The Relationship between Serum Zinc, Copper and Vitamin A Concentration and the Severity of Lesions in Sarcoptic Mange of Goat

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**ABSTRACT**

The present study evaluated the serum levels of zinc, copper and vitamin A in caprine sarcoptic mange. Fifteen goats were purposively selected in the field and assigned into 3 groups (A, B and C) of 5 goats each, based on the severity of the mange skin lesions. Another 2 groups (D and E) of 5 goats each were used as the contact (exposure) infested and the uninfested control groups, respectively. Serum zinc and copper levels were assayed by atomic absorption spectroscopy while serum vitamin A level was determined by calorimetric method. Data generated were analyzed using one way analysis of variance. Serum zinc, copper and vitamin A concentration decreased with increased severity of lesions in infested goats in which the naturally infested goats (groups A, B, C) had significantly (p ≤ 0.05) lower serum levels of zinc, copper and vitamin A compared to the contact (exposure) infested goats (D) and uninfested control (E). Results of this study suggests a possible role of zinc, copper and vitamin A in the pathogenesis of sarcoptic mange, as the deficiency of these elements in the serum may lead to an increase in susceptibility to infestation with mange parasite.

**Keywords:** histopathology, trace elements, Vitamin A, sarcoptic mange, goat.

**Running Title: Serum mineral and Vitamin A in Goats**

1. **INTRODUCTION:**

Sarcoptic mange caused by the mite, *Sarcoptes scabiei*, inflicts severe damage to the hosts’ skin by forming tunnels within the upper epidermal layers (Morris, 1996). These extra dermal lesions are thought to be due to the adverse effects of pro-inflammatory cytokines or secretion of some toxins by mites that affects the epidermal structures. Furthermore, the role of the highly reactive oxygen species (ROS) in the pathogenesis of ectoparasitic diseases has been emphasized by many authors, and excess free radical generation due to *Sarcoptes scabiei* infestation have been reported in dogs and goats (Camkerten *et al.,* 2009: Ujjwal and Dey 2010). It is speculated that the combined effects of these free radicals and products of mite activity may be associated with organs dysfunction in scabies-infested animals (Ujjwal and Dey 2010). Sarcoptic mange thrives mainly in animals with underlying immunosuppression (Walton and Currie 2007: Currie *et al.,* 2004). Micronutrients, like zinc and copper, have been shown to play a role in immunomodulation and modification of the risk of infection (Chandra,1992).Zinc deficiency is associated with alteration in thymic function with resultant loss of T-cell-mediated responses and increased susceptibility to infectious diseases (Failla, 2003). Copper is also an essential trace element in both humans and animals and is involved in a number of biological processes including antioxidant defense, neuropeptide synthesis and immune function (Bonham *et al.,* 2002; Uriu-Adams and Keen, 2005). Both Zinc and copper are components of the cytosolic erythrocyte superoxide dismutases (Cu/Zn-SOD), an essential component of the body’s antioxidant defense mechanism that plays an important role in the prevention of free radical – induced damage to tissues for maintenance of health and production (Beigh *et al*., 2013). Vitamin A also is known to protect the integrity of the integumentary system and support the immune system through its anti-infective, antioxidant and anti-inflammatory properties (Aydogan *et al.,* 2013). It acts as an antioxidant by quenching singlet oxygen, neutralizing thiyl radicals, and acting in chain breaking by combining with peroxyl radicals (Palace *et al*., 1999).The objective of this study is to evaluate the serum levels of zinc, copper, and vitamin A in goats infested with sarcoptic mange in order to evaluate the status and possible role of these trace elements and vitamin in the pathogenesis of the disease.

1. **MATERIAL AND METHODS**

**2.1. Selection of animals**

The fifteen (15) naturally infested WAD goats were purposively selected and assigned into three (3) groups (A, B and C) of five (5) goats each, based on the degree of skin lesions and another ten (10) healthy goats were randomly divided into two groups (D and E) of five (5) goats each as groups A (mild-lesions, affecting ≤ 1/3 of body surface), B (moderate- lesions affecting > 1/3 but ≤ 2/3 of body surface, C ( severe- lesions affecting more than 2/3 of body surface, D (goats infested by contact), E (healthy uninfested control).

**2.2 Experimental Infestation of Goats with Sarcoptic Mites by Contact (Exposure).**Two female goats with severe sarcoptic mange were purchased from an infested farm. The goats were used to infest healthy goats of group D by housing them together in the same pen (Elbers *et al*., 2000; Tarigan *et al*., 2002).

**2.3 Parasitological Examination**

Skin scrapings from infested WAD goats were examined for mites. Briefly, a dull scapel blade was held perpendicular to the area of affected skin and used with moderate pressure to scrape the edges of lesions into a petri dish. The scraped samples were then transferred into a test-tube, and 10% KOH was added. Samples were mildly heated for 5-6 minutes until they dissolved. They were, thereafter, centrifuged at 10,000*g* for 5 minutes. Obtained precipitates were examined under a light microscope at low and high power for presence of mites or their eggs. Positive cases were classified as clinical sarcoptic mange based on the presence of mites with long unsegmented pedicels.

**2.4. Clinical Monitoring of contact animals**The in-contact animals in group D were monitored once every week for clinical manifestations, such as pruritus, after which they were individually restrained and examined closely for the presence of skin lesions such as erythema, papules, crusts, and alopecia before sample collection.

**2.5. Blood Sample Collection**

By external jugular venipuncture, 3ml of blood was collected from each of the goats in groups A, B, C and E at the beginning of the experiment (week 0) and from group D at week 6. The blood was allowed to clot. Supernatant sera were centrifuged at 10,000*g* for 10 minutes. Clean sera were extracted and used for the determination of the studied parameters.

**2.6. Determination of Serum Zinc and Copper**

Serum zinc and copper concentration were measured using atomic absorption spectroscopy according to the method previously described (Delves, 1987; Kassu, *et al*., 2008). The sample was thoroughly mixed by shaking and, thereafter, aspirated into the oxidizing air-acetylene flame or nitrous oxide acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

**2.7. Determination of Serum Vitamin A**

The serum vitamin A concentration was determined calorimetrically according to the method described by Neeld and Pearson, 1963.

**2.8. Histopathology**

The skin biopsy samples from different regions of the body of mite infested goats were fixed in 10% neutral buffered formalin for 72 hours and then processed and sectioned at 5µm thickness. They were then stained using haematoxylin and eosin (H&E) for light microscopy.

**2.9. Statistical Analysis**

Data obtained were subjected to one-way analysis of variance (ANOVA) and variant means were separated by the least significant difference (LSD) method, using SPSS statistical package. A *p* value of < 0.05 was considered significant.

1. **RESULTS**

Skin scrapping gotten from both the naturally and contact (exposure) infested goats revealed *Sarcoptes scabiei var capri*, identified on the basis of body morphology such as short legs, oval to round, dorsally convex, tortoise-like body covered with triangular scales (Fig.1).The skin lesions in the infested goats were characterized by areas of alopecia, crusts and lichenifications. The mean serum zinc concentrations in the naturally infested group C (severely infested) was significantly (p < 0.05) lower than all the groups. Although the mean values of group A and B were not significantly different from each other, they were significantly (p < 0.05) lower than groups D and E. Thus, the mean values obtained for the naturally infested goats (groups A – C) were significantly (p < 0.05) lower than those of groups D and E, with values in group C (severely infested) been the lowest (Fig. 2).The naturally infested goats also had significantly (p < 0.05) lower values for serum copper concentration compared to groups D and E, but only that of the severely infested goats (group C) was significantly (p < 0.05) lower compared to all the groups (Fig. 2).

Mean serum vitamin A concentration of the naturally infested goats (A-C) decreased with increased severity of infestation and were significantly (p < 0.05) lower than the contact (exposure) infested group D and uninfested group E. Also, the mean serum vitamin A concentration of the exposure of the contact infested group (D) was significantly (p < 0.05) lower than those of uninfested group E (Fig.3). The microscopic lesions observed were mainly hyperkeratosis, mononuclear cellular infiltration in the papillary dermis, and epidermal pustules (Fig 4), with sections of mites within the stratum corneum as seen in the moderately infested goats (Fig. 5). The severely infested goats in addition showed epidermal hyperplasia and more pronounced cellular infiltration in the papillary dermis (Fig. 6). However, the contact exposed goats showed vacuolation of keratinocytes and epidermal parakeratosis (Fig.7) while the un-infested control group had normal skin histology characterized by a thin layer of epidermis overlying a vascular dermis with intact hair follicles (Fig.8).

1. **DISCUSSION**

A lot of researchers have been trying to understand the pathogenesis of sarcoptic mange for years. However, different factors have been found to be responsible for lesions, but the most common hypothesis include malnutrition, immunodeficiency and oxidative stress (Camkerten *et al.,* 2009: Ujjwal and Dey 2010). As observed in this study, infestation of goats with sarcoptic mange showed a reduction in serum vitamin A as well as low zinc and copper concentrations. This was more pronounced in the naturally infested goats compared to the contact infested group. This may be due to the duration of the infestation in the naturally infested goats especially the severely infested group, which had significantly lower serum copper levels compared to the un-infested control. Although, there is no report available on the status of vitamin A, zinc and copper concentration in *Sarcoptes scabiei* infested goats, available information on mange infested dogs and cattle indicates that zinc, copper and vitamin A concentrations in serum reduced significantly (Kozat *et al.,* 2005; Khatak and Khurana, 2011; Beigh *et al.,* 2016). The decreased level of vitamin A was attributed to a concurrent decrease in zinc concentration in serum because zinc is required for the metabolism of vitamin A (Christian and West 1998; Beigh *et al.,* 2014).Vitamin A stimulates fibroblast, the cell response for developing tissues that keeps skin firm and healthy. Moreover, vitamin A, zinc and copper are well known nutritional antioxidants in addition to their other functions in the body (Bickers and Athar 2006; Dimri *et al.,* 2008). It appears that decrease in their serum levels in mange infested animals may be due to over utilization arising from increased synthesis of antioxidant enzymes to counter the effects of mange-induced oxidative stress (Ewans and Halliwell, 2001, Beigh *et al.,* 2016).The observed microscopic lesions in the skin of the infested goats were consistent with previous reports (Morris and Dunstan, 1996). It appears that prolonged irritation caused by the activities of the mange mites on the skin contribute also to the microscopic lesions observed such as acanthosis, hyperkeratosis and pustular epidermitis, which may have resulted from microbial contamination of the scratch wounds. Previous reports also suggest that skin damage, blood loss as a result of parasitic activity and subsequent loss of copper binding proteins (ceruloplasmin) from skin lesions may lead reduction of zinc and copper concentration in blood (Boosalis *et al.,* 1986; Stehbens, 2003). Zinc and copper play a role in skin formation and wound repair at the stage of cell proliferation, re-epithelization and keratinization (Borkow *et al.,* 2008; Pickart, 2008). Thus, zinc is found in cells throughout the body where it helps to boost the body's defensive (immune) system and play a role in cell division, cell growth and wound healing. The local utilization and increased need for these nutrients following skin lesions of mange is also a contributory factor for the observed serum concentration. From the results of the present study, it was concluded that disturbances in the serum levels of zinc, copper and vitamin A occur in caprine sarcoptic mange and were proportional to the duration and severity of the disease. Alterations in these nutrients may negatively influence the immune system and ensure that the disease thrives in affected hosts. Thus; it is important to consider nutritional deficiency of vitamin A, copper and zinc as one of the factors that predispose goats to sarcoptic mange.Therefore, it is advised to add these nutrients within the program of treatment and prevention of sarcoptic mange.  
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2. **Author contribution:** The investigations and data gathering were carried out by RI Onoja while the manuscript was written by RI Onoja and DC Anyogu.  
   The study was supervised by SVO Shoyinka.
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**Fig 1:** *Sarcoptes scabiei* mite isolated from the infested goats, identified by its round shape, short legs and long non-jointed pedicels (10% potassium hydroxide preparation, x400 magnification)

**Fig.2**: Serum concentrations of copper and zinc (mg/L) in experimental goats (n=5), groups A (mild-lesions, affecting ≤ 1/3 of body surface), B (moderate- lesions affecting > 1/3 but ≤ 2/3 of body surface, C ( severe- lesions affecting more than 2/3 of body surface, D (experimentally infested goats -by contact), E (healthy uninfested control).\* p < 0.05 vs A, B, D, E. #p<0.05 vs E. &p < 0.05 vs C, D and E.

**Fig.3**: Serum concentrations vitamin A (µmol/l) in experimental goats (n=5), groups A (mild-lesions, affecting ≤ 1/3 of body surface), B (moderate- lesions affecting > 1/3 but ≤ 2/3 of body surface, C ( severe- lesions affecting more than 2/3 of body surface, D (experimentally infested goats -by contact), E (healthy uninfested control).\* p < 0.05 vs A, B, D, E. #p<0.05 vs E. &p < 0.05 vs C, D and E.

**Fig.4**: Photomicrograph of the skin section from group A- (mildly infested) goats showing hyperkeratosis (D) and epidermal pustules (EP). HE x 400

**Fig.5**: Photomicrograph of skin section from group B (moderately infested) goats showing degenerating section of the mite (black arrow), pustule (P), rete peg formation (Rr) and mononuclear cellular infiltration of the papillary dermis (PD).HE x 100.

**Fig. 6**: Photomicrograph of skin section from group C (severely infested) goats showing a section of the parasite (black arrow), hyperplastic epidermis (H), severe mononuclear cell infiltration of the dermis (PD) and pustules (asterisk).HE x 100

**Fig. 7**: Photomicrograph of skin section from group D (in-contact exposure) goats showing vacuolation of keratinocytes (white arrows) and epidermal parakeratosis (asterisk).HE x 400

**Fig. 8**: Photomicrograph of skin section from group E (control) goats showing normal epidermis (black arrow), the dermis (D) and intact hair follicles (asterisk).HE x 100

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