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Evaluation of serum mineral status in dermatological disorders of dogs[#]

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Abstract

The study was undertaken to evaluate serum mineral status, specifically zinc, copper and selenium in dogs affected with dermatological disorders in comparison to normal healthy dogs. The study included 40 dogs of 2-5 years of age with skin diseases presented to University Veterinary Hospitals at Mannuthy and Kokkalai under the Kerala Veterinary and Animal Sciences University, and ten apparently healthy dogs brought to the hospital for routine health check-up which served as control. Serum concentrations of the three minerals were compared not only between normal and dogs with skin diseases as a whole but also between individual skin diseases- six different skin diseases- with the control group. On comparing the dogs with skin ailments (n=40) and control group, the serum mineral levels analysed through Atomic Absorption Spectrophotometry (AAS) revealed that the concentration of zinc was significantly lower ($p<0.01$) in the diseased group (0.53 ± 0.04 mg/L) of dogs (n=40) than the control group (1.11 ± 0.09 mg/L) and the serum copper concentration was also significantly reduced ($p<0.05$) in diseased dogs (0.91 ± 0.08 mg/L) with respect to the control animals (1.23 ± 0.11 mg/L). The serum selenium concentration of diseased group of animals(n=40), however failed to show any significant difference from the control group of animals. The outcome of the study suggests a need for supplementation of zinc and copper in dogs with skin ailments like canine demodicosis that relapse even after prolonged therapy with acaricides.

Keywords: *Dog, skin diseases, deficiency, minerals, serum levels, copper, zinc, selenium*

The skin is the largest, metabolically active organ system that serves to protect the body from injury and infection, aids in temperature control, immunoregulation, and acts as storage

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reservoir for certain nutrients. Because of its high metabolic needs, the skin has a relatively high requirement for energy, protein, minerals, vitamins and other essential nutrients. Nutrition plays a significant role in maintenance of skin health in animals. A supply of dietary zinc is needed for the maintenance of epidermal integrity, biosynthesis of fatty acids, and zinc also has a role in regulation of keratinisation (Miller, 1989). Copper is needed in the skin for the conversion of amino acid tyrosine to melanin. Selenium is an essential component of enzyme glutathione peroxidase, and like vitamin E, functions to protect cell membranes from oxidative damage.

Nutritional imbalances can occur consequent to feeding a poorly formulated or improperly stored commercial food, or as a result of metabolic or functional disorders that affect the ability to digest, absorb, or use nutrients (Watson, 1998). Failure of the skin's immunity can also lead to a variety of problems, ranging from a low-grade skin infection to severe microbial disease and life-threatening neoplasia. Zinc deficiency in dogs is characterized by scaling, crusting skin lesions, hyperkeratosis, and secondary skin infections responsive to dietary change or zinc supplementation (Schwartz *et al*., 2005). Deficiency of copper can cause depigmentation of the hair coat in dogs. Selenium deficiency can cause reduced hair growth in adult dogs. This study was conducted to analyse the variation in serum mineral status, specifically zinc, copper and selenium in dogs affected with dermatological disorders in comparison to normal healthy dogs

Materials and methods

Selection of animals

Dogs of different sex and breed, in the age group between 2 to 5 years, presented to the University Veterinary Hospitals at Mannuthy and Kokkalai under the Kerala Veterinary and Animal Sciences University with clinical signs of skin disorders like alopecia, pruritis, hyperpigmentation, erythema and pustules were included in the study. A total of 40 dogs with various skin ailments (Table 1) were studied, and ten healthy dogs which were brought to the hospital for routine checkup, vaccination and/or for deworming were used as control group. Based on clinical findings and laboratory results of the cases, diagnoses were arrived at (Table 1) and blood samples were collected from these animals**.** Animals of both control and diseased groups were selected based on feeding and management history collected from owners so as to ensure that the all selected dogs were till then maintained on apparently balanced diet consisting of meat based petfood or home-made food or their combination.

Mineral estimation

Three milliliters of blood sample were collected in serum separation vials from each animal for the estimation of minerals. After holding the collected blood samples in clot activator vials for 30 min at room temperature, they were subjected for serum separation by centrifuging at 3000 rpm for 15 minutes. Serum samples were stored in Eppendorf vials at -20^{o}C till further estimations.

Serum levels of zinc (ppm), copper (ppm) and selenium (ppm) were analysed by AAS. The majority of current flame atomic absorption spectroscopy methods for zinc, copper and selenium of plasma or serum were direct techniques in which the sample was diluted either with deionized water or 1 per cent nitric acid. Three hundred microliters of serum were diluted in 3mL of one per cent nitric acid. The contents were vortex mixed in vortex mixer for 30 sec and then fed to AAS for analysis. The flame atomizer with a temperature of 2400^{o}C was set for the measurement. Fuel and oxidant gases used were acetylene (2.5L/min) and air (10 L/min) respectively. Calibration curve was plotted with standards ranging from 0.5 to 2 ppm with a calibration equation nonlinear through zero and slit width of 0.7nm ensured accurate analysis. For zinc analysis, hollow cathode lamp of wavelength 213.86 nm, with minimum energy level 58 per cent and lamp current of 15 mA were used. For copper analysis, hollow cathode lamp of wavelength 324.75 nm, with minimum energy level 81 per cent and lamp current of 15 mA were used. For selenium analysis, hollow cathode lamp of wavelength 196.03 nm, with minimum energy level 61 per

cent and lamp current of 15 mA were used.

Statistical analysis

Results were expressed as mean±S.E. The statistical significance of difference or relationship between two groups were analysed by independent T test using the software statistical product and services (SPSS) version 24.0.

Results and discussion

Animals which exhibited clinical signs of skin disorders like alopecia, pruritis, erythema, and pustules were selected for the present study. The total number of diseases and cases taken for the study are presented in Table 1.

Table 1. Dogs with dermatological disorders taken for the study

Sl. No.	Diseases	Number of cases
1.	Demodicosis	6
2	Fungal infection	7
3	Yeast + Cocci infection	7
4	Yeast infection	7
5	Flea bite dermatitis	6
6	Fungal + Yeast + Cocci infection	7
Total		40

Table 2. Serum zinc, copper and selenium concentration (mg/L) in control and skin diseased group

Variable	Control (n=10)	Diseased (n=40)	p-value
Zinc	1.11 ± 0.09	0.53 ± 0.04	<0.001**
Copper	1.23 ± 0.11	0.91 ± 0.08	0.030*
Selenium	0.17 ± 0	0.16 ± 0	0.215 ns

MMean ±S.E. ** Significant at 0.01 level, * Significant at 0.05 level, ns non-significant

Table 3. Serum zinc, copper and selenium concentration in different skin disease Conditions

Serum zinc (mg/L)			
Disease Condition	**Control**	**Diseased**	**p-value**
Demodicosis	1.11 ± 0.09	0.37 ± 0.04	<0.001**
Fungal infection	1.11 ± 0.09	0.84 ± 0.01	0.002**
Yeast + Cocci infection	1.11 ± 0.09	0.39 ± 0.01	<0.001**
Yeast infection	1.11 ± 0.09	0.84 ± 0.01	0.002**
Flea bite dermatitis	1.11 ± 0.09	1.06 ± 0.14	0.548ns
Fungal + Yeast + Cocci infection	1.11 ± 0.09	0.35 ± 0.01	<0.001**
Serum copper (mg/L)			
Disease Condition	**Control**	**Diseased**	**p-value**
Demodicosis	1.23 ± 0.11	0.43 ± 0.02	<0.001*
Fungal infection	1.23 ± 0.11	0.41 ± 0.02	<0.001**
Yeast + Cocci infection	1.23 ± 0.11	0.37+0.01	<0.001**
Yeast infection	1.23 ± 0.11	0.41 ± 0.02	<0.001**
Flea bite dermatitis	1.23 ± 0.11	1.20 ± 0.12	0.825ns
Fungal + Yeast + Cocci infection	1.23 ± 0.11	0.43 ± 0.01	<0.001**
Serum selenium (mg/L)			
Disease Condition	**Control**	**Diseased**	**p-value**
Demodicosis	0.17 ± 0	0.17 ± 0.0	0.386ns
Fungal infection	0.17 ± 0	0.16 ± 0.02	0.154ns
Yeast + Cocci infection	0.17 ± 0	0.17 ± 0.0	0.638ns
Yeast infection	0.17 ± 0	0.16 ± 0.01	0.696 ns
Flea bite dermatitis	0.17 ± 0	0.16 ± 0.02	0.220ns
Fungal + Yeast + Cocci infection	0.17 ± 0	0.17 ± 0.0	0.851ns

Mean ±S.E. ** Significant at 0.01 level, * Significant at 0.05 level, ns non-significant

The results of statistical analysis comparing the serum zinc, copper and selenium concentration in control and diseased dogs are presented in Table 2.

Over and above the comparison of serum mineral levels between the control/ normal dogs with overall diseased animal group (n=40), we also compared the serum mineral status between dogs with individual skin diseases and control group (Table 3).

Zinc and copper

Serum zinc ($p<0.01$) and copper ($p<0.05$) concentrations were significantly lower in overall diseased (n=40) group (Table 2) as well as in dogs with other individual skin ailments except flea bite dermatitis (Table 3) when compared to control group of normal animals. The reduced levels of zinc and copper in overall diseased group (n=40) as well as the five (of microbial origin and with more systemic involvement) out of six individual skin ailments were evident except in flea bite dermatitis affected group. The deficiencies could be attributed to the increased oxidative stress associated with inflammatory conditions. This in turn might have overutilised or exhausted the available serum zinc and copper levels for the biosynthesis of various antioxidant enzymes like copper/ zinc superoxide dismutase (Cu-Zn SOD), to neutralize reactive oxygen species generated (Altobelli *et al*., 2020). Similar observations as obtained in the present study have been reported by earlier workers. Dimri *et al*. 2008 observed significantly lower ($p<0.01$) blood zinc and copper levels by atomic absorption spectrophotometry in dogs with localized demodicosis (Zn - 0.91±0.01 µg/mL, Cu - 0.61 ± 0.02 µg/ml) and generalized demodicosis (Zn - 0.64 ± 0.017 µg/ml, Cu - 0.42 ± 0.01 µg/ml) than healthy control (Zn- 1.19 ± 0.17 µg/ml, Cu - 0.83 ± 0.02 µg/ml) animals. Beigh *et al*. (2013) and Ragheb (2019) also observed significantly lower serum zinc and copper levels ($p<0.01$) in dogs with generalised demodicosis by atomic absorption spectrophotometry. The reason was attributed to the increased oxidative stress observed in demodicosis which might have led to the overutilisation of zinc and copper for the synthesis of antioxidant enzymes to counter oxidative stress (Dimri *et al*. 2008). Beigh *et al*. (2014) observed significantly decreased plasma zinc and copper levels ($p<0.01$) in dogs with dermatophytosis compared to normal healthy counter parts. The authors stated that dermatophytosis in dogs is associated with significant alteration in trace elements and this might be secondary to such inflammatory changes caused by dermatophytosis. In contrary to our observations, Ural *et al*. (2009) determined serum zinc concentrations by atomic absorption spectrophotometry and found no significant difference between control (14.05 ± 0.93 µmol/L) and dogs with dermatophytosis (13.68 ± 1.18 µmol/L).

According to Stehbens (2003) the reduced levels of zinc in blood might be an aftermath of associated skin injuries, parasite blood loss etc. Svenson *et al*. (1985) stated that interleukins are released by activated phagocytes during infection, causing an increase in the synthesis of metallothionein in the liver, which removes zinc from the circulation**.** Morevover, zinc is essential for the integrity of immune system. Deficiency of zinc reduces immunocompetence by lowering humoral, cell-mediated, and nonspecific immunity. The profound effect of zinc on the immune system suggests that low levels of these mineral in the body can cause skin lesions.

Copper is required for skin formation and wound repair by affecting process like cell proliferation, re-epithelisation and keratinisation. Thus, the increased need of skin for these elements and their local utilisation in skin lesions could also be considered as one of the reasons for their observed low plasma concentration (Borkow, 2014). Similarly, Shyma and Vijayakumar (2011) observed decreased serum copper levels in dogs affected with bacterial dermatitis (49.5 ± 3.49 µg/dL) in comparison to that of control animals (115 ± 20.08 µg/dL). Stehbens (2003) noticed that skin wounds, parasite blood loss, and the loss of ceruloplasmin, a copper-binding protein, from skin lesions etc might be the reason behind lowered copper levels in blood in dogs with skin ailments. Svenson *et al*. (1985) stated that immune dysfunction is strongly correlated to an increased incidence of infection in trace element deficient animals. Copper is essential for the integrity of immune system. Copper

deficiency reduces immunocompetence by lowering humoral, cell-mediated, and nonspecific immunity. This profound effect of copper on the immune system suggests that low levels of these mineral in the body can cause skin lesions.

Therefore, multiple factors might have played significant roles in considerably reducing serum zinc and copper levels in dogs with skin ailments (except flea bite dermatitis) in our study as well, in contrast to normal healthy dogs even if they are fed with diet adequate in copper and zinc.

The contradictory finding in flea bite dermatitis (Table 3) where in the affected dogs did not exhibit reduced serum levels of copper and zinc might be due to the skin affliction which is often superficial in nature as against the deeper wounds, intense systemic involvement and wide spread inflammatory processes observed in other skin ailments of microbial origin. Flea bite dermatitis is often transient and does not lead to involvement of deep underlying tissues unless complicated by secondary bacterial infections (Udayasree and Usha., 2005) leading to further complications like pyoderma. Thus, unlike in other five types of skin ailments (Table 3) of microbial origin, where there are widespread inflammatory changes with more of systemic involvement, the available serum zinc and copper might have got spared in flea bite dermatitis from participating in antioxidant or other anti-inflammatory processes**.** However, no specific conclusion could be drawn with regard to serum levels of copper and zinc in cases of flea bite dermatitis.

Selenium

Selenium, like zinc and copper is a strong antioxidant, which is actively involved in neutralization of the oxidative free radicals released consequent to skin inflammatory conditions, However, unlike reduced serum copper and zinc levels in dogs affected with various skin ailments, we failed to observe any significant reduction in the serum selenium levels in diseased dogs (Tables 2 and 3) compared to normal healthy control dogs**.** Though this cannot be substantiated fully, it might be probably due to higher dietary requirement of zinc (10 mg/ 100 g dry matter) and copper (1.1 mg/ 100 g dry matter) when compared to selenium requirement (0.035 mg/ 100 g dry matter) for dogs (ICAR, 2013). Hence the selenium content in pet and/ home food (Davies *et al*., 2017) would have been well above the normal requirements so that the likelihood of selenium deficiency becomes remote even in dogs with skin ailments. The dry and canned dog foods usually contain inorganic type of selenium *i.e*., sodium selenite or sodium selenate. Dairy products, whole grains, sea food, meat, poultry etc. are rich sources of selenium. Over and above the selenium contained in these ingredients of pet food, additional sources of selenium are also incorporated in commercial diet formulations which are enough to successfully prevent the occurrence of selenium deficiency in companion animals (Sharadamma *et al*., 2011) maintained on these diets.

Conclusion

From the present study it is concluded that significantly decreased serum zinc and copper levels were observed in the common skin diseases in dogs, except flea bite dermatitis, with respect to that of control, suggesting that the deficiencies of copper and zinc encountered in diseased dogs might be playing significant roles in either causation and/or consequence of skin ailments. Serum selenium however, did not differ significantly between diseased and control dogs possibly because the dietary requirement of selenium in dogs is much lower compared to that of zinc and copper (per 100 g dry matter). Thus, unlike zinc and copper with much higher dietary requirements, the selenium supplied in meat-based dog food and /or home-made food would have been sufficient to maintain the normal serum levels, even after meeting the nutritional requirements and performing antioxidant activities during adverse inflammatory conditions. The study suggests the need for supplementation of zinc and copper along with specific treatment as it would possibly augment the recovery.

Conflict of interest

The authors declare no conflict of interest.

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