

# Blood Levels of Zinc in Crioulo Horses Used in Sera Production

Tatyana S. Baptista<sup>a,b</sup>, Cibele B Zamboni<sup>a</sup>, Luciana Kovacs<sup>a</sup>, Monica G. Freitas<sup>b</sup> and José R. Marcelino<sup>b</sup>.

<sup>a</sup>*Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)*

*Av. Professor Lineu Prestes 2242*

*05508-000 São Paulo, SP- Brazil*

<sup>b</sup>*Instituto Butantan*

*Av Vital Brasil 1500*

*05503-900 São Paulo, SP – Brazil*

Using Neutron Activation Analysis Zn concentrations were determined in blood of horses (Crioulo breed). No significant difference was observed between male ( $0.0029 \pm 0.0007 \text{ gL}^{-1}$ ) and female ( $0.0031 \pm 0.0011 \text{ gL}^{-1}$ ) animals. These data are an important support to understand the physiological functions of Zn in blood during the process of sera production at Butantan Institute (São Paulo, Brazil) using Crioulo horses.

**Keywords:** horse, blood, Zinc, hyperimmune sera, NAA

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## INTRODUCTION

The body of the horse consists of approximately 60 to 65% water, 30 to 35% of proteins, fats and carbohydrates and 4% minerals. Although in lower concentration, minerals are essential for the development, growth and immune function of horses. According to OTT [1] a diet based on grass is sufficient to maintain the physical condition of these animals when it is rich in minerals. However, if necessary, for short periods, hay, ration (rich in minerals and vitamins) and legumes can be incorporated into the diet to increase or complement the essentials nutrients.

Most nutrients correspond to the macro minerals, such as, calcium, phosphorus, sodium, chlorine, potassium, magnesium and sulfur and trace minerals that include: selenium, iodine, copper, zinc, manganese, iron and cobalt. Most of these trace minerals are component of enzymes involved in many biological reactions[2]. Zinc is a constituent of enzymes related to metabolism of carbohydrates, protein and keratin, and it is also involved in the immune system and resistance to stress. This trace mineral is essential for the development, growth, immune function and differentiation of tissues of all species[3].

Zinc deficiency causes rapid atrophy of the thymus and functional changes of T cells and natural killers (NK), diminishing mainly the production and activity of thymic hormones and certain classes of antibodies[3], while in excess can impair the absorption of copper[4]. According to the literature, the value of serum zinc in horses

show quite disparate results regarding breed, age, sex, type of activity and pregnancy: 30 to 40  $\mu\text{g/dL}$ [5] for ponies ; 173 to 271  $\mu\text{g/dL}$ [6] for PSI horses (1 to 6 months); 82 to 372  $\mu\text{g/dL}$ [7] (5 to 10 years old) and 101 to 428  $\mu\text{g/dL}$ [7] (11 to 15 years old) for Mangalarga's horses; 102 to 307  $\mu\text{g/dL}$ [7] (5 to 10 years old) and 150 to 409  $\mu\text{g/dL}$ [7] for Quart Horses (11 to 15 years old). For Crioulo breed there is still a lack of such data for blood.

In this study the reference value of zinc in the blood of Crioulo breed horses used in the production of hyperimmune sera (types of antivenom)[8] were determined using NAA. This technique has been applied in our clinical researches with success, for blood investigations in several laboratory animals, in function of simplicity in the sample preparation and agility in the analyses [9-11].

This investigation is important to understand the physiological functions of Zn in blood during the process of sera production[12] using Crioulo breed horses at Butantan Institute (São Paulo, Brazil).

## EXPERIMENTAL PROCEDURE

For this study 17 equines from Crioulo breed not submitted to the hyperimmunization process yet (Control Group) with 12-36 months-old, (9) females and (8) males animals, from São Joaquim Farm at Butantan Institute (São Paulo city, Brasil) were investigated. All the animals are without clinical signs of disease.

For sample preparation about 2 ml of whole blood was taken from jugular vein in vacuum plastic tube, without anticoagulants, using needles of 25 x 8mm. Aliquots of 500 $\mu\text{l}$  were collected in plastic cylinders (Eppendorf) and dried for few minutes using an infrared lamp. Reference material (IAEA-13) was prepared following the same procedure. Sample and standard were irradiated in the nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type) for 8 hours in a thermal flux of  $3.3 \cdot 10^{12} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$  allowed to decay time (7days) and count time for 2 hours. A HPGe detector (ORTEC Model GEM-60195, FWHM = 1.87keV) connected to an ADCAM multichannel analyzer (ORTEC Model 919E MCA) and to a PC computer was used to measure the induced gamma-ray activity. The concentration of zinc element was obtained using in-house software[12].

## RESULTS AND DISCUSSION

The concentration of the Zn in the blood samples of Crioulo horses are shown in Table 1. All the results are a mean of duplicate analyses. The reference interval considering  $\pm 1$  SD (Standard Deviation), the median, the minimum and maximum values were also presented. All the Zn concentration results for male and female, considering  $\pm 1$  and  $2 \pm \text{SD}$ , are shown in figure 1.

**TABLE 1:** Concentration of Zn in whole blood of horses (Crioulo race) used for sera production.

Zn, gL <sup>-1</sup>	Mean ± 1SD	Median	Min value	Max value
Male range [±1SD] (n=8)	0.0029 ± 0.0007 [0.0022- 0.0036]	0.0028	0.0017	0.0039
Female range [±1SD] (n=9)	0.0031 ± 0.0011 [0.0020 – 0.0042]	0.0029	0.0021	0.0053

n: number of animals  
Min: minimum value  
Max: maximum value

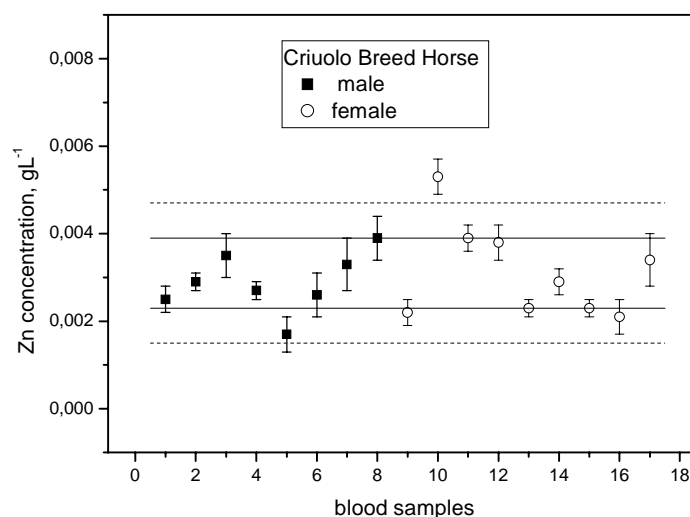


FIGURE 1. The Zn concentrations data for blood of Crioulo breed horses.

According to Table 1 no significant difference related to sex of the animals was observed although only males are used for sera production. Another aspect that can be emphasized from these results is relate to the possibility of checking the clinical status of the animals (Crioulo horses) during the immunization[12]: no damage in their clinical chemistry performance can be associated to zinc in blood, since its concentration is in a confidence interval of 95% (0.015-0.043gL<sup>-1</sup>) usually adopted as reference for clinical practice.

## CONCLUSION

The concentration for Zn was determined in blood of Crioulo breed horse using NAA. The result for the male control group give us parameters to interpret, in the future, the behavior of this element in blood since the first immunization as well as during the all the immunizations process established for an year (at least four) permitting to investigate in more details the evolution of the immunization process in sera production .

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