Sudan University of Science and Technology

College of Veterinary Medicine

A Study of Some Trace Minerals Status in Serum of Sick Animals

دراسة لمستويات المعادن النادرة في مصل الحيوانات المريضة

By:

A research submitted in partial Fulfillment of the requirement of the Vet. Med. Sur. (Honours)

Maawia Mohammed Haroun Mohammed

Maha Mohammed Abd Alshacour Makoush

Noon Azhury Awd ALseed Mohammed

Rayan Ahamed Osman Ahamed

Remah Dafa Allah Mohammed Aibed

Tagwa Fatah Alrhman Omer Ahamed

Supervised by

Dr. Hala Ali Mohammed Ibrahim

Department of Pathology, Parasitology and microbiology

October 2016

الإستهلال

قال تعالى: شَهِدَ اللَّهُ أَنَّهُ لَا إِلَهَ إِلَّا هُوَ وَالْمَلَائِكَةُ وَأُولُو الْعِلْمِ قَائِمًا بِالْقِسْطِ لَا إِلَهَ إِلَّا هُوَ الْعَزِيزُ الْحَكِيمُ.

آل عمران : الآية 18

DEDICATION

TO OUR MOTHERS, TO OUR FATHER,S, To BROTHERS , SISTERS AND FRIENDS, TO ALL OF THEM WITH LOVE

Acknowlegement

First of all, our sincere thanks to Allah who gave us the power and patience to begin and complete our study.

We would like to express our most sincere thanks to our supervisor, Dr. Hala Ali Mohammed Ibrahim, for her guidance, help and encouragement throughout this study.

Limitless thanks and gratitude to Mrs. Rawda Hassan at The Department of Biochemistry-Soba Central Veterinary Labrotary, for her great help in minerals analysis.

Thanks and gratitude to Dr. Abu Obieda Alsheekh at Sharg Alneel Hospital for his great help in collection of samples.

Many thanks for professor Mohammed Tag Eldein for his help in statistical analysis.

List of Contents

Content **Page**

List of Tables

Abstract

Aim: The present study was carried out with the main objective to investigate the serum copper, zinc and magnesium status in sick animals of different species (goat, sheep and cattle)

Materials and Methods: Nineteen animals of different species (Goats, sheep and cattle) with clinical signs of sickness were selected randomly from Sharg ALneel Hospital - Elseleit , East Nile- Khartoum State. Serum minerals were estimated by atomic absorption spectrophotometry.

Results: The mean serum Cu concentrations in goat, sheep and cattle were (2.014 ± 0.23, 2.163± 0.30 and 1.950 ± 0.24 mg/L) respectively. The mean serum Zn concentrations in goat, sheep and cattle were (0.264 ± 0.05, 0.262 ± 0.04 and 0.261 ± 0 .03 mg/L) respectively, whereas the mean serum Mg concentrations in goat, sheep and cattle were 1.407 ± 0 $.13^{b}$, 1.751 ± 0.10^{a} and 1.894 ± 0.22^{a} mg/dl respectively. The result showed that no significant (P>0.05) differences were found in the mean serum Cu and Zn concentrations between the different animals species, while the mean serum Mg concentration was significantly (P<0.01) different between animals species. The mean Cu concentration in serum of the different animal species was significantly (P<0.01) high (2.063 ±0.26 mg/L), whereas the means of Zn and Mg concentrations in serum of the different animal species were significantly (P<0.01) low (0.262±0.04 mg/L and 1.654±0.24 mg/dl respectively) as compared to the normal value.

Conclusion: The result showed that the serum concentrations of Cu and Zn are not different between goat, sheep and cattle, whereas the serum concentration of Mg is different between this species. There are changes in the levels of serum Cu, Zn and Mg in the sick animals.

Key words: Cu, Zn, Mg, goat, sheep, cattle, sickness

الخلاصة

الهدف من هذه الدراسة هو بحث مستويات تراكيز النحاس والزنك والماغنسيوم في مصل الحيوانات المريضة من الماعز والضأن والبقر.

تم إختيار تسعة عشرحيواناً عشوائياً من الماعز (7)، الضأن (8) والبقر (4) التي أظهرت أعراض مرضية مختلفة.

وتم أخذ أمصال هذه الحيوانات المريضة من مستشفى شرق النيل بالسليت ، وتم قياس مستوي النحاس، الزنك والماغنسيوم بواسطة قياس شدة موجات الطيف للامتصاص الذري .

النتائج أوضحت أن متوسط تركيز النحاس في مصل الماعز ، الضأن والبقر كان (2.014±0.23 ،2.163±0.30 و 1.950±0.24ملجم/ليتر) علي التوالي.

ومتوسط مستوي تركيز الزنك في مصل الماعز، الضأن والبقر (0.264±0.05،0.262±0.04 و0.261±0.03 ملجم/ليتر) علي التوالي.

بينما كان متوسط تركيز الماغنسيوم في مصل الماعز ، الضأن والبقر (1.407±0.13 ،1.751± 0.16 و 1.894±0.22ملجم/ديسليتر) علي التوالي ، النتيجة أظهرت عدم وجود فروقات معنوية في تركيز النحاس والزنك بين الانواع المختلفة من الحيوانات ، بينما كان هناك إختلاف معنوي ($P<0.05$) في تركيز الماغنسيوم بين الماعز، الضأن والبقر .

كان مستوي النحاس، الزنك والماغنسيوم في مصل الحيوانات من الانواع المختلفة 2.06±0.26 ملجم/ليتر ، 0.04 ±0.262 ملجم/ليتر ، 0.24 ±1.654ملجم/ديسليتر علي التوالي.

النتائج أوضحت وجود إنخفاض معنوي ($P<0.01$) في متوسط تركيز الزنك والماغنسيوم في مصل الحيوانات المريضة بينما كان هناك ارتفاع معنوي ($P<0.01$) في مستوي النحاس في مصل هذه الحيوانات مقارنة بالمستويات الطبيعية لهذه المعادن في مصل الحيوانات السليمة.

Introduction

The prevention of animal diseases is important for many different reasons. First of all, the animal itself has to be protected and the spread of disease must be restrained to guarantee a healthy animals population (Bren, 2016). The majority of the animal population in Sudan is raised under range management and nomadic conditions hence mineral investigations are of great importance. On the other hand, there are few reports on occurrence of nutritional disorders and there is underestimation of problems associated with these disorders because their incidence is masked by the infectious diseases. Nutrition and more specifically, minerals and trace elements play an important role in immune function and health. Even moderate deficiencies can adversely impact animal performance (Prashanth *et al.*, 2015). It is well known that trace minerals (Cu, Mg, and Zn, among others) are required for the normal functioning of basically all biochemical processes in the body. They are part of numerous enzymes and coordinate a great number of biological processes, and consequently they are essential to maintain animal health and productivity (Suttle, 2010). Although minerals such as copper, magnesium and zinc have been researched in human and animals, data of their status in animal's serum during diseases are still lack. **Therefore the objectives of this study were designed to**

1- To investigate the levels of serum copper, magnesium and zinc in sick animals of different species.
2- To evaluate the differences of serum levels of minerals between the different animal species.
3- To estimate the effect of sickness in minerals status.
4- To give a light on the effects of copper, magnesium and zinc in immune response against diseases.

Chapter One

Literature Review

1.1. Trace Elements (Micronutrients)

Trace elements copper, magnesium and zinc, are elements that are required in minute amounts for optimal growth, development and physiology. Trace elements are indispensable for life and play an important role in essential functions, including immune function (Brent, 2016). Low intakes of micronutrients have been shown to suppress immune function by affecting the innate T-cell mediate immune response and antibody response, which, in turn, increases the susceptibility to infections. Further, infections especially recurring infections can further worsen existing micronutrient deficiencies by interfering with nutrient intake, increasing losses, and impeding utilization (Bondestam *et al.*, 1985).

1.1.1. Copper (cu)

Is one of the essential trace elements in animals, and disorders associated with its deficiency and excess have been reported, Cu deficiency has also been reported to develop after gastrointestinal surgery, intractable diarrhea, and prolonged parental or intra nutrition (Nezu *et al*., 1992).

1.1.1.1. Copper metabolism

The inhibitory effect of copper on glycine receptor mediated currents is voltage independent, cu acts at a specific neuromodulatory site rather than as channel blocker, the increase in neurotransmission in response to prolonged exposure to copper has been ascribed to a copper induced enrichment of the receptor at the plasma membrane (Aoki. *et al.*, 1996).

1.1.1.2. Copper Sources and bioavailability

Cu availability in most feed stuff fed to farm animals range between 1% and 15%. Grains are lower in Cu than are forages. Most forage contains Cu at levels equal to or above the National Research Center (NRC) requirements. However, as plant mature and the phytate and lignin content increases, bioavailability of the Cu decreases rapidly (Hemken *et al.*, 1993; Clark *et al.*, 1993). Two types of soils have a marked influence on copper uptake by the plants or cu absorption by the animal. Muck soils, because of their high organic matter bind copper which in turn makes forage grown on these soils deficient in copper. Soils high in molybdenum can produce forages with high molybdenum content which can interfere with copper metabolism in animal grazing these high molybdenum soils (NRC, 1980).

1.1.1.3. Copper physiology

Cu is an essential component of several physiologically important metalloenzymes; hence, Cu deficiency leads to effect on multiple organ systems. These enzymes include cytochrome c oxidase, ceruloplasmin, lysyl oxidase, phenyl oxidase (tyrosinase), superoxidase dismutase, dopamine-beta-hydroxylase, and thiol pxidase (Jones *et al.*, 1997).

1.1.1.4. Copper physiological effects

In general copper is poorly absorbed by the digestive tract. Copper absorption is influenced by the age and breed of cattle (NRC, 1980). Cu has role in iron metabolism, (Philip *et al.*, 2005), connective tissue formation (Sherry, 2004), pigmentation and keratinization of hair and wool (Ibrahim, 2006), central nervous system as in sway back (enzootic ataxia) of lambs (Howell *et al.*, 1969), reproductive system (Ahmed *et al.*,

2002). Also Cu has effects on immune system and effect on cardiovascular system (Sherry, 2004).

1.1.1.5. Copper concentration in blood serum

Blood serum Cu concentration is a more reliable and consistent measure of Cu status than is whole blood, but neither reflects dietary intake unless the liver is depleted of its Cu stores. Faye and Grillet, (1984) established a range for normal Cu status in animals: mean serum Cu at 24.7 mg/100 ml (5-55) in sheep, 37.2 mg/100 ml (15-60) in cattle and 41.8 mg/100 ml (20-60) in goats.

1.1.1.6. Copper and immunity

Cu metabolism affects T and B cells, neutrophils, and macrophages. The relationship of Cu to the immune system is through superoxidase dismutase, a Zn-Cu, and manganese (Mn)-dependent enzyme that plays a role in the microbial system of phagocytes. An impaired humoral immune response (i.e. decreased number of antibody-producing cells) was observed in mice with hypocuprosis. The magnitude of this impairment was highly correlated with the degree of its functional deficiency (Sherry, 2004). Some of research showed that interleukin 2 is reduced in copper deficiency and is likely the mechanism by which T cell proliferation is reduce (Susan, 1998).

1.1.2. Magnesium

Magnesium is the fourth most common action in the body, and the second most common intracellular action after potassium.

In many of its actions it has been likened to a physiological calcium antagonist (Abraham *et.al.*, 1977).

1.1.2.1. Magnesium metabolism

The first steps in magnesium metabolism through this pathway are breakdown by the mechanical action of chewing, and the digestive action of gastric acids found in the stomach. Following digestion, magnesium is largely absorbed in the small intestine. There magnesium passes from tiny “villi”, finger-like surfaces inside the small intestine, into capillaries, tiny blood vessels surrounding the small intestine. Digestive factors unique to the individual can also influence the amount of magnesium absorbed in the GI tract. These include the ability to breakdown magnesium containing foods in the stomach, and the ability to absorb magnesium in the small intestine (Adams and Mitchell, 1979).

1.1.2.2. Magnesium sources and bioavailability

Less than 1% of total body magnesium is found in serum and red blood cells. It is distributed principally between bone (53%) and the intracellular compartments of muscle (27%) and soft tissues (19%). Ninety percent of this intracellular magnesium is bound to organic matrixes. Serum magnesium comprises only approximately 0.3% of total body magnesium, where it is present in three states ionized (62%). Protein bound (33%), mainly to albumin, and complexes to anions such as citrate and phosphate (5%). Equilibrium between tissue pools is reached slowly with a half-life for the majority of radiolabelled magnesium varying between 41 and 181 days (Elin, 1994). Green plants are an excellent dietary source of Mg for animals because of the presence of Mg in chlorophyll Rich magnesium sources include green vegetable, nuts, seeds and tape water (Aikawa, 1981).

1.1.2.3. Magnesium physiology

It is an important electrolyte needed for proper muscle, nerve and enzymes. It also helps the body use energy and is needed to moves other electrolytes (potassium and sodium) into and out of cell (Fischbach and Duning, 2009).

1.1.2.4. Magnesium physiological effects

Magnesium is required for many of the major organs to function and plays a crucial role in human and mammalian physiology. Magnesium is essential for the structure of bones and teeth, acts as a cofactor for more than 300 enzymes in the body, including binding to ATP for kinase reactions, and affects permeability of excitable membranes and neuromuscular transmission. Despite these essential roles, much is still unknown about magnesium physiology and homeostasis (Samantha and Andrea, 2014).

1.1.2.5 Magnesium concentration in blood serum

Electrolyte concentrations are similar whether measured in serum or plasma. Values can be expressed in terms of weight per unit volume (mg⁄ deciliter) or in the number of molecules in volume, molarity (moles or mill moles⁄ liter). The range of normal values sometimes varies slightly between different species and different analytical laboratories (Puls, 1994). The serum magnesium estimations may not provide representative information on the status of other stores.

1.1.2.6. Magnesium and immunity

Magnesium is involved in immune function, both in innate and acquired immune response according to researchers (Tam *et al.*, 2003). According to Leo Galland, magnesium acts as a cofactor for immunoglobulin synthesis, immune cell adherence, antibody dependent cytolysis, IgM lymphocyte binding, macrophage response to lymphokines, T helper and B cell adherence, and additional responses (Galland, 1988). (Cojocaru *et al.*, 2009) Mention that magnesium (Mg) deficiency seems to be implicated in immune dysfunction, including acute and chronic infections and they concluded that, the measurement of Mg serum in bacterial infections is useful and physicians should maintain a high index of suspicion for hypomagnesemia and implement Mg therapy

1.1.3. Zinc (Zn)

Zinc is an essential trace element because of its fundamental role in gene expression, cell development and replication. It plays structural, regulatory, and catalytic roles in the body (Hambridge, 2000).

1.1.3.1. Zinc metabolism

Only two genetic defects of zinc metabolism are known in animals. One is associated with lethally inadequate concentration of of zn in the milk of mice, the other with the A46 trait in Friesian cattle. A46 is a recessively inherited defect of Zn absorption which is lethal in the absence of major Zn supplementation of the diet. The characteristics of the disease are very similar to those of acrodermatitis (Andresen, 1994).

1.1.3.2. Zinc sources and bioavailability

The study of zinc bioavailability in foods is important because this mineral intake does not meet the recommended doses for some population groups During 42 days, rats were divided into four groups and fed with diets containing two different sources of Zn fortified with zinc oxide, or control diet: zinc carbonate (ZnCO3), supplying 50% or 100%, respectively, of the recommendations of this mineral for animals. Weight gain, food intake, feed efficiency ratio, weight, thickness and length of femur; retention of erythrocyte and plasmatic Zn between groups. Although rice fortified with zinc oxide showed a lower bioavailability compared to ZnCO3, this food can be a viable alternative to be used as a vehicle for fortification (Ceres *et al.,* 2014). the total zinc content of the meal and the amount and source of protein of these, the most critical factor affecting the bioavailability of zinc is the phytic acid content of the diet. Phytate or phytic acid is a main storage form of phosphate and is ubiquitously distributed in plant foods, especially cereal, grains and legumes (Cuong *et al.*, 2015).

1.1.3.3. Zinc Physiology

Zinc is an essential component of over 70 enzymes found in mammalian tissues. Enzymes that require zinc are involved in protein, nucleic acid, carbohydrate, and lipid metabolism. Zinc is also important for normal development and functioning of the immune system, in cell membrane stability, and gene expression (Brent, 2016).

1.1.3.4. Physiological effect of zinc

Zinc plays a significant role in cytotoxic events in single cells. Here, zinc influences apoptosis by acting on several molecular regulators of programmed cell death, including caspases and proteins. where zinc is prominently involved in cell death is the brain, and cytotoxicity in consequence of ischemia or trauma involves the accumulation of free zinc, and has a detrimental impact on growth, neuronal development, and immunity, it is deficiency caused by malnutrition and foods with low bioavailability, aging, certain diseases, or deregulated homeostasis is a far more common risk to human health than intoxication (Brent, 2016).

1.1.3.5. Zinc concentration in blood serum

Trace minerals must be provided to livestock in optimal concentrations and according to requirements that change during the rapid growth and development of the animal and the production cycle (Javier *et al.,* 2013).

1.1.3.6. Zinc and immunity

Nutrient “zinc” is a relevant micronutrient involved in maintaining a good integrity of many body homeostatic mechanisms, including immune efficiency, owing to its requirement for the biological activity of many enzymes, proteins and for cellular proliferation and genomic stability (Javier *et al*., 2013).

Chapter Two

Materials and Methods

2.1. Experimental Animals

Nineteen animals of different species; goats (7), sheep (8) and cattle (4) with clinical signs of sickness were selected randomly to be used for this study. All the animals were from ElSeleit - East Nile- Khartoum State.

2.2. Samples

2.2.1. Blood Collection

Jugular blood samples (5ml) were collected from all animals. The blood was drown into clean dry vials without addition of anticoagulant, allowed to clot at room temperature for one hour, and kept overnight at 4ºC, centrifuged at 3000 rpm for 15 minutes. Clear unhaemolyzed serum was separated and then stored at -20 ºC until used.

2.2.2. Determination of serum copper, zinc and magnesium concentration

For the determination of serum copper and zinc the serum sample was diluted with an equal volume of deionized water and then analyzed for copper and zinc by atomic absorption spectrometer (Perkin- Elmer modle-3110, USA), using air/ acetylene, fuel rich flame (air 5.0 liter min^{-1}, C_2H_2 1.0 liter min^{-1}) as described previously by(Camas *et al.*, 1999).
Serum Mg was measured by using commercially available kit (Biomedical Systems, Barcelona, Spain). The analyses were carried out according to the manufacturer's prescriptions.
Laboratory analysis was done at the department of biochemistry-Soba Central Veterinary Labrotary.

2.3. Statistical analysis

Data were analyzed with SPSS (Statistical package for social sciences) statistical software, version 22. Data are presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) and T-test (one sample) were used. A level of *P* value of less than 0.05 was considered statistically significant.

Chapter Three

Results

3.1. Cu concentration in serum of different species

The mean values of Cu concentration in the serum of the different species are shown in table1. There was no significant different (P>0.05) of serum Cu concentration between the different species.

3.2. Zn concentration in serum of different species

The mean values of Zn concentration in the serum of the different species are shown in table2. There was no significant different (P>0.05) of serum Zn concentration between the different species.

3.3. Mg concentration in serum of different species

The mean values of Mg concentration in the serum of the different species are shown in table3. There was significant different (P<0.01) of serum Mg concentration between the different species. The mean concentration of serum Mg in goat was different (P<0.05) from that in sheep and cattle, while there was no significant different (P>0.05) of serum Mg concentration between sheep and cattle.

3.4. Serum Cu, Zn and Mg concentrations of different species compared with the normal value

The mean values of Cu, Zn and Mg concentrations in the serum of the different species compared with the normal value are shown in table4. There were significant changes in the levels of Cu, Zn and Mg in the serum of the animals of the different species. Serum Cu concentration of the different species is higher (P<0.001) than the normal value, while the serum Zn and Mg concentrations of the different species are lower (P<0.001) than the normal value.

Table1. Means of serum Cu concentration of different species

Species	Number of animals	Cu (mg/L)	Significant
Goat	7	2.014 ± 0.23 (1.800 - 2.400)	NS
Sheep	8	2.163 ± 0.30 (1.700 - 2.600)	
Cattle	4	1.950 ± 0.24 (1.700 - 2.200)	

Results are mean ± SD.

NS: Not significant (P>0.05).

Table2. Means of serum Zn concentration of different species

Species	Number of animals	Zn (mg/L)	Significant
Goat	7	0.264 ± 0.05 (0.188 - 0 .310)	NS
Sheep	8	0.262 ± 0.04 (0.19 7- 0.300)	
Cattle	4	0.261 ± 0 .03 (0.225 - 0.285)	

Results are mean ± SD.

NS: Not significant (P>0.05).

Table3. Means of serum Mg concentration of different species

Species	Number of animals	Mg (mg/dL)	Significant
Goat	7	1.407 ± 0.13^{b} (1.210-1.640)	**
Sheep	8	1.751 ± 0.10^{a} (1.600 - 1.900)	
Cattle	4	1.894 ± 0.22^{a} (1.570 - 2.060)	

Results are mean ± SD

**: significant at (P<0.01)

a, b: means within the same column followed by different superscript are significantly (P<0.05) different.

Table4. Means of serum Cu, Zn and Mg concentration of different species compared with the normal value

Mineral	Mean concentration	Normal value*	Significant
Cu	2.063±0.26 mg/L	0.548 mg/L	**
Zn	0.262±0.04 mg/L	0.864 mg/L	**
Mg	1.654±0.24 mg/Dl	2.11 mg/dL	**

Results are mean ± SD (Number of animals=19)

**: significant at (P<0.01)

*: The Merck Veterinary Manual

Chapter Four

Discussion

The present study was carried out to investigate serum levels of Cu, Zn and Mg in sick goat, sheep and cattle.

There are two general classes of abnormalities associated with trace elements: specific deficiency from dietary inadequacies, imbalances, or a secondary to other diseases. Both kinds of abnormalities can be diagnosed by analyses of trace elements in serum or other tissues (Klassing, 1988). The trace element concentrations in serum samples change during infection (Beisel, 1976; Montaya, 2002). These changes are part of defense strategies of the organism, induced by IL-1, IL-6, and TNF-$\propto$5 (Klassing, 1984, 1988).

There are a lot of studies about these essential trace elements in a various infectious diseases. It was claimed that low serum Zn levels was observed in parasitic diseases and also immune system was adversely affected during these infections (Tasci, *et al*., 1995). In the present study, we showed that the serum Zn levels were significantly decreased in sick animals of different species as compared to the normal value described by (Merk, 2016). Because Zn is a functional component of several enzyme systems such as carbonic anhydrase, carboxypeptidase a and b, phosphateses, several dehydrogenases. We can postulate that in the case of sickness the whole organs of the animals affected adversely.

The role of certain inflammatory products in regulation of the Zn balance has been well documented. Thus, leukocyte endogenous mediators (interleukins), released from activated phagocytic cells, causing a lowering of Zn levels, resulting from increased synthesis of metallothionine in liver and other tissues (Klassing, 1984; Svenson, *et al*.,

1985). It has also been known that Zn is an essential trace element for immune function that plays a role in immune response against infection (Kargin, 2004**)**. In view of the fact that decreased Zn levels found in our study may be related to immune response against disease. Interestingly, in our study, we found that serum Cu levels were significantly higher in the diseased sera of animals of different species than the normal values. It has been found that serum Cu values are increased by infection (Etzel, *et al*., 1982). Serum Zn decreased and serum Cu increased during market-transit morbidity or after stressed by bovine respiratory disease and infectious bovine rhino tracheitis (IBRV) (Orr, *et al*., 1990). Our result is also agreed with the finding of (Kamil, *et al*., 2004). Unfortunately, we were not able to determine the serum ceruplasmin the main Cu carrier in serum concentrations. The increased serum Cu may be in the form of serum ceruplasmin which formed in response to inflammation associated with the disease**,** On the other hand Mg concentrations in serum from sick animals of different species were significantly decreased in sick animals of different species as compared to the normal value described by (Merk, 2016).

Mg deficiency seems to be implicated in immune dysfunction, including acute and chronic infections. (Cojocaru, *et al.*, 2009). Our finding is agreed to the observations of (Yazar, *et al.*, 2003). Who reported decreased Mg concentrations in patients with chronic toxoplasmosis. In other words, according to our finding Mg-linked enzyme systems such as ATPase, alkaline phosphatase seem to be affected by disease.

Conclusion

In conclusion, Animals of different species with clinical signs of sickness showed decreased serum Zn and Mg levels and increased Cu concentrations as compared to the normal values. This may affect many of the enzyme systems.

Recommendations

1- Large scale surveys can overcome these limits of experimental data, because the large number of animals and herds makes it possible to evidence moderate modifications of sickness incidence, and because multifactorial analysis makes it possible to take into account in the same database the effects of several trace elements.
2- Establishment of mineral reference ranges is important for such species, as their health and productivity are managed

References

Abraham, A.S., Eylath, U., Weinstein, M., Czaczkes, E.(1977). Serum magnesium levels in patients with acute myocardial infarction. N Engl J Med ; 296: 862–3

Adams, J.H., Mitchell, J.R.A. (1979). The effects of agents which modify platelet behaviour and magnesium ions on thrombus formation in vivo. Thrombi Headmost ; 42: 603–10

Ahmed, M.M., Fadlalla, I.M. and Barri, M.E. (2002). Possible association between dietary intake of copper, zinc and phosphate and delayed puberty in heifers in Sudan. Tropical Animal Health and production. 34: 75-80.

Aikawa, J.K. (1981). Magnesium: Its Biological Significance. Boca Raton, Florida: CRC Press.

Aoki,T.,Yamaguchi,Y.,Shimizu,N. (1996) Copper deficiency and it treatment from the point of view of copper metabolism in the body. Pediatrics of Jaban 317-329 (in Japanese).

Anderson , K.A., and Talcoh, P.A.(1994) . Magnesium.In "Hand book on metals in clinical and anal chemistry .pp.453—466. Marcel Dekker,New York.

Beisel, R.W. (1976). Trace elements in infectiosis processes. Med Clin North Am; 60: 831-849.

Brent, H. (2016). Diagnosing Trace Mineral Deficiencies and Excesses in Transition Dairy Cows WCDS Advances in Dairy Technology, Volume 28: 287-297.

Bondestam, M. Foucard, T. Gebre - Medhin, M. (1985). Subclinical trace element deficiency in children with undue susceptibility to infections. Acta Paediatr Scand. 1985; 74(4):515 - 20.

Camas, H., Bildik. A., Gülsever, F. (1999). Investigation of some trace elements (Cu, Mo, Zn, Co, Mn) and sulphate in soil, grass and sheep's blood. Y.Y.Ü. Vet Fak Derg ; 10(1-2): 87-91).

Ceres Mattos, Della lucia, luiza Menezes Santos, kellen Cristina da Cruz Rodrigues, Vivian Cristinada ,Cruz Rodrigues,Hercia Stampini Duarte Martin and Helena Maria Pinheiro Sant Ana. (2014) . Bioavailabity of zinc in wistar Rats fed with Rice fortified with zinc oxide nutrients. (6): (2279 - 2289).

Clark, T., Xin, Z., Du, Z. and Hemken, I. (1993). A field trail comparing copper sulfate, copper proteinate and copper oxide as copper sources for beef cattle. Journal Dairy Science. 76: 334.

Cojocaru, I.M.1, Cojocaru, M. Tanasescu, R. Iacob, S.A. Iliescu, I. (2009) **.**Changes of magnesium serum levels in patients with acute ischemic stroke and acute infections. Rom J Intern Med. ;47(2):169-71.

Cuong, D.T.Geethal,G.Elissa,K. (2015). The potential for zinc stable isotope techniques and modeling to determine optimal zinc supplementation nutrients'7(6):4271_ 4295.

Elin, R.J. (1994). Magnesium: the fifth but forgotten electrolyte. Am J Clin Pathol; 102: 616– 22.

Etzel, K. R., Swerdel, M. R., and Cousins, R. J.(1982), Endotoxin-induced changes in copper and zinc metabolism in the Syrian hamster. J. Nutr. 112:2363-2373.

Faye, B. and Grillet, C. (1984). Copper deficiency in domestic ruminants in the Awash region (Ethiopia). 1: Plasma copper and ceruloplasmin content in sheep, goats, cattle and camels of the Wash region (Ethiopia). Revue d Elevage et de Medecine Veterinaire des pays Tropicaux 37: 42-60.

Fischbach, F.T. Dunning, M. B. (2009). Manual of Laboratory and Diagnostic test, 8th ed, philadephia:lippincott Williams and wilkins.

Galland, L. (1988). Magnesium and immune function: an overview.Magnesium ; 7(5):290 ‑ 9).

Hambridge, K.M. (2000). Human zinc deficiency. Journal of Nutrition, 130:S1344–1349.

Hemken, R.W., Clark, T.W. and Du, Z. (1993). Copper: Its role in animal nutrition. In: Tlyons (Ed.) Biotechnology in the feed industry. PP 35-39 Altech Technical Publications.

Howell, J., Davison, A. and Oxberry, J. (1969). Observations on the lesions in the white matter of the spinal cord of sway back of sheep. Acta Neuropathol. 12: 33-41.

Ibrahim, A.M. Hala. (2006). Clinico-pathological effect of copper deficiency on goat. M.VSc. Theis, University of Khartoum.

Javier Romeo, Marco Malavolta, Laura Costarelli, Robertina Giacconi, Ligia-Esperanza Diaz, and Ascension Marcos.(2013). The merck veterinary manual (11th edition). Serum Biochemical Reference Ranges. MERCK& CO., INC. KENILWORTH, NJ, USA 2016 Vol. 21, No. 5 : 685 – 691.

Jones, T.C, Hunt, R. D and king, N.W. (1997). Hyperlipidemia disorders in dogs. Compendium on Continuing Education for the Practicing Veterinarian 27, 361–364.

Kamil Seyrek, Serdar Pasa, Funda Kiral. (2004). Levels of Zinc, Copper And Magnesium In Sheep With Toxoplasmosis. Uludag Univ. J. Fac. Vet. Med. 23 ,1-2-3: 39-42

Kargin, K. F., Seyrek. K., Bildik, A. (2004). Determination of the Levels of Zinc, Copper, Calcium, Phosphor and Magnesium of Chios Ewes in the Aydın Region. Turk J Vet Animal Science 34: 75-80.

Klassing,C.K.(1984). Effect of inflamatory agents andInterleukin I on iron and zinc metabolism. Am J. Physiol ; 247: 901-904.

Klassing,C.K. (1988). Nutritional aspects of leukocytic cytokins. J. Nut ; 118: 1435-1443.

The merck veterinary manual (eleventh edition)

(2016). Serum Biochemical Reference Ranges. MERCK & CO., INC. KENILWORTH, NJ, USA (Editor-in-Chief: Susan E. Aiello, DVM, ELS , Executive Editor: Michael A. Moses).

Montaya, J.G. (2002) Laboratory diagnosis of Toxoplasma gondii infection and Toxoplasmosis. J. Infectious Disease ; 185(15): 73-82.

National Research Center (NRC). (1980). Medical and biological effects of environmental pollutants copper. Academic press. 1: 391-403.

Nezu , R, Takagi, Y.,Okada, A. (1992). Intra venous nutrition, enteral nutrition and trace elements. The pediatrics quarterly; 25 (2) : 283-298 (in Japan)

Orr,C.L., Hutcheson,D.P., Grainger,R.B., Cummins,J.M., Mock,R.E. (1990). Copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J Animal Science. 68(9):2893-900.

Prashanth Lingamaneni, Kiran Kumar Kattapagari1, Ravi Teja Chitturi, Venkat Ramana Reddy Baddam1, Lingamaneni Krishna Prasad. (2015). A review on role of essential trace elements in health and disease. Journal of Dr. NTR University of Health Sciences 2015;4(2) 75-85

Puls , R. (1994) .Mineral levels in Animal health :Diagnostic Data . 2nd edition.

Philip, G., Lana, C.S., De Mars, W., Johnson, T. and Henry, C. (2005). The effect of copper deficiency on iron absorption and duodenal enterocytic hephaestin protein in rats. Lukaski Journal Nutrition. 135: 92-98.

Samantha Long and Andrea MP Romani (2014). Role of Cellular Magnesium in Human Diseases. Austin J Nutr Food Sci. 18; 2(10): 1051.

Sherry, A.R. (2004). Copper: The missing link in your diet. Tired or toxic? A blue print for health.1st edition, Prestige Publishing, New York, 13220, U.SA.

Susan, S. P. (1998). Copper and immunity, Am J Clin Nutr . Printed in USA. © American Society for Clinical Nutrition, ;67(suppl):1064S–8S.

Suttle, N. F. (2010). Mineral Nutrition of Livestock, Cabi Publishing.

Svenson, K.L.G., Hallgren, R. Johansene, E., Lindth, U. (1985). Reduced zinc in peripheral blood cells from patients with inflammatory connective tissue disease. Inflammation ; 9: 189-199.

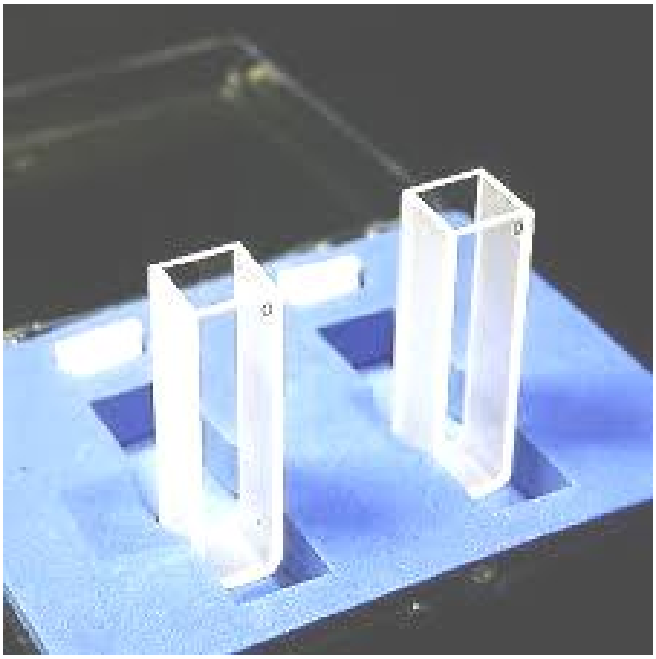
Tasi ,S. Sengil, A.Z., Altindis, M., Arisoy, K.(1995). The effect of zinc supplementation in experimentallyinduced Toxoplasma gondii infection. J. Egypt Soc Parasitol ; 25: 745-755.

Tam, M., Gomez, S., Gonzalex‐Gross, M., Marcos, A. (2003). Possible roles of magnesium on the immune system. Eur J Clin Nutr. 57(10):1193‐7).

Yazar, S,. Kilic, E., Saraymen, R. (2003) Changes of total content of magnesium and zinc status in patients with chronic toxoplasmosis. Biologyof trace element; 92: 11-15.

Appendices

Appendix I Atomic absorption spectrometer



Appendix II Spectrophotometer