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# Measurement of Zinc in Arabian Horse's Serum by Spectrophotometric Method in Tabriz

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**Abstract:** Zinc is an essential mineral that is naturally present in some foods, added to others and available as a dietary supplement. Zinc is also found in many cold lozenges and some over-the-counter drugs sold as cold remedies. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis and cell division. Zinc also supports normal growth and development during pregnancy, childhood and adolescence and is required for proper sense of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system. The aim of this study was to measurement of zinc in Arabian horse's serum by Spectrophotometric Method in Tabriz. In this study about 186 blood samples from apparently healthy Arabian horses were obtained and blood samples taken near the ice and sent to the laboratory and after serum preparation were freezing inside the micro tube. In lab, blood samples centrifuged at 2000 rpm for 15 min.

**Key words:** Zinc, Arabian horses, serum, spectrophotometry, Tabriz, Iran

## INTRODUCTION

Zinc is a trace element of essential biological importance. Zinc serves as structural ions in transcription factors and is stored and transferred in metallothionein, it is found in nearly 200 metalloenzymatic systems or zinc containing proteins. Three basic functions of relevance of this study have been demonstrated: catalytic, structural and as regulator for keratinocytes proliferation and differentiation, Marycz found morphologic improvement of hair after feeding with zinc enriched food.

Alcohol dehydrogenase possess antioxidant properties and also helps speed up wound healing and zinc deficiency has been shown to play a role in increased susceptibility to infections and delayed wound healing Wound healing of the skin and the cell metabolism both depend on zinc as a catalyst enzyme in DNA synthesis and in metallothionein mRNA. Zinc indirectly activates part of a cellular differentiation process: the keratinization that transforms live epithelial cells into corneous cells that are structurally stable and with no metabolic activity (Iwata *et al*., 1999). Pories *et al*. (1967) found that zinc sulphate significantly accelerates wound healing. In proteins, Brandt *et al*. (2009) studied the structural site of horse liver alcohol dehydrogenase and found that zinc ions often were coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine. Zinc ions are effective antimicrobial agents even at low concentrations (McCarthy *et al*., 1992). Studies of zinc deficiency including hair zinc levels have been reported in man and normal zinc status trough hair analysis seems potentially useful in experimental medicine but its use in clinical medicine will remain limited until validation by the standard methods of clinical investigation is achieved (Klevay *et al*., 1987). Clinical zinc deficiency has not been unequivocally described so far in the horse (Kienzle and Zorn, 2006) but several studies have been conducted in order to quantify the normal mineral status in orses. Zinc concentration in hair and serum are so far the most well studied paraclinical parameters to equine zinc status.

## MATERIALS AND METHODS

This study was conducted to measurement of zinc in serum of Arabian horses during two seasons of 1 year Summer and Winter. In this study, about 186 blood samples from apparently healthy Arabian horses were obtained and blood samples taken near the ice and sent to the laboratory and after serum preparation were freezing inside the micro tube. In this inspection, age, body condition score and pregnancy status of animals was investigated. Simultaneous inspection of animals,

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attempting to obtain blood samples of 10 mL of jugular vein was done by venoject. In lab, blood samples centrifuged at 2000 RPM for 15 min. Measurement of zinc in serum was followed by Spectrophotometric Method. In this study to analyzing and comparison of data were used of ANOVA test and to evaluate the relationship between the variables together, correlation test was used.

## RESULTS AND DISCUSSION

Many studies in various species have been made regarding the influence zinc has on skin, in acceleration of wound healing (Pories *et al*., 1967; Senapati *et al*., 1990) and a part of a cellular differentiation process: the keratinization that transforms live epithelial cells into corneous cells that are structurally stable and protective (Iwata *et al*., 1999) (Table 1-4 and Fig. 1 and 2). Chesters and Petrie (1999) showed that zinc has influence on both cell replication and differentiation. Others have shown effect of treatment on skin problems such as alopecia, superficiel flaking of dried epidermis, poor healing of abrasion and recurrent infections with edema, parakeratosis, seborrhea sicca and crusting dermathosis (Harrington *et al*., 1973; Van den Broek and Thoday, 1986; Sousa *et al*., 1988). When choosing the zinc source to equine feed, absorption must be considered.

The absorption of zinc occurs primarily in the small intestine (Weigand and Kirchgessner, 1976; Hambidge *et al*., 1998). Once absorbed in plasma, zinc is bound to and transported by albumin and transferrin (Chesters and Will, 1981; Duchateau *et al*., 1981). Since transferrin also transports iron, excessive iron reduces zinc absorption and vice-versa. The intake of excessive amounts of iron induces a decrease of plasma zinc levels in ponies. The intake of iron must be >800-2000 mg $kg^{-1}$ feed/day/horse before effects are detectable (Lawrence *et al*., 1987).

Table 1: Zn distribution in the samples studied in the Summer

| Variable | No. | Mean | SEM | SD | Min | Max |
|---|---|---|---|---|---|---|
| Zn | 90 | 111.42 | 1.75 | 16.65 | 73 | 140 |

Table 2: Zn distribution in the samples studied in the winter

| Variable | No. | Mean | SEM | SD | Min | Max |
|---|---|---|---|---|---|---|
| Zn | 96 | 43.52 | 0.98 | 9.62 | 30 | 72.22 |

Table 3: Comparison of zinc in serum in two season

| Season | No. | Mean±SD | F-value | p-value | t-test | df |
|---|---|---|---|---|---|---|
| Winter | 96 | 43.52±9.62 | 27.32 | 0.000 | 33.74 | 140.55 |
| Summer | 90 | 111.42±16.65 | | | | |

Table 4: Comparison of zinc in serum by sex

| Sex | No. | Mean±SD | F-value | p-value | t-test | df |
|---|---|---|---|---|---|---|
| Male | 162 | 76.01±63.53 | 0.041 | 0.84 | 0.26 | 184 |
| Female | 24 | 78.24±37.67 | | | | |

A similar reaction occurs with copper, as metallothionein absorbs both zinc and copper. In intestinal cells, metallothionein is capable of adjusting absorption of zinc by 15-40%. However, inadequate or excessive zinc intake can be harmful.

Zinc supplementation with 161 mg zinc $kg^{-1}$ of Dry Matter (DM) feed along with excessive amount of calcium 1.25% DM feed showed a statistically significant fall of zinc concentration in blood being 1.03 mg $L^{-1}$ before and 0.86 mg $L^{-1}$ after (Danek *et al*., 1999). Crozier *et al*. (1997) found that hay containing the amount of zinc recommended by American National Research Council (1989) still may require supplementation with phosphor, sulfur, cupper and crude protein to optimize the zinc absorption. Oral zinc preparations contain zinc in different types of salts and chelates which in turn affects

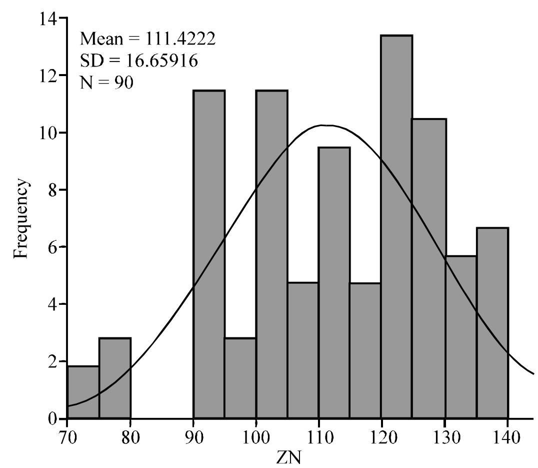


Fig. 1: Zn distribution in the samples studied in the Summer

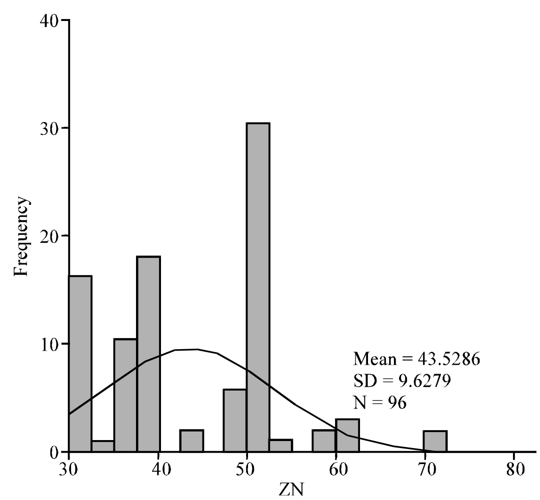


Fig. 2: Zn distribution in the samples studied in the Winter

the bioavailability of zinc. Bioavailability can be defined as a measurement of the rate and extent of a nutrient that reaches the systemic circulation and is available at target tissue level (Kienzle and Zorn, 2006). Wichert *et al.* (2002a, b) showed that the oral supplementation with zinc as zinc sulphate and zinc sulphate chelates to horses resulted in the highest increase in plasma zinc concentration compared to zinc oxide and zinc lactate.

The same tendency has been shown in chicks (Edwards and Baker, 1999; Wedekind *et al*., 1992). Although, an increase in serum zinc levels after long term oral supplementation of zinc oxide to ponies has been shown (Schryver *et al*., 1980), the tendency has later been shown to be of lower significance (Wichers *et al*., 2002). Lowe *et al*. (1994) showed that the rate of growth of hair and the amount of zinc deposited in the hair was significantly higher in dogs fed diets containing zinc as the amino acid chelate than in dogs fed zinc as zinc oxide or as a zinc polysaccharide complex. The 1989 NRC recommend that all classes of horses require 40 mg zinc $kg^{-1}$ feed of dry matter per day. Jackson suggests the intake to be 400 mg Zn $day^{-1}$ for horses at light work and 500 mg $day^{-1}$ for a horse at moderate or heavy work, due to the zinc loss in sweat in working horses that Meyer found in 1986 (20-21 mg zinc $L^{-1}$ of sweat).

Meyer *et al*. (2002) found that zinc requirement seems to be higher if the diet contains high levels of phytate, calcium or copper. When the diet is supplemented with as high as 1000 or 2000 mg zinc $kg^{-1}$ feed the copper metabolism is affected adversely in foals (Bridges and Muffit, 1990). The ratio of zinc to copper should not exceed 4:1 to 5:1 (Cymbaluk *et al*., 1986). Distal limb skin disease is a common debilitating problem in horses and to know whether the zinc status is below or above the normal range is essential in planning of treatment. This pilot study was conducted in order to observe the clinical effect of 2 months of oral organic zinc compound supplementation to seven horses with distal limb skin disease. Furthermore to observe the distribution of zinc in serum, hair and skin before and after oral zinc supplementation to horses with distal limb skin disease and hence to evaluate the paraclinical methods used to estimate equine zinc status.

Evaluation of the clinical effect is initially straightforward. There is sufficient improvement in all the horses, to validate the results. However, a control groups would have provided stronger evidence. It would exclude the possibility that the improvement could be due to weather, seasons, breed, age or gender. Assessment of the clinical results would quite evidently be stronger if the data material was constituted by a larger number. It would be beneficial to design more specific inclusion criteria about etiologies of the disease and more standardized feeding before and during the study. Regarding SEM-EDS analysis of hair, it's easy to collect mane hair samples and it is without major inconvenience to the horse. When it comes to hair from the affected area many of the horses were fairly annoyed at the collection and precautions to avoid being kicked had to be taken.

Since, there also had to be taken biopsies, there was made perineural nerve block. The photo of magnifications of hair shows clearly the improvements. Regarding SEM-EDS analysis of skin and especially looking at the histopatological examination, it requires more in depth knowledge and experience to interpret. A good laboratory with experience in the field is recommended. The histopatological findings in this study match closely with the results of Sanecki *et al*. (1985) in puppies with skin problems related to zinc deficiency. Collection of the skin biopsy is more laborious and yields are not entirely worth the effort. The results of this study showed however as previous studies (Pories *et al*., 1967; Harrington *et al*., 1973; Van den Broek and Thoday, 1986; Sousa *et al*., 1988; Senepati, 1990; Iwata *et al*., 1999; Chester and Petrie, 1999; Rostan *et al*., 2002) that zinc therapy promotes healing and improves coat health.

Looking at Table 5, the positive effect is easy to spot. Assessment of the different paraclinical techniques to estimate the nutritional zinc status in the horses of this study has been limited by the lack of patients. It makes no sense to perform statistical calculations; they would at best be able to point to trends more than any significant evidence. However, the current results are of interest for further study. Regarding zinc serum concentration, it's easy to collect blood samples and it is without major inconvenience to the horse. The analysis of serum zinc concentrations of serum can be performed in a local laboratory and is not very time consuming. This paraclinical parameter is the most investigated and normal values are recognized to be applicable (National Research Council, 1989; SCAN, 2003).

The results must however be evaluated with caution since many variables come into play when estimating zinc concentration. The fact that all the horses were in the lower range of the reference values both before and after the treatment could be due to the fact that they were chronic infected or vice-versa, the low zinc concentration leading to chronic infection. Others had shown correlation between infection and serum zinc concentration. Dede *et al*. (2008) shoved a decrease in plasma zinc in horses with piroplasmose and a correlation between a decrease in plasma zinc levels and increased activity of carbonic anhydrates. Carbonic anhydrates is the zinc containing enzyme that regulates the homeostasis of erythrocytes (Mafra and Cozzolino, 2004). Located in the lower end of the reference values or below, the diseased horse can certainly benefit from organic zinc supplements. Attempts to achieve a more accurate reflection of the tissue zinc and hence a precise estimate of horse zinc status using blood samples is done by Milne *et al.* (1985). They described a method for cell separation and analysis of separated platelets, mononucleated cell, polymorphnucleated cell and erythrocytes and measured individual zinc levels in each cell. Magneson *et al.* (1987) described the enzyme phosphoglucomutase as an indicator to measure free zinc in equine plasma. However, both these two methods are not accessible at laboratories.

## CONCLUSION

In this study, results showed that there are significant differences in serum zinc levels in Summer and Winter.

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