Influence of Haemolysis

Subjects: Medical Laboratory Technology

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Haemolysis of serum samples is the leading cause of preanalytical errors in clinical laboratories.

haemolysis mineral elements serum

1. Introduction

Minerals are inorganic elements that participate in almost all biochemical processes in living organisms and play a critical role in animal health and production [1]. Minerals have structural, physiological, catalytic and regulatory functions and are involved in tissue growth, cell replication and differentiation, energy and oxidative metabolism and immunity, among other vital processes [1][2]. Twenty-five mineral elements are considered essential to animals [3] and must be obtained through the diet.

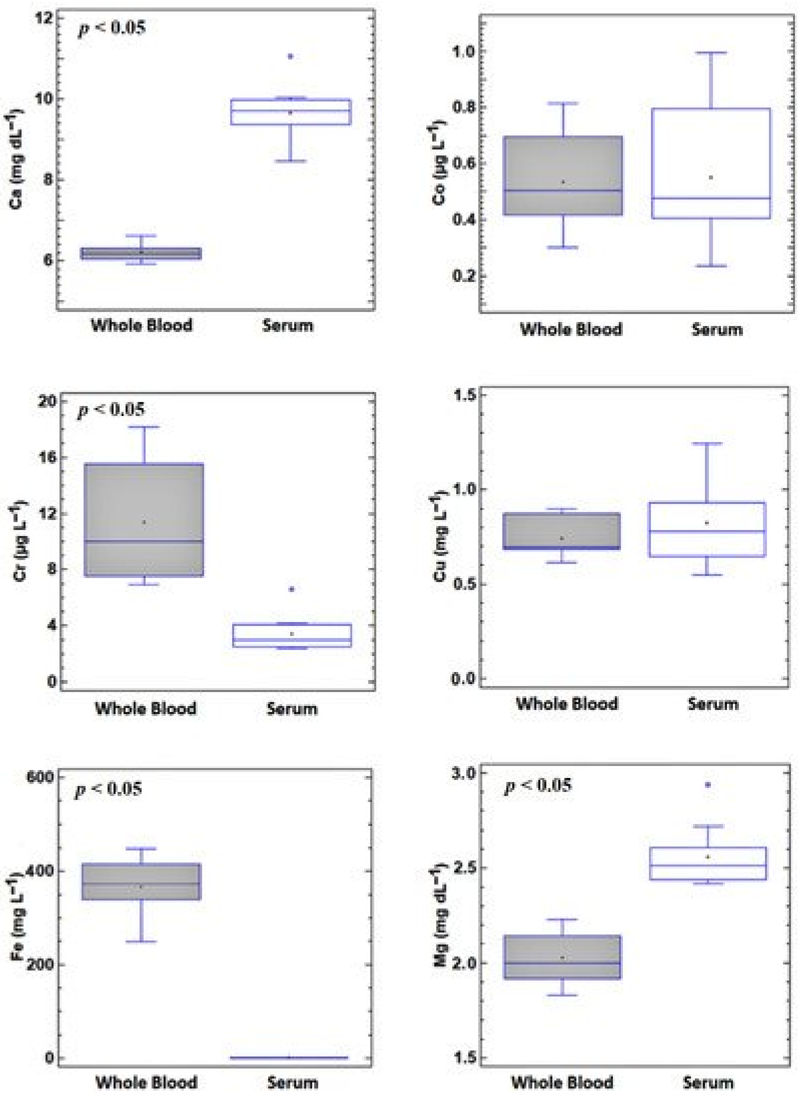
Mineral deficiencies, imbalances and toxicities can lead to obvious clinical disorders or can be manifested as subclinical processes that affect the profitability of livestock through decreased growth, reproduction and production rates [4][5]. Therefore, optimal mineral intake is important to maintain cattle health and also to maximize production. As most essential mineral elements have large safety margins, supplementation in feed has become routine practice. Therefore, mineral deficiencies and imbalances are less frequent nowadays and are almost exclusively reported in pasture-based farming in which concentrate feed is limited or avoided, as in the emergent sustainable production systems [6]. However, excessive mineral supply causes environmental pollution and has become of concern in the European Union, especially in relation to copper (Cu) and zinc (Zn) [7][8]; thus, mineral supplements must be carefully adjusted to meet the physiological needs of livestock. These requirements should be tailored according to the species, breed, sex, age, production phase, food properties and total intake [1][9][10]. Moreover, monitoring the mineral profile can provide useful information in relation to human nutrition and health, as animal products are one of the main sources of essential mineral intake and toxic element residues in the human diet [11].

Blood is the most commonly used and convenient sample for preliminary determination of the mineral profile of herds, as collection is simple and non-lethal and modern atomic spectrometry techniques provide precise and inexpensive mineral determinations at low concentrations [12][13]. Whole blood is seldom used and plasma and serum are considered suitable and interchangeable for most elements [14][15]. Almost all available data regarding physiological mineral concentrations in cattle refer to serum [16][17].

Haemolysis is the leading cause of sample rejection in laboratories and, although little is known about its influence in mineral determination, it is widely recognized to bias the results of most routine analyses [18][19][20]. Blood cells may be disrupted for pathological reasons or due to sample handling, with the latter being the most frequent cause of preanalytical errors [21]. The intracellular and serum concentrations of some mineral elements are known to differ; thus, mineral profiles can potentially be altered by haemolysis. However, as broad comparative studies of mineral concentrations in cattle whole blood and serum/plasma are scarce, the influence of haemolysis cannot be accurately predicted. While the release of blood cell contents could increase the concentrations of some minerals, as is known to occur with iron (Fe), Zn, selenium (Se), magnesium (Mg) and manganese (Mn) [13][17][22][23][24], the concentrations of other elements, such as Cu [25], may decrease due to dilution of the sample if the intracellular concentration is lower. Nonetheless, if the variation in mineral concentration is not significant, the haemolyzed sample may be suitable for analysis and unnecessary sample rejection could be avoided.

2. Mineral Concentrations in Paired Blood and Serum Samples

The mineral concentrations in paired whole blood and serum samples (*n* = 10) are summarised in **Figure 1**. Iron was the most abundant element in whole blood (364 ± 64 mg L^{-1}, ranging from 211 to 381 mg L^{-1}), in concentrations approximately 300 times higher than those in serum. Whole blood Fe is mainly represented by Hb, which contains four Fe atoms per molecule [1]. The variations in the ratio of Fe in whole blood:serum can almost exclusively be attributed to the number and volume of erythrocytes. Physiological variations in red blood cells are observed among individuals, as well as in numerous pathological conditions, particularly those causing anaemia. Overall, the results obtained indicate that haemolysis can strongly influence the determination of the Fe status of cattle and could lead to the overestimation of Fe, although this error can potentially be corrected if the HD is calculated by considering that 1 g of haemoglobin contains 3.47 mg of Fe [26].



p < 0.05
Ca (mg dL-1)
Whole Blood
Serum
Co (µg L-1)
Whole Blood
Serum
p < 0.05
Cr (µg L-1)
Whole Blood
Serum
Cu (mg L-1)
Whole Blood
Serum
p < 0.05
Fe (mg L-1)
Whole Blood
Serum
p < 0.05
Mg (mg dL-1)
Whole Blood
Serum

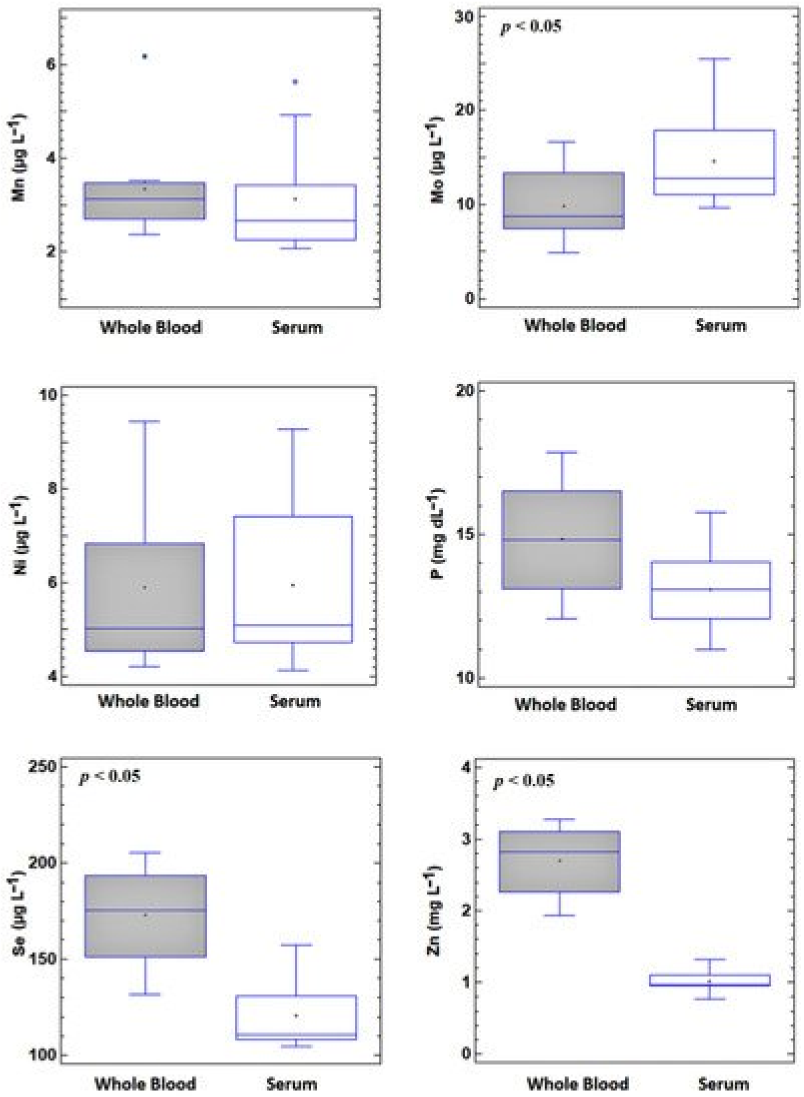


Figure 1. Box-and-whisker plots showing macro and microminerals in paired whole blood and serum samples (n = 10). $p < 0.05$ indicates a statistically significant difference between whole blood and serum values. Cr, Fe, Se and Zn are significantly higher in whole blood and Ca, Mg and Mo are significantly higher in serum.

Chromium, Zn and Se concentrations were also significantly higher in whole blood than in serum, with whole blood:serum ratios of 3.73, 2.71 and 1.43, respectively. For all of these elements, the largest interindividual variations were found for the whole blood samples (**Figure 1**). Both Se and Zn are cofactors or essential intra erythrocyte enzymes that are closely involved in the immune system, which could explain the large individual variation within subjects. Selenium is associated with GPX1, the most abundant glutathione peroxidase in the body, and is also responsible for most of the blood Se concentration (erythrocyte Se usually represents 60–73% of the Se in whole blood in cattle [1][22]). Zinc is also essential for multiple intraerythrocytic enzymes such as superoxide dismutase, carbonic anhydrase and lactate dehydrogenase (LDH) [1][3]. The high concentration of LDH in red blood cells leads to its overestimation when measured in serum with HD values lower than 0.5 g Hb L^{-1} [23]. Regarding Cr, when present as Cr (III), it constitutes an essential mineral with beneficial influence on immune and antioxidative activity and energy metabolism [27][28] and is transported in serum due to its inability to penetrate red blood membranes [29]. By contrast, the hexavalent form Cr (VI) is highly toxic and carcinogenic [30] and has a high affinity for erythrocytes, where it is reduced to Cr (III) and bound to the β-chain of Hb [29]. In the present study, the higher Cr concentration in the whole blood samples than in the serum probably reflected a slight exposure of the subjects to environmental Cr (VI).

The whole blood concentrations of Mg, Mo and Ca were significantly lower than in serum (whole blood:serum ratios of 0.79, 0.65 and 0.66 respectively). The intraerythrocytic concentrations of these elements are low and, as they are mainly transported bound to plasma proteins in the blood, sample haemolysis would lead to underestimation of the concentrations in serum due to a dilution effect. The Mg and Mo concentrations showed similar inter-subject variability in whole blood and in serum, making it difficult to precisely predict its influence. However, the Ca concentrations in whole blood revealed very low inter-individual variation, suggesting that the effect of haemolysis could potentially be estimated by the analysis of haemolyzed serum. For the other elements, there were no significant differences between the concentrations in whole blood and serum, suggesting that haemolysis would have a negligible effect on the concentrations measured in serum.

The essential role of mineral elements in animal health and production, together with the high prevalence of mineral imbalances worldwide, has led to a large body of research on mineral element metabolism in the last few decades, including the establishment of precise reference values in serum and plasma (for reviews, see [1][16][17]). However, information of mineral element concentrations in whole blood is scarce, with the exception of Se. This is probably because Se deficiency is one of the most prevalent and costly trace element imbalances in livestock and the determination of Se in whole blood provides accurate, long-term information about the Se status of the animal [1][13]. Information about the effect of haemolysis on the determination of mineral status in cattle and other livestock species is also very limited. In a comprehensive review by Herdt and Hoff [17] on the use of blood to evaluate the mineral status in ruminants, apart from Fe, possible leakage from erythrocytes is only mentioned in relation to Zn and Mn. According to these authors, haemolysis or prolonged contact of the serum with the clot leads to the escape of Zn and Mn from red blood cells into the serum, thus producing a false increase in serum concentrations. However, these suggestions are based on studies in humans, in which the whole blood:serum ratios are different from those in bovine species. In humans, concentrations of both Mn [31] and Zn [24] in erythrocytes are 10–20 times higher than in plasma or serum, i.e., a ratio whole blood:serum in the range 5–10. In this study, whole blood Zn concentrations were around three times higher than in serum and no difference was observed for Mn. These results indicate that haemolysis may have less influence on the determination of Zn and particularly Mn in cattle than in humans. For all these reasons, it is important to known how and the degree to which haemolysis affects serum concentrations of minerals.

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