

# THE DETERMINATION OF SERUM COPPER AND ZINC CONCENTRATIONS IN THOROUGHBRED FOALS AND THEIR DAMS AFTER PARTURITION

Achara Tawatsin\* Panumas Ruantongdee Prachak Poomvises

## Abstract

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## THE DETERMINATION OF SERUM COPPER AND ZINC CONCENTRATIONS IN THOROUGHBRED FOALS AND THEIR DAMS AFTER PARTURITION

Serum copper (Cu) and zinc (Zn) concentrations in 5-35 thoroughbred foals and their dams during first 24 weeks after parturition were determined. Mean serum Cu in foals increased significantly ( $p < 0.01$ ) with age, from  $10.4 \pm 1.1 \mu\text{mol/l}$  at week 1 to  $25.9 \pm 0.8 \mu\text{mol/l}$  at week 24, whereas those in mares decreased significantly  $p < 0.05$ , from  $26.2 \pm 1.3 \mu\text{mol/l}$  at week 1 to  $22.0 \pm 0.5 \mu\text{mol/l}$  at week 24 after parturition. There was a large negative correlation between the mean serum Cu concentrations in foals and their dams ( $r = -0.86, p < 0.01$ ). Serum Zn concentrations in both foals and mares on the other hand, gradually decreased after parturition, ranging from and  $17.7 \pm 0.3 \mu\text{mol/l}$  at week-1 to  $13.8 \pm 0.5 \mu\text{mol/l}$  at week 24, in foals and from  $16.2 \pm 1.2 \mu\text{mol/l}$  at week 1 to  $14.4 \pm 0.5 \mu\text{mol/l}$  at week 24 in mares. The correlation coefficient between the mean serum Zn concentrations in foals and their mares was high ( $r = 0.895, p < 0.01$ ).

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**Keywords :** Copper, zinc, serum, mare, foal

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## บทคัดย่อ

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### ระดับของทองแดงและสังกะสีในลูกม้าพันธุ์โรโรเบรดและแม่ม้าภายหลังคลอด

ในการศึกษาตั้งแต่สัปดาห์ที่ 1 ถึงสัปดาห์ที่ 24 หลังคลอด ระดับทองแดง (Cu) และสังกะสี (Zn) จากซีรัมในลูกม้าและแม่ม้าพันธุ์โรโรเบรด จำนวน 5-35 ม้า พบว่าระดับ Cu ในซีรัมของลูกม้าเพิ่มขึ้นเรื่อยๆ อย่างมีนัยสำคัญทางสถิติ ( $p < 0.01$ ) ตั้งแต่สัปดาห์ที่ 1 ( $10.4 \pm 1.1 \mu\text{mol/l}$ ) จนถึงสัปดาห์ที่ 24 ( $25.9 \pm 0.8 \mu\text{mol/l}$ ) ในขณะที่ระดับ Cu ในแม่ม้าลดลงอย่างมีนัยสำคัญ ( $p < 0.05$ ) ตั้งแต่สัปดาห์ที่ 1 ( $26.2 \pm 1.3 \mu\text{mol/l}$ ) ถึงสัปดาห์ที่ 24 ( $22.0 \pm 0.5 \mu\text{mol/l}$ ) ค่าเฉลี่ยของระดับ Cu ในซีรัมของลูกม้าและแม่ม้ามีความสัมพันธ์สูง ( $r = -0.86, p < 0.01$ ) ส่วนระดับ Zn ในซีรัมทั้งในลูกม้าและแม่ม้าจะค่อยๆ ลดลง จากสัปดาห์ที่ 1 ( $17.7 \pm 0.3 \mu\text{mol/l}$ ) ถึงสัปดาห์ที่ 24 ( $13.8 \pm 0.5 \mu\text{mol/l}$ ) ในลูกม้า และจากสัปดาห์ที่ 1 ( $16.2 \pm 1.2 \mu\text{mol/l}$ ) ถึงสัปดาห์ที่ 24 ( $14.4 \pm 0.5 \mu\text{mol/l}$ ) ในแม่ม้า ค่าเฉลี่ยของระดับ Zn ในซีรัมของลูกม้าและแม่ม้ามีความสัมพันธ์สูง ( $r = 0.895, p < 0.01$ )

คำสำคัญ : ทองแดง สังกะสี ซีรัม แม่ม้า ลูกม้า

## Introduction

Copper (Cu) and zinc (Zn) are the two most intensively investigated nutritive trace elements (Solomons, 1979). The importance of Cu and Zn in human and animal health was well described by Underwood (1977) and in the health of horses, as reviewed by Cymbaluk and Smart (1993). Cu deficiency was implicated to be associated with several clinical diseases in horses, including osteochondrosis, because Cu is a required co-factor of lysyl oxidase, an enzyme needed for the synthesis and maintenance of collagen (Messer, 1981; Gunson et al., 1982, Cymbaluk and Smart, 1993; Suttle, 1993; Bathe and Cash, 1995; Suttle et al., 1996). While Zn has been shown to have a potent immunomodulation capacity, particularly

influencing T helper cell organization and cytokine secretion (Stark et al., 2001). Zn concentration in serum or plasma is normally expected to be lower when the animal is in a defensive stage. Auer et al.(1989) indicated that plasma Zn concentration was influenced by the severity of inflammation. Similarly, Barton and Embury(1987) found that thoroughbred foals infected with *Rhodococcus equi* had lower serum Zn concentration in comparison to those of healthy foals. Contrary, plasma Cu increased during inflammatory reaction process (Suttle, 1994). Ruantongdee et al.(1997) found that serum Cu concentrations in equine infectious anemia (EIA) infected horses were significantly higher than those of un-infected horses but there was no difference in serum Zn concentrations

between the EIA infected and uninfected horses. Also, Zn appears to be the most important cation inhibitor of Cu absorption in the equine intestine, where Zn competes directly with Cu for the same transport mechanism (Cymbaluk and Smart, 1993).

Dietary Cu and Zn imbalances have been implicated as the cause of many health problems, including musculoskeletal abnormalities in young horses (Messer, 1981; Bridges and Harris 1988; Bridges and Moffitt, 1990). The determination of serum Cu and Zn levels has been increasingly utilized in disease diagnosis as well as prognosis, in both human and veterinary medicine (Hartoma et al., 1979; Solomons, 1979; Sullivan et al., 1979; Messer, 1981; Suttle, 1993; Bathe and Cash, 1995; Suttle et al., 1995). However, diagnosis of Cu deficiency in horses both in the field and in experimental situations needs more information in order to establish the diagnostic criteria (Cymbaluk and Smart 1993).

Even though there were studies of Cu and Zn concentrations in mares and their foals after parturition, the information was from either a few animals or from limited study intervals. A more thorough study is needed especially in Thailand where lameness in foals is one of the major problems. Only less than 60% could race when they were 2 years old. Cu deficiency has been thought of as one of the factors associated with lameness, due to osteochondrosis (Bridges et al., 1984; Bridges and Harris, 1988) and the first six months of the foals' life is apparently the critical period for either deficiencies or excesses of both Cu and Zn (Bridges et al., 1984). This study is the first report in

Thailand on Cu and Zn levels in mares and their foals, presenting more thorough information by following the changes in serum concentrations of Cu and Zn through the first six months of life of the thoroughbred foals and their dams.

## Materials and Methods

The study was carried out in 5-35 thoroughbred mares, ranging in age from 5 to 17 years, during the first 24 weeks after parturition with their foals at foot, in a stud farm in Nakorn Rachasima province. Both mares and foals were clinically normal, based upon an assessment of their health history and physical examination, prior to each blood sample collection.

Jugular blood samples of 10 ml were collected directly through an 18 gauge needle into a vacuum blood collection tube (B-D Vacutainer; Becton Dickinson, New Jersey, USA). The serum was separated by centrifugation at 2500 rpm, for 10 minutes and kept at -20°C until tested.

The serum concentration of copper was determined as described by Landers and Zak (1958) by using the colorimetric method (Bathocuproin). The serum zinc concentration was analysed by atomic absorption spectrophotometer (Model AA-775, Varion Techtron Pty, Ltd., Springval, Australia) after dilution with deionized water, as described by Varian Ltd., (1978).

Statistical analyses conducted on the data included descriptive statistics, student *t*-test and simple correlations. Differences were considered significant at  $p < 0.05$ .

## Results

The serum Cu and Zn values in foals and mares after parturition are presented in Table 1 and 2. The mean serum Cu concentrations in foals increased significantly with age ( $p < 0.01$ ), ranging from the lowest level of  $10.4 \pm 1.1 \mu\text{mol/l}$  at week 1 after parturition to the highest level of  $25.9 \pm 0.8 \mu\text{mol/l}$  at week 24. In a contrary manner, the mean serum Cu concentrations in mares decreased significantly ( $p < 0.05$ ) from the highest level of  $26.2 \pm 1.3 \mu\text{mol/l}$  at week 1 after parturition to the lowest level of  $22.0 \pm 0.5 \mu\text{mol/l}$  at week 24. There was a large negative correlation between the mean serum Cu concentrations in foals and their mares ( $r = -0.86$ ,  $p < 0.01$ )

The highest level of serum Zn concentrations in foals was observed at weeks 1 and 2 at  $17.7 \pm 0.3$  and  $17.7 \pm 0.8 \mu\text{mol/l}$ , with a gradual decline to the lowest level of  $13.8 \pm 0.5$  at week 24 after parturition ( $r = -0.871$ ,  $p < 0.01$ ). Similarly, serum Zn concentrations in mares were highest at  $17.3 \pm 0.8 \mu\text{mol/l}$  on week 2 after parturition and declined to the lowest level of  $14.4 \pm 0.5$  at weeks 20 and 24 ( $r = -0.808$ ,  $p < 0.05$ ). There was no difference in the decline pattern of serum Zn concentrations in foals and their dams from week 1 to week 24 and the correlation coefficient was high ( $r = 0.895$ ,  $p < 0.01$ ).

## Discussion

Although technical factors may affect the determination of Cu concentration in tissues, Eamens et al. (1984) found that there was no difference when using plasma or serum for the

determination of Cu concentrations in horses. Even though daily dietary Cu intake in the mares studied was not measured, it was considered adequate, as the same mineral supplements were maintained and the mares were in healthy condition. The levels of serum concentration in these mares were found to be higher than the normal values suggested by Suttle (1993) or in the normal range suggested by Bathe and Cash (1995) and by Mee and McLanghlin (1995). In addition, the growth of the foals from these mares was normal as determined during the routine examinations when blood samples were collected. A significant increase in serum Cu concentrations and a gradual decrease in serum Zn concentrations in foals, when age increased, were similar to those reported by Cymbaluk et al. (1986), who studied plasma Cu and Zn concentrations in foals of several breeds, at four intervals; at birth, one week, 6 months, and one year old and who found the Cu levels increased while the Zn levels decreased during the period from one week to 6 months of age. Pearce et al. (1998), who studied thoroughbred foals up to 150 days old, also reported an increase in plasma Cu concentrations, from the lowest level at birth which gradually increased with age. However, he did not determine the Zn concentration.

Newborn foals are hypocupremic because the early neonatal liver has a low protein synthetic capacity which limits the supply of apoceruloplasmin to bind with Cu to form ceruloplasmin (Cp). Cp acts as a Cu exchange protein interacting with Cp receptors in various tissues. Ceruloplasmin Cu stimulates lysyl oxidase synthesis and acts as a cofactor in lysyl oxidase reaction which is needed

for collagen synthesis, especially in rapidly growing foals (Cymbaluk and Smart, 1993). Moreover, Stowe (1968) reported increases in plasma Cu concentrations in mares before parturition suggesting an influence of estrogen-induced synthesis of Cp (Cymbaluk and Smart, 1993). In the present study, the mare serum Cu concentrations were found to be highest while those of the foals were lowest at wk 1 after parturition. A significant decline in serum Cu concentration in mares in this study was highly correlated to a significant increase of serum Cu concentration in foals over a period of 24 weeks after parturition. The serial serum Cu concentration measurements in this study clearly demonstrated a reduction of serum Cu concentration in mares which is provided to their foals via milk, which is the main source of Cu for neonatal foals. However, based on milk Cu concentration measurements, the loss of Cu by this route only represents about 10% of the daily dietary intake (Cymbaluk et al., 1986) and mares, milk, Cu concentration is not responsive to Cu supplementation (Breedveld et al., 1987). Since, the liver is the main organ of Cu homeostasis and near-term foetal liver can contain up to 300 mg Cu, deficits in the suckling foal's Cu needs may be met by mobilising hepatic Cu (Cymbaluk et al., 1993).

While serum Zn concentration was suggested to be lower during an inflammatory process (Auer et al., 1989) and during infections with *Rhodococcus equi*, Ruantongdee et al. (1997) found no difference between the serum Zn concentrations in the equine infectious anemia infected horses and normal un-infected horses.

The decline in serum Zn concentrations in foals in this study was similar to that reported by Shaw (1979), who found a decrease in serum Zn concentrations in new born foals which coincided with a decline in milk Zn concentration over the period of six months after parturition. Cymbaluk et al. (1986) found that plasma Zn concentrations were higher in the neonatal foal than those in weanlings and yearlings. The decline of serum Zn concentration in this study was not influenced by daily dietary Zn intake, because the same ration of mineral supplements was given throughout the study.

Cu and Zn are trace minerals required for normal growth and maintenance of domestic animals (Underwood, 1977). During the first 6 months, rapidly growing foals need Cu for normal skeletal and joint development (Bridges et al., 1984). However, Zn is one of the antagonists for Cu absorption, either by competing for common transport mechanisms or by inducing cellular Cu-binding ligands for excretion (Harris, 1991). The appropriate Zn : Cu ratios recommended by NRC (1989) are at 4:1 or 5:1 for all breeds of horses.

From the present study, a series of simultaneous measurements of serum Cu and Zn concentrations for 24 weeks after parturition demonstrated a strong relationship between serum Cu and Zn concentrations in mares and their foals. Serum Cu concentrations in foals increased significantly during 24 weeks after parturition and mare's milk appeared to be the main source of the Cu demanded by rapidly growing foals. A gradual decline of serum Zn concentrations both in mares and foals were highly correlated. Possibly the

**Table 1** Serum copper concentrations ( $\mu\text{mol/l}$ ) in Thoroughbred foals and their mares during 24 weeks after parturition

| Post partum | Foals |                              |           | Mares |                              |           |
|-------------|-------|------------------------------|-----------|-------|------------------------------|-----------|
|             | No.   | Mean $\pm$ SE                | Min-Max   | No.   | Mean $\pm$ SE                | Min-Max   |
| Week-1      | 5     | 10.4 $\pm$ 1.1 <sup>a</sup>  | 7.9-13.2  | 5     | 26.2 $\pm$ 1.3 <sup>a</sup>  | 22.7-29.6 |
| Week-2      | 5     | 13.6 $\pm$ 1.6 <sup>a</sup>  | 9.9-19.7  | 8     | 24.5 $\pm$ 1.3 <sup>ab</sup> | 19.7-31.5 |
| Week-4      | 8     | 21.7 $\pm$ 2.1 <sup>b</sup>  | 15.7-31.5 | 8     | 24.0 $\pm$ 1.1 <sup>ab</sup> | 18.9-27.6 |
| Week-8      | 33    | 23.7 $\pm$ 1.0 <sup>bc</sup> | 15.7-45.4 | 35    | 24.0 $\pm$ 0.6 <sup>ac</sup> | 17.8-31.5 |
| Week-12     | 31    | 24.5 $\pm$ 0.8 <sup>bc</sup> | 15.7-40.2 | 31    | 23.9 $\pm$ 0.6 <sup>ac</sup> | 15.7-29.6 |
| Week-16     | 27    | 24.8 $\pm$ 0.9 <sup>bc</sup> | 18.9-39.4 | 27    | 23.6 $\pm$ 0.8 <sup>ab</sup> | 18.6-35.4 |
| Week-20     | 35    | 25.6 $\pm$ 0.7 <sup>bc</sup> | 18.9-37.0 | 35    | 22.4 $\pm$ 0.5 <sup>bc</sup> | 18.6-28.3 |
| Week-24     | 31    | 25.9 $\pm$ 0.8 <sup>c</sup>  | 18.9-35.9 | 32    | 22.0 $\pm$ 0.5 <sup>b</sup>  | 15.8-26.5 |

<sup>abc</sup> Values in the same column with different superscripts differ ( $p < 0.05$ )

**Table 2** Serum zinc concentrations ( $\mu\text{mol/l}$ ) in Thoroughbred foals and their mares during 24 weeks after parturition

| Post partum | Foals |                |           | Mares |                |           |
|-------------|-------|----------------|-----------|-------|----------------|-----------|
|             | No.   | Mean $\pm$ SE  | Min-Max   | No.   | Mean $\pm$ SE  | Min-Max   |
| Week-1      | 5     | 17.7 $\pm$ 0.3 | 16.8-18.3 | 5     | 16.2 $\pm$ 1.2 | 13.8-19.9 |
| Week-2      | 8     | 17.7 $\pm$ 0.8 | 15.3-19.9 | 8     | 17.3 $\pm$ 0.8 | 15.3-19.9 |
| Week-4      | 8     | 15.9 $\pm$ 1.8 | 10.7-27.5 | 8     | 15.3 $\pm$ 1.7 | 10.7-24.5 |
| Week-8      | 34    | 14.7 $\pm$ 0.5 | 9.2-18.3  | 35    | 15.1 $\pm$ 0.5 | 9.2-24.5  |
| Week-12     | 31    | 14.5 $\pm$ 0.6 | 10.7-26.0 | 31    | 14.5 $\pm$ 0.5 | 10.7-21.4 |
| Week-16     | 27    | 13.7 $\pm$ 0.3 | 10.7-18.3 | 27    | 15.1 $\pm$ 0.5 | 10.7-22.9 |
| Week-20     | 35    | 14.1 $\pm$ 0.3 | 12.2-18.3 | 35    | 14.4 $\pm$ 0.5 | 10.7-22.9 |
| Week-24     | 32    | 13.8 $\pm$ 0.5 | 10.7-19.9 | 32    | 14.4 $\pm$ 0.5 | 10.7-21.4 |

antagonistic effects of Zn on Cu metabolism may be avoided and an appropriate Zn : Cu ratio could be maintained resulting in a physically normal musculoskeletal development in these foals.

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