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Effect of selenium and zinc supplementation in serum Total protein concentration of Vrindavani cattle during heat stress

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Abstract
In order to compare the effects of heat stress on serum total protein concentration, the current study examined the effects of selenium and zinc supplementation on total protein concentration during heat stress in Vrindavani cattle. In the study, 12 male animals of age 1 to 1.5 years were randomly allocated into 5 groups (n=6); NHSC (non-heat-stressed control), HS (heat-stressed), HSZ (Heat stressed with zinc @ 100 ppm supplemented), HSS (Heat stressed with selenium @ 1 ppm supplemented) and HSZS (Heat stressed both zinc @100 ppm and selenium @ 1 ppm supplemented).The heat exposure at 38ºC in the psychrometric chamber to heat exposed groups (HS, HSZ, HSS and HSZS) daily for 6 hours (9:00AM-3:00 PM) with duration of 25days washout period prior to subsequent exposure. Blood samples were collected at the end of heat exposure on days 1, 7, 14, 21 and on day 28 (as recovery) for total protein analysis. Two way repeated measure ANOVA followed by Tuckey's multiple range test was performed between groups at different experimental periods. It was concluded that heat stress negatively affects the oxidative status and effect total protein concentration in serum which can be ameliorated through dietary selenium and zinc supplementation.

Keywords: Total protein, heat stress, cattle, Vrindavani

Introduction

Livestock contributes significantly to global food production and food security by providing additional nutrients, proteins, fats, and other necessary micronutrients through milk, meat, and eggs (Mottet *et al.,* 2017) [20]. In the following three decades, the demand for livestock products is anticipated to double globally (Yitbarek, 2019) [33]. This rise in demand can be attributed to a number of factors, including an improved standard of living, a growing population, socio-cultural trends, and a shift away from staple foods towards calorie and flavor dense food items worldwide (Kearney, 2010) [14]. According to Gudev *et al.* (2007) [10], albumin, globulins, and fibrinogen (which are only present in plasma and not serum) are the components of total protein. The main roles of proteins include regulating oncotic pressure, acting as carriers of calcium, lipids and hemoglobin, promoting inflammatory responses, and participating in the complement cascade. Since albumin makes up a larger portion of total protein, changes in albumin levels are the primary cause of changes in total protein levels (Moman *et al.*, 2017) [19]. According to Bertoni *et al.*, (2008) [5], variations in albumin concentration signify impaired liver function, which may be brought on by inflammatory diseases of the body. Numerous variables, such as breed, nutrition, climate, season, and health, must be taken into consideration in order to interpret the serum protein concentration.

Prolonged heat stress to the animals increases the concentration of serum proteins in farm animals and possible variation seen within the species (Belhadj Slimen *et al.*, 2016) [3]. According to Tabiri *et al.*, (2000) [30], short heat stress decreases plasma concentrations of aspartic acid, serine, tyrosine, and cysteine increases protein catabolism (indicated by an increase in plasma uric acid level), and reduces protein synthesis and nitrogen retention. However, prolonged exposure to heat reduces protein synthesis in a variety of muscles, reduces protein breakdown, and raises serum levels of aspartic acid, glutamic acid, and phenylalanine in addition to lowering plasma amino acid levels (particularly sulphur and branched-chain amino acids) (Temim *et al.*, 2000) [31].

Calamari *et al.* (2011) [6] reported a significant increase in the albumin level in heat-stressed cattle. During short-term heat stress, a significant increase in total protein and albumin was

reported by Helal *et al.* (2010) [12] in Baladi and Zarabi goats. At different high-temperature exposure of malpura ewes showed significantly higher total protein and albumin (Sejian *et al.*, 2013) [28]. Some investigations have reported animals under stress had lower total plasma protein levels and have established plasma protein levels and the temperature of the environment are inversely correlated (Sejian *et al*., 2010) [29]. Glucocorticoids are also seen in higher concentrations during period of thermal stress, and one of their main roles is to encourage protein catabolism, which breaks down proteins into amino acids to enable gluconeogenesis (Sejian *et al.*, 2010) [29]. The purpose of thermal-stress in lowering proteins to promote hepatic gluconeogenesis via glucocorticoids, which raises glucose levels and enables the animal to be resistant to heat stress to some extent (Korde *et al*., 2007) [15]. At cellular level, heat stress is one of the factor that can causes the induction of oxidative damage through the excess production of reactive oxygen species (ROS) which results in oxidative stress in animals (Belhadj Slimen *et al.*, 2014) [4].
Selenium is a powerful antioxidant that fights oxidative stress. Selenium in conjunction with Vitamin E, serves primarily as a biological antioxidant in the animal as it contains the enzyme glutathione peroxidase that protects cells from oxidative damage caused by free radicals (Pisoschi and Pop, 2015) [22]. Glutathione peroxidase is a crucial enzyme that catalyses removal of hydrogen peroxide and shields cell membranes from oxidative damage contains selenium as one of its components (Lubos *et al.*, 2011) [17].
Zinc is involved in a variety of biological processes, as a structural, catalytic, and intracellular and intercellular signaling component (Kambe *et al.*, 2015) [13]. Zinc (Zn) is an essential trace element that is a potent enhancer of protein metabolism due to its numerous roles in metabolic processes (Papet *et al.*, 2008) [21].
Zinc acts as a co-factor for important enzymes involved in the proper functioning of the antioxidant defense system. In addition, zinc protects cells against oxidative damage, acts in the stabilization of membranes and inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase) (Marreiro *et al.*, 2017) [18].
Zinc also induces the synthesis of metallothioneins, which are proteins effective in reducing hydroxyl radicals and sequestering reactive oxygen species (ROS) produced in stressful situations (Chiaverini and De Ley, 2010) [7]. The antioxidant capabilities of zinc can help protect muscle cells from the deleterious action of free radicals (Fang *et al.*, 2002) [9]. Zinc is a structural component of the enzyme superoxide dismutase present in the cytoplasm of cells which promotes the conversion of two superoxide radicals to hydrogen peroxide and molecular oxygen, reducing the toxicity of ROS because it converts a highly reactive species to a less harmful one (Marreiro *et al.*, 2017) [18]. Therefore, the present experiment was designed to test effect of the dietary Selenium and zinc supplementation on serum total protein levels during heat stress.

Materials and methods

Total protein in serum was estimated using a total protein kit (Coral Clinical System). Protein, in an alkaline medium, binds with the cupric ions present in the biuret reagent to form a blue-violet colored complex which is measured by 550nm. The intensity of the color formed is directly proportional to the amount of protein present in the sample.

Results

In serum, the Mean±SE of Total protein (g/dl) during study period has been shown in Fig. 1. The lowest total protein levels were observed in NHSC group. There was significant (p<0.05) difference between non heat stressed (NHSC) group and heat stress (38 °C) treatment groups (HS, HSZ, HSS and HSZS). The total protein levels increased significantly (p<0.05) up to day 14th of study period. However no significant (p>0.05) differences between heat stress exposed (38 °C) groups (HS, HSZ, HSS and HSZS) were observed. In all the groups' significant difference was observed on 1st, 14th and 28th day of study period.

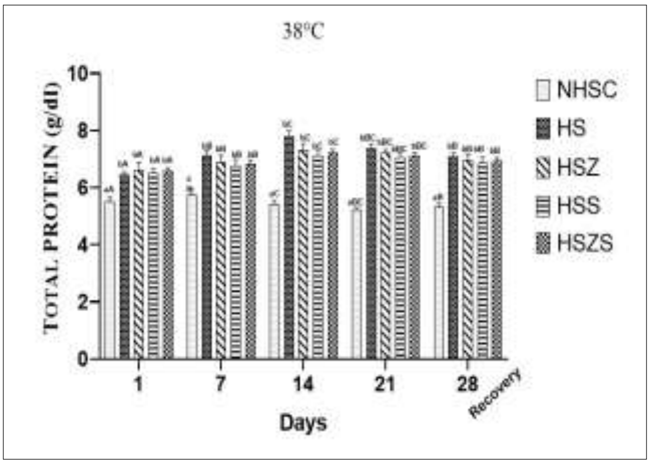

Fig 1: Mean ±SE of Total protein (g/dl) level in Vrindavani cattle during various phases of study period.

Discussion

Several species have reported that heat stress changed protein metabolism, which is evident by decreased lean tissue accretion (Lu *et al.*, 2007) [16]. Since glucose becomes the preferred fuel for heat-stressed animals, the breakdown of skeletal muscle is likely a mechanism to supply precursors for gluconeogenesis (Rhoads *et al.*, 2011) [27]. As a result, our study's findings indicated the total protein level increased during the Heat Stress but was not significant. According to El-Nouty *et al.* (1980) [8], dehydration brought on by heat stress caused a fast rise in ADH levels, which in turn caused a considerable decrease in urine output and increased plasma protein in cattle. According to earlier studies (Beatty *et al.*, 2006) [2], cattle and buffalo with high ambient temperatures had higher total plasma protein levels. Heat-stressed dairy cows had higher plasma total amino acid concentrations. The ability of amino acid catabolism is presumably strengthened in the liver due to the nutritional shortage causing elevated levels of plasma amino acids (Guo *et al.*, 2018) [11]. When compared to winter, Rasooli *et al.* (2004) [25] found that the serum total protein and albumin concentrations in Holstein heifers were significantly higher in the summer. This rise in serum protein levels showed a loss of extracellular fluid from heat exposure. Additionally, they discovered a strong positive correlation between ambient temperature and the serum levels of total protein and albumin.
Gudev *et al.* (2007) [10] found a substantial decrease in plasma total proteins in lactating buffaloes exposed to direct solar radiation in the afternoon compared to morning values. The observed decline suggests that the annuals were in the process of haemodilution. Al-Haidary *et al.*, 2012 [1] discovered that Najdi rams exposed to heat load during the hot summer

season had significantly higher serum total protein concentrations (g/dl) and globulin concentrations (g/dl) than rams exposed during the winter season.

In the present experiment it could be observed that the serum total protein was significantly higher ($p<0.05$) on day 16 ($p<0.05$) and day 21 in HS group with respect to NHSC during the research period. According to reports (Podar and Oroian, 2003; Rasooli *et al.*, 2004; Beatty *et al.*, 2006) [24, 2, 25], ruminants had higher total plasma protein levels when the environment was hot. This is explained by the fact that heat-stressed animals exhibit elevated proteolysis in skeletal muscle (Wheelock *et al.*, 2010) [32], which could result in a decrease in lean tissue incorporation in various species (Rhoads *et al.*, 2013) [26]. This phenomenon is due to the systemic metabolism of amino acids derived from skeletal muscle, which provides substrates for the metabolism of energy (Wheelock *et al.*, 2010) [32]. Additionally, El- Nouty *et al.* (1980) [8] claimed that ruminants' total plasma protein levels significantly increased as a result of the heat and dehydration-induced increase in ADH level.

Conclusion

The results revealed that heat stress significantly effects serum total protein concentration. In summary, our study shows role for selenium in regulating protein metabolism during heat stress through the differential responses of selenoproteins. Zinc's antioxidant properties can help to protect from the damaging effects of free radicals. These investigations provide new insights into the protein metabolism to heat stress and contribute to a better understanding of the adverse effects of heat stress on cattle and role of selenium and zinc supplementation.

Declaration

Conflict of interest: The authors declare no competing interests

Data availability

The data will be made available and would be shared on reasonable request.

Ethics approval

All the animal experiments had prior approval from the animal ethics committee of the Indian Veterinary Research Institute (IVRI), Izatnagar, UP, India.

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