basal diet containing 185 ppm Zn). Revy et al. (2002) found that an increased amount of Zn methionine complex supplementation in the diet resulted in increased daily Zn absorption and excretion. Carlson et al. (1999) reported that pigs fed high concentrations of Zn (3000 ppm) diet increased the production of metallothionein in the body tissues, including the intestinal mucosa, where metallothionein regulates Zn absorption by binding and temporarily storing the excess Zn in the mucosal cells until the mucosal cells are later sloughed. In chicks, Cao et al. (2000) found that mucosal metallothionein increased ($P < 0.0001$) with increasing dietary Zn concentration (added 200 and 400 ppm Zn as ZnAAC to basal diet containing 59 ppm Zn). Results from these reports may be illustrated that the amount of Zn absorption increased with increasing metallothionein production. McDowell (2003) reported fecal Zn increases with increased Zn intake. However, the amount of Zn absorption by dogs from both experiments were in accordance to Hellman and Carlson (2003) studies with pigs, indicated that the amount of Zn absorption is usually much less than 50% of the intake. Gropper et al. (2005) reported that Zn absorption varies from approximately 10 – 59%.

In conclusion, the present study demonstrated that Zn supplementation in the form of ZnMG gave the better results than $ZnSO_4$. Supplementation of ZnMG resulted in greater hair growth rate, level of Zn deposition in hair, serum ALP activity,

amount of Zn absorption, and smoother and less fragmented hair than $ZnSO_4$ supplementation. Increasing amount of Zn supplementation as ZnMG from 80 to 160 ppm DM to the diet resulted in increased hair growth rate, level of Zn deposition in hair, plasma Zn concentration, level of serum ALP activity, and amount of Zn absorption, and also improved hair coat condition. This study found that the appropriate level of ZnMG supplementation in commercial dog foods could possibly be at 160 ppm DM. Further investigations shall be performed that the effects of Zn supplementation in commercial dog foods over 160 ppm DM to improve hair coat and skin quality of dogs.