Journal of Animal and Veterinary Advances 8 (11): 2223-2227, 2009
ISSN: 1680-5593

The Effect of Transport Stress on Erythrocyte Glutathione Peroxidase Activity, Plasma Concentrations of Lipid Peroxidation and Some Trace Element Levels in Beef Cattle

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Abstract: This study was performed to investigate, the effect of transport on erythrocyte glutathione peroxidase activity, plasma lipid peroxidations concentrations and some mineral substances levels in feedlot beef cattle. The study material consisted of male beef cattle (n = 30; 1-2 years old), transported from Adana to Sanliurfa. Blood samples were taken before and after transport. Decrease in erythrocyte glutathione peroxidase activity (from 12.70±2.0-9.67±1.1 IU g^{-1} Hb) and increase in plasma lipid peroxidations concentrations (from 1.627±0.14-2.196±0.26 nmol mL^{-1}) were found in cattle exposed to transport stress. These differences were not statistically different. In addition; transport stress caused significant ($p<0.01$) decreases in the levels of zinc (from 2.034±0.15-1.503±0.05 μg mL^{-1}), cupper (from 1.608±0.095-1.330±0.072 μg mL^{-1}) and increase in the levels of iron (from 1.60±0.136-2.05±0.125 μg mL^{-1}). Transport stress did not affect Magnesium levels.

Key words: Cattle, transport, glutathione peroxidase, lipid peroxidation, trace elements, oxidant, antioxidant

INTRODUCTION

Transportation of cattle is a routine management practice. However, mixing groups of unfamiliar animals and loading, unloading and driving the cattle are associated with psychological stress, physical damage and injury.

These stresses have adverse effects on animal welfare and lead to reduced meat quality and economic losses to the farmer (Knowles, 1999; Eicher, 2001).

Studies to determine the amount of stress on farm animals during transport often have highly variable results and are difficult to interpret. The reaction of animals to stressors depends on the duration and intensity of the stressors, previous experience of the animals, their physiological status and the immediate environmental restraints (Fazio and Ferlazzo, 2003).

Trace elements function as cofactors to antioxidant enzymes. Stress can affect antioxidant enzyme activities, thereby indirectly affecting trace element metabolism. Trace element deficiency may therefore, compromise the defense against oxidative damage (Magalova, 1994; Panemanglore and Bebe, 1996).

Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) are well-known scavenger enzymes preventing the cell from oxidative stress. Some trace elements such as Selenium (Se), Zinc (Zn), Manganese (Mn), Copper (Cu), iron (Fe) are involved in the antioxidative system (Saglam *et al*., 2002).

Trace elements are required in small concentrations as essential components of antioxidative enzymes (Panemanglore and Bebe, 1996). While, cytoplasmic Cu-Zn SOD enzyme contains copper and zinc metals as cofactors, GSH-Px enzyme contains selenium and Catalase (CAT) contains iron (Ceballos-Picot *et al*., 1992).

Iron and oxidative stress are closely linked, as iron in combination with oxygen mediates the generation of Reactive Oxygen Species (ROS) like superoxide anions (O_2^-) peroxides (RO_2) and Hydroxyl radicals (OH) through the Haber-Weiss and Fenton reactions (Miller and Britigan, 1999; Touati, 2000).

Copper and zinc are two of the most abundant trace elements found in the human body and are intricately involved in the metabolism of oxygen and the biochemistry of redox reactions. Copper ions are involved in both the generation of and the defense against ROS in

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cells. The generation of superoxide and hydrogen peroxide is due to the interaction of intracellular copper ions with thiols such as GSH and oxygen, which is present intracellulary in high micromolar concentrations (Rotruck *et al*., 1973).

The essential role of zinc has been shown in a wide range of cellular processes including electron transport, cell proliferation, reproduction, immune functions and defense against free radicals (Bray and Bettger, 1990; Powell, 2000) as well as genetic stability and function (Oteiza *et al*., 1995, 1996). Zinc deficiency causes oxidative DNA damage and chromosome breaks have been reported in animals fed a zinc-deficient diet (Golub *et al*., 1985; Hainaut and Milner, 1993).

Zinc may exert this protective antioxidant effect by stabilizing lipid membranes and preventing lipid peroxidation by free radicals. In humans, several studies suggest that zinc has a role in protecting against oxidative damage (Rostan *et al*., 2002).

Mg does facilitate free radical scavenging, it does so at only a minimal level compared with other scavengers such as the transition metal manganese. Thus, the mechanism for Mg's attenuation of free radicals may be through inhibition of free radical production upon reperfusion and not by direct scavenging of radicals already present (Chirase *et al*., 2004).

The antioxidant system and trace elements were investigated in many different studies, but the effects of transport on antioxidant status in cattle have not been investigated.

The aim of study was to measure the alterations in serum Copper (Cu), Zinc (Zn), Magnesium (Mg) and iron (Fe) concentrations and in erythrocyte GSH-Px activities and plasma Malondialdehyde levels (MDA) in beef cattle under stress condition loaded 7 h road transport.

MATERIALS AND METHODS

This study included 30 male beef cattle, 1-2 years old, with body weights ranginig from 200-300 kg. They were transported from Adana, Turkey, to a special feedlot in Sanliurfa, Turkey.

Distance between two cities is approximately, 420 km transfer time took about 7 h. Cattle with body weights ranging from 200-300 kg were carried on a truck on a day of May with the average temperature of 22.2°C. No fodder or water was given to the animals during transportation.

Two blood samples, one before and the other after transportation, were drawn from the jugular veins of the animals. Test tubes containing EDTA and mineral-added jelly substance were used to measure plasma MDA and erythrocyte GSH-Px activities.

Collected samples were immediately transferred to the laboratory for analysis. Plasma was separated by centrifugation of blood samples at 3000 rpm for 10 min and plasma MDA levels were measured within 3 days. After centrifugation and separation, serum samples prepared for analysis of minerals were stored in deep freeze (-80°), until the day of analysis. Erythrocyte GSH-Px activity was measured by Beutler (1975) method and plasma levels of MDA were measured by spectrophotometry using modified Satoh (1978) and Yagi (1984) method. Amount of minerals were measured by atomic absorption spectrophotometry (brand name SOLAAR AA) in the Central Laboratory of Yuzuncu Yil University.

Statistical analysis of the data was performed by Minitab (1998) software using dependent t-test.

RESULTS AND DISCUSSION

We were informed that problems such as slumping, inability to stand up and sometimes death were observed among the animals that were brought to this farm from other provinces. Biochemical results of the study group are shown in the Table 1.

Transportation of the beef cattle has become an important issue in the recent years. Almost, all adult cattle experience transportation in a period of their life. Transportation of the animals may lead to psychologic stress as well as physical damage and injury. Together with the absence of fodder and water, this stress may lead to weight loss and reduce the quality of the meat (Halliwel and Gutteridge, 1990; Grandin, 1997; Knowles, 1999; Kanan *et al*., 2000; Pregel *et al*., 2005). Kenny and Tarrant (1987) reported that for Irish cattle, the actual journey was more stressful than loading and unloading.

Antioxidant mechanisms that protect metabolically active cells against free radical damage include enzymatic systems such as SOD, CAT and GSH-Px. Among these enzymes GSH-Px and CAT take part together in reduction of H_2O_2 and protect the cells against free radical damage (Halliwel and Gutteridge, 1990; Gurgoze *et al*., 2005).

Table 1: Erythrocyte glutathione peroxidase activity, plasma concentrations of lipid peroxidation and same trace element levels before and after transport

	Transport		
Variables	Before	After	p-value
GSH-Px (IU g^{-1} Hb)	12.70±2.0	9.67±1.1	-
MDA (nmol mL^{-1})	1.627±0.14	2.196±0.26	-
Zinc (μg mL^{-1})	2.034±0.15	1.503±0.05	*
Copper (μg mL^{-1})	1.608±0.095	1.330±0.072	*
Iron (μg mL^{-1})	1.60±0.136	2.05±0.125	*
Magnesium (mg dL^{-1})	2.199±0.17	2.052±0.16	-

-: Insignificant, *: $p<0.01$. Data are represented as mean±SEM

Pregel *et al.* (2005) have reported very low level of antioxidants after transportation in their study on calves that experienced transportation. Similarly, Chirase *et al.* (2004) have determined significant decrease of total antioxidant capacity in their study that assessed the effect of transportation on beef cattle.

In the study, erythrocyte GSH-Px activity of the beef cattle that experienced transportation showed a noticeable, but not statistically significant decrease after transportation when compared to before transportation. This may be explained by higher standard deviation or inadequate number of the animals.

After transportation, plasma MDA levels, which is an indicator of lipid peroxidation and oxidative stress, was noticeably higher, but not statistically significant, compared to before transportation.

Chirase *et al.* (2004) have determined significant increase in MDA concentrations among the beef cattle that have experienced transportation. Ishida *et al.* (1999) have also reported significantly increased concentrations of lipid peroxidation in their study related to exercise and transport stress among horses.

Similar to the GSH-Px activity, increase of MDA concentrations that do not reach statistical significance may be explained by higher standard deviation or insufficient number of animals in the study. Above mentioned studies have enrolled a very large population of animals.

Iron, copper, zinc and manganese are trace elements with antioxidant properties. These trace elements have key roles in the antioxidant defense system (Magalova, 1994; Magalova *et al.*, 1997; Lawrence *et al.*, 1998).

Iron and oxidative stress are closely linked, as iron in combination with oxygen mediates the generation of ROS like superoxide anions (O_2^-), peroxides (RO_2) and Hydroxyl radicals (OH) through the Haber-Weiss and Fenton reactions (Miller and Britigan, 1999; Touati, 2000).

Some researchers (Kocyigit *et al.*, 2001) state that cigarette that cause stress in humans does not cause a change in plasma Fe concentration, whereas some report changes in Fe concentration among the calves that experience transportation (Cole *et al.*, 1988) and some other report reduction in Fe concentration after the stress caused by acute infection (Chesters and Will, 1981). Similarly, serum Fe concentrations after transportation were significantly higher compared to the levels before transportation ($p<0.01$).

Copper ions are involved in both the generation of and the defense against Reactive Oxygen Species (ROS) in cells. The generation of superoxide and hydrogen peroxide is due to the interaction of intracellular copper ions with thiols such as GSH and oxygen, which is present intracellulary in high micromolar concentrations (Klotz *et al.*, 2003).

In this study, Cu concentrations showed statistically significant decrease after transportation compared to before transportation ($p<0.01$). This decrease in copper concentrations may be explained by its utilization for the antioxidant enzyme SOD.

Multiple studies in humans suggest that zinc may have a protective effect against free radical generation and oxidative stress. Zinc levels influence many conditions mediated by oxidative damage, including cutaneous and rheumatologic inflammatory diseases, alcoholism and liver cirrhosis and cardiovascular diseases (Magalova, 1994; Rostan *et al.*, 2002).

In the study, serum Zn concentrations showed significant decrease after transportation compared to before transportation ($p<0.01$). This decrease of serum Zn concentration is probably caused by utilization of Zn element by numerous antioxidant enzymes.

Mg body stores are associated with total antioxidant capacity of blood, dependent on albumin and GSH concentrations, but do not influence lipid susceptibility to *in vitro* peroxidation (Lawrence *et al.*, 1998). In the present study, no effect of transportation on serum Mg concentration was determined.

CONCLUSION

Transportation caused significant changes in serum levels of zinc, copper and iron and also caused changes to some extent in erythrocyte glutathione peroxidase and plasma lipid peroxidation activities, which were not statistically significant. We believe that administration of trace elements with antioxidant properties such as (copper, zinc, selenium etc.) to the cattle before transportation will help to reduce the effects of transportation stress.

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