Veterinary Research 5 (4): 91-96, 2012
ISSN: 1993-5412

Use of Serum Magnesium as a Biomarker of Oxidative Stress in Marwari Goat from Arid Tracts in India

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Abstract: An investigation was carried out in 1235 male and female goats of Marwari breed belonging to arid tracts in India to find out the role of serum magnesium as a biomarker of oxidative stress. The samples were collected during moderate and hot ambiences from apparently healthy animals and from the animals having gastrointestinal parasites, Peste des Petits Ruminants (PPR), mange and those affected by drought. The mean value recorded during moderate ambience was considered as control. The mean value of serum magnesium during hot ambience of animals having gastrointestinal parasites, PPR, mange and those affected by drought were significantly ($p \leq 0.05$) lower than control mean value. Minimum value was observed in drought affected animals. The control mean value of serum magnesium was 1.24±0.003 m mol L^{-1} and the lowest value in drought affected animals was 0.49±0.002 m mol L^{-1}. Simultaneously, serum catalase was also determined in each case to assess oxidative stress because it is considered as a potent marker of oxidative stress. Significantly higher ($p \leq 0.05$) values of serum catalase in each case revealed the presence of oxidative stress in animals. A concomitant decrease in serum magnesium reflected towards its antioxidant property. It was concluded that oxidative stress resulted in lowering in the serum magnesium levels however, impact varied. Low serum magnesium in gastrointestinal parasitised, PPR, mange and drought affected animals could be the outcome of oxidative stress to the animals and therefore it can be opined that dietary deficiency of magnesium is not the only reason behind the low magnesium levels but oxidative stress mechanism also play pivotal role. Serum magnesium can be used effectively as one of the biomarkers to assess oxidative stress.

Key words: Ambience, drought, gastrointestinal parasites, goat, magnesium, Marwari, serum

INTRODUCTION

Levels of different serum electrolytes indicate the physiological status of the animal. Due to wide spread genetic variation in these electrolyte levels, an interest has recently arisen in determining the possibility of utilizing these variations for improving animal production (Das *et al*., 2010). This knowledge is also important in supplementation of minerals as one electrolyte may affect the concentration of other. Goats are considered as multipurpose animals that produce meat, milk and skin to enhance the economy of marginal farmers in rural areas. They are unique in their ability to adapt and maintain themselves in harsh tropical environments however, optimum levels of minerals are essential to maintain the health conditions. The most tedious work in goat raising is to provide proper mineral compositions as most goat producers are not knowledgeable enough to formulate balanced ration. Due to staggeringly complex interaction of minerals, identification of mineral of significance is essential as no single mineral can be singled out as more important. Magnesium is one of the essential minerals in feeding management of goat. As compared to other minerals, magnesium is poorly absorbed from concentrate and forage feeds in the alimentary tract. Therefore, availability to animal can be best assessed by determining the level in the blood (Khan *et al*., 2008). Further collection of data in various environmental conditions can help in supplementing magnesium rich mineral mixture during deficit periods as mineral composition of forages is also affected by environments (Chicco *et al*., 1972).

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The physiological functions of magnesium are very wide starting from its involvement in fuel metabolism, energy storage and biochemical reactions to maintenance of functioning of genes, nucleic acid stability, cell cycle, channel regulation and as a cofactor for several enzymes. Since, the indispensability of magnesium in biological system is because of its participation at cellular levels, an understanding of the physiology of magnesium has undergone considerable changes in the recent years with the fact that cellular magnesium is tightly controlled and stable under physiological conditions and that it plays a major role in regulating cardiovascular and renal functions (Schiffrin and Touyz, 2005), in addition to contribution to the physical structure as a part of the bones crystal lattice and its scaffolding and help to regulate nerve and muscle tone.

Catalase is considered as an antioxidant enzyme found in nearly all the cells that are exposed to oxygen where it catalyses decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is a harmful by product of many normal metabolic processes. To prevent damage it must be quickly converted into other, less dangerous substances. Higher serum catalase activity in stressed animals indicate the higher rate of formation of hydrogen peroxide therefore it is considered as one of the markers to assess oxidative stress in animals (Kataria *et al*., 2010a, 2012). As the connection of magnesium with oxidative stress has been evidenced (Wolf *et al*., 2008), the awareness to relate hypomagnesaemia with stress and inflammatory processes is gaining interest in scientific community. Since cellular redox state is influenced directly by magnesium status its deficiency is associated with the increased production of reactive oxygen species and the induction of immune and inflammatory reactions (Schiffrin and Touyz, 2005). Poor nutritional practices combined with less than desirable conditions of low-magnesium forage often have lethal results. These situations can be prevented with proper supplementation. High nitrogen and potassium levels from chemical fertilizers or manure can also inhibit magnesium absorption by the plants. A long-term chronic magnesium deficiency is related to development of oxidative stress (Martin *et al*., 2008). In animals, deficiency of magnesium is associated with many clinical dysfunctions like staggering, excitation, lowered urine and milk production and anorexia. In humans, research based on magnesium status of stressed cases has come out with the need for magnesium supplementation along with antioxidant vitamins for those living in conditions of chronic stress (Cernak *et al*., 2000) however, studies to correlate the magnesium levels with the oxidative stress conditions are rare in ruminants.

Owing to great variation in the biochemical parameters between breeds of goats it is wise to establish appropriate physiological baseline values for various native breeds in arid tracts which could help in realistic evaluation of the management practice, nutrition and diagnosis of pathological condition. Marwari is a breed of goat in arid tracts from India playing an important role in the economy of marginal farmers and land less labourers (Kataria *et al*., 2011). Looking towards the paucity of research on this native breed of goat, the present investigation was envisaged with the aim to generate data of healthy animals at a large scale and to compare them with various stress conditions in order to find out the pattern of changes in magnesium concentration and to explore the possibility of its use as a potential biomarker of stress.

MATERIALS AND METHODS

Animals: In the present investigation 1235 goats of Marwari breed of both sexes and varying age groups were screened and investigation was divided into two phases. In first phase, blood samples were collected from jugular vein during slaughtering from private slaughter houses (Rajasthan, India) in moderate and hot ambiences. In each ambience 400 blood samples were collected from male and female animals. The maximum temperature ranged from 25-27°C during moderate ambience and from 45-47°C during hot ambience. The data generated during moderate ambience were used as control.

In second phase blood samples were collected from the goats affected with gastrointestinal parasitism (150), Peste des Petits Ruminants (PPR) (95), mange (81) and drought affected goats (109), each irrespective of sex and age. They belonged to farmers' stock managed in similar conditions of feeding and watering.

Samples and sampling protocol

Blood samples: Blood was collected directly into the clean, dry test tubes without any anticoagulant to harvest sera. Supernatant non-haemolysed sera were pipetted out into sterilised vials for analysis.

Adopted methods

Serum magnesium: It was determined by Titan Yellow Method (Varley, 1988). Titan yellow gives a red colour with magnesium. The colour density is read at 520 mu wave length using a spectrophotometer.

Serum catalase: It was determined by basic colorimetric method as described by Goldblith and Proctor (1950) with little modifications (Kataria *et al*., 2010b). The serum was allowed to react with H_2O_2 for a specific period of time.

The reaction was then stopped by using H_2SO_4 Then, excess of $KMnO_4$ solution was added to allow to react with the peroxide not decomposed by the catalase. Within 1 min, the excess in $KMnO_4$ was determined photometrically.

Statistical analysis: The data were analyzed using a spilt-plot completely randomized design (Steel and Torrie, 1980). Differences among means were ranked using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The mean values of serum magnesium and catalase of goats during hot ambience and those affected with gastrointestinal parasites, PPR, mange and drought (Table 1) have been compared with the control or moderate mean value. The range of serum magnesium value uring moderate condition was from 0.94-1.44 m mol L^{-1}. The mean value of each case was significantly ($p\leq 0.05$) lower than control. Minimum value was observed in drought affected animals. Few of these animals also showed the signs of magnesium deficiency. Serum catalase activity showed the reverse trend. The mean value of each case was significantly ($p\leq 0.05$) higher than control. Maximum value was observed in drought affected animals. The pattern of decrease in serum magnesium was concomitant to the increase in value of serum catalase. Maximum mean value of serum catalase was observed in drought affected animals.

Hot ambience associated variations in serum magnesium (Kataria *et al*., 1993, 2002) could be due to stress (Healy and Falk, 1974). About 70% of the magnesium is relatively tightly bound in the skeleton and can only be released during general bone absorption. An efficient homeostatic mechanisms for maintenance of a steady state blood magnesium concentration lacks in animals such as those which maintain calcium levels. Magnesium levels are therefore also more likely to be low in large numbers of animals in adverse climatic conditions than other electrolytes. When harsh environmental condition couples with short period of starvation, low level of magnesium suddenly exacerbates resulting in hypomagnesaemia. Scientists suggest that extreme ambience related stress may also increase urinary excretion of magnesium (Shiga *et al*., 1979). The earlier reportings although, few have shown comparative higher concentration of serum magnesium in cold stress conditions than in heat stress (Nazifi *et al*., 2003).

Table 1: Serum magnesium and catalase profile of Marwari goat

Effects	Serum magnesium (m mol L^{-1})	Serum catalase (CAT, kU L^{-1})
Moderate ambience (400)	1.24±0.003*	65.00±2.00*
Hot ambience (400)	0.73±0.004*	80.66±2.01*
Gastrointestinal parasiticised (150)	0.58±0.003*	120.66±2.98*
PPR affected (95)	0.53±0.003*	170.00±8.00*
Mange affected (81)	0.66±0.002*	101.21±2.22*
Drought affected (109)	0.49±0.002*	232.43±9.11*

Figures in the parenthesis indicate number of animals and all the means* differed significantly ($p\leq 0.05$) from each other within a column. PPR = Peste des Petits Ruminants

Based upon the findings of earlier reports (McCoy, 2004) serum magnesium level during hot ambience could be considered as hypomagnesaemia as it was <0.8 mmol L^{-1}. Stress intensification of magnesium inadequacy could be due to elevated levels of catecholamines during extreme hot ambience (Seelig, 1994). Extreme ambience associated prolonged higher secretion of cortisol and aldosterone (Kataria *et al*., 2000) could be attributed to low serum magnesium levels. Environmental stress is considered as one of the aetiological factors for hypomagnesaemia. When it combines with poor availability of magnesium and improper absorption, magnesium lowering deepens. This phenomenon potentiates catecholamine release, further exacerbating the magnesium deficit culminating into lower feed intake, decrease rumen cellulose digestion and lower gut motility (McCoy, 2004).

Magnesium deficiency enhances stress sensitivity of organism while stress increases magnesium deficit and favours occurrence of decreased immunity on infections. Magnesium is one of the macroelements that is the best known due to its stabilising influence on nervous system. Scientists are trying to link the magnesium changes and blood antioxidants levels which could be of both diagnostic and prognostic value (Healy and Falk, 1974). Antioxidant enzyme activity has been observed to be increased significantly with magnesium supplementation (Hans *et al*., 2003) and low magnesium is found to be related with free radical generation (Schiffrin and Touyz, 2005). Harsh environmental conditions stimulate free radical generation and magnesium level is probably depleted to combat them. Magnesium is considered as one of the efficient antioxidants (Hasebe, 2005) and its deficiency enhances circulating levels of factors that promote free radical generation (Shivakumar and Prakash, 1997). Low magnesium in serum has been found to be associated with oxidative stress (Wolf *et al*., 2008). These discussions enlighten the role of magnesium as a potent biomarker to assess the stress.

Drought affected animals showed lowest serum magnesium which could be due to poor availability of nutrition. Scientists have observed that various types of stressors in goats result in higher level of cortisol and low magnesium (Ali *et al*., 2006). Improper nutrition under the condition of stress may intensify magnesium deficiency (Garcia-Gomez and Williams, 2000). Sub normal serum magnesium level may not cause immediate deficiency signs but may prove an indicator of serious metabolic disorders. It is important to discuss that low serum magnesium during drought also indicates towards free radical generation and poor absorption of magnesium. Magnesium is transported across the ruminal mucosa by an active sodium-linked process (Fontenot, 1979). Generally, drought periods are accompanied by low rainfall and scorching summer heat resulting in poor availability of nutrients along with imbalances in nutrients. The latter can affect magnesium absorption tremendously. Sodium deficiency can result in depressed magnesium absorption (Martens and Schweigel, 2000). Feeding high levels of potassium generally has decreased concentration of blood serum magnesium due to reduced magnesium absorption (Newton *et al.*, 1972). Imbalance between protein and carbohydrate in the rumen may lead to a deficiency of absorbable energy from the rumen. Lack of carbohydrates result in decreased production of volatile fatty acids and carbon dioxide (Martens and Schweigl, 2000). Decreased volatile fatty acid production may account for reduced blood flow to rumen resulting in low magnesium absorption (Ammerman *et al.*, 1971). Recurring droughts bring stress to livestock. Researchers have attributed lowering of serum magnesium to isolated stress (Healy and Falk, 1974).

Peste des Petits Ruminants (PPR), an office international des epizooties list a disease of sheep and goats caused by morbillivirus is characterised by high morbidity and high mortality rates resulting into heavy economical losses. Inflammation, pneumonia and gastroenteritis are few of the important symptoms. Researchers have recorded significant ($p \leq 0.05$) changes in serum biochemical parameters including electrolytes like sodium and potassium in PPR affected animals (Kataria *et al.*, 2007) but availability of literature on serum magnesium value is lacking. In present study a depletion of serum magnesium was observed which could be due to immense stress observed by the animals during the course of disease. Scientists have reported immunosuppression with PPR virus infection (Emikpe *et al.*, 2010). Magnesium also has a strong relation with the immune system in both non specific and specific immune responses and magnesium deficit has been shown to be related with impaired cellular and humoral immune function. Magnesium deficiency leads to immunopathological changes that are related to the initiation of a sequential inflammatory response (Laires and Monteiro, 2008). Low magnesium and immune system activation seem to cooperate to promote endothelial dysfunctions (Wolf *et al.*, 2008). The variation in susceptibility with this infection (Kataria *et al.*, 2007) arises a need to determine antioxidant status so that supplementation can be carried out to provide relief to affected animals. Several lines of experimental evidence have also suggested that magnesium intake may have beneficial effects on endothelial function (Shechter *et al.*, 2000). Stress together with lack of magnesium causes an increased secretion of catecholamines resulting in increased cellular permeability. These factors result in lowering of immune status of animals (Seelig, 1994).

The forestomachs are the main sites of magnesium absorption in ruminants which is essential for maintaining its normal blood concentration. Hypomagnesaemia in parasitised goats can be due to poor absorption of this element from forestomach. There are several factors affecting magnesium absorption in the rumen. Both potassium and rapidly degradable protein have a negative effect on magnesium absorption. Sodium, Vitamin D and possibly saponins have a positive effect on magnesium absorption (Garcia-Gomez and Williams, 2000). The deficiency of macro elements with parasitic diseases causes clinical disorder, loss of yield and death. Because of inflammation in gut wall in gastro-intestinal parasitosis, absorption of some minerals can be changed (Tanritanir *et al.*, 2009). Although, magnesium is an essential mineral but its homeostasis is not regulated by a hormonal feedback system and simply depends on absorption from the gastrointestinal tract and outflow in the form of endogenous secretion, requirement for milk production and uptake by tissues. Internal or external parasites may induce blood loss in certain occasions. Such situations may cause anaemia and low magnesium and influence the productivity of goats, particularly during gestation and lactation (Abdalla and Abdelatif, 2008). As magnesium is directly linked with metabolism, scientists have observed low magnesium values in metabolic disorders and attributed it to poor absorption in rumen and large intestines (Haenlein, 1992). Parasitism can disturb normal metabolism of the animals. External parasitic infestations cause severe health problems in livestock that may be accompanied by a decrease in some blood biochemical parameters, blood trace elements and mineral levels (Tanritanir *et al.*, 2009).

Halliwell and Gutteridge (1999) described several lines of defense against reactive oxygen species in animals and antioxidant enzyme catalase is one of them which can be used effectively as a biomarker of oxidative stress. Further it can be inferred that higher catalase activities showed activation of defense system as it is considered as an endogenous antioxidant enzyme. Earlier researchers have pointed out that the increased levels of serum catalase are indicative of oxidative stress (Kataria *et al*., 2010c). On this basis it can be reiterated that catalase activity showed the generation of free radicals in stressed animals whereas magnesium acted as an endogenous antioxidant therefore its low levels showed depletion to combat the free radicals.

CONCLUSION

The strengths of the present study include the large size of data and the relatively homogeneous nature of the stress effects. The assessment of multiple stress factors using statistical analysis increased the validity of the results. Lowering of magnesium concentration can be due to insufficient intake, poor magnesium absorption from forestomach, immunological compromise, free radical generation, alterations in metabolism or excess excretion. It can be summarised that dietary deficiency of magnesium is not the only reason behind low serum magnesium levels but oxidative stress mechanism also play pivotal role in lowering magnesium levels through hormones like cortisol and aldosterone. Low serum magnesium in parasitised, PPR, mange and drought affected animals could be the outcome of oxidative stress to the animals. The fact regarding role of magnesium as a biomarker of oxidative stress was well supported by the simultaneous findings of serum catalase. Therefore, serum magnesium can be used as a biomarker of oxidative stress in the goats. On the basis of results, antioxidants supplementation is recommended in the stressed animals.

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