Bulgarian Journal of Agricultural Science, 20 (No 1) 2014, 210-214
Agricultural Academy

TRACE ELEMENTS CONTENT OF *M. BICEPS FEMORIS* AND LIVER IN RABBITS EXPOSED TO DIRECT SOLAR RADIATION

M. DYAVOLOVA
Institute of Animal Science, BG - 2232 Kostinbrod, Bulgaria

Abstract

DYAVOLOVA, M., 2014. Trace elements content of *m. biceps femoris* and liver in rabbits exposed to direct solar radiation. *Bulg. J. Agric. Sci.,* 20: 210-214

This investigation was initiated to study the effect of solar radiation on the content of some trace elements in liver and muscle tissue as potential heat stress indicators. Twelve New Zealand rabbits at the age of 4 months were allocated into 2 groups – control (no direct solar radiation, temperature range 24-27°C) and experimental (heat stressed) rabbits. Experimental rabbits were exposed to direct solar radiation for 4 hours (30 - 35°C) without any access to food and water. Blood samples were taken immediately before the start of heat exposure and 2 hr following the heat stress by ear venepuncture. At the end of the stress period, the animals of both groups were slaughtered and liver and *m. biceps femoris* samples were taken and analyzed for trace elements. Rectal temperature was measured prior to blood sampling. Exposure of rabbits to heat caused significant increase in liver content of Na ($p<0.01$), Cr ($p<0.01$) and Cu ($p<0.01$), but had no effect on muscle concentration of Na, K, Cr, Fe, Zn, Cu and Se. Control rabbits had higher concentration of Na, Fe, Zn, Cu and Se in liver than in muscle ($p<0.001$). Muscle potassium concentration exceeded that in the liver ($p<0.001$) of control rabbits. Muscle sodium to potassium ratio was almost 3 times lower than that in the liver of control rabbits. Heterophil to lymphocyte ratio increased significantly in experimental rabbits ($p<0.01$) thus showing that rabbits under heat experienced significant stress load. The results were interpreted to assume that heat induced liver lipid peroxidation and the possible increase in the portion of the unbound copper are the main metabolic changes which have adverse effect on liver function.

Key words: trace elements, heat stress, rabbits, liver, muscle

Introduction

Trace elements depletion and excess can both lead to metabolic disturbance. Many of the trace elements serve as cofactors to variety of enzymes which are taking part in carbohydrate and protein metabolic paths. Some of them (Se) are cofactors of enzymes with antioxidant effect. Copper participates in the structure of many enzymes, such as dopamine-6 -monooxygenase and monoaminooxygenase, which play role in the metabolism of neuroaminotransmiters norepinephrine, epinephrine and dopamine.

High temperature stress is known to induce redistribution of trace elements in the tissues (Belay et al., 1992). Oxidative stress induced by exposure to heat may be a contributory factor to increased disease susceptibility. Reactive oxygen species can initiate lipid peroxidation and cause cellular damage to tissues. A number of trace minerals are involved in the antioxidant defense system (Spears, 2008). The available literature provides little data about the content of trace elements in tissues and blood. The present knowledge about metabolism of the trace elements and their interaction is highly insufficient.

Having in mind the great significance of trace elements for normal growth and development and their toxic effect during metabolic disturbance, we set ourselves the task to assess the normal values of some microelements in rabbit's liver and muscle and the effect of heat stress on the level of these elements. Our preference of those organs is based on the fact that metallothioneins are concentrated mostly in the kidney and the liver.

Rabbits are very sensitive to heat stress. Therefore, the aim of our study was to establish what the limits of normal variation of some microelements are and how they change after heat stress as well as to assess their potential use as a stress markers.

E-mail: m.dyavolova@gmail.com

Materials and Methods

The experiment comprised 12 New Zealand White male rabbits (*Oryctolagus cuniculus*) at the age of 4 months and average weight of 3 kg, divided in two groups (control and experimental),consisting of 6 rabbits each. The groups were paired on the basis of sex and body weight. Rabbits were reared in an enclosed building under summer conditions with variable natural temperatures within the range of 24° to 27°C. The outside temperature ranged from 30 to 35°C. Rabbits were housed individually in wire-floor cages, provided with feeders and automated drinkers – feed and drinking water were supplied *ad libitum*.

The experimental group was exposed to direct sunlight for 4 hours at ambient temperature 35.5°C without any access to food and water. Blood samples were collected by ear venepuncture at the start of the experiment (basal level) and at 2 h following heat exposure. Rectal temperature was measured by digital thermometer at the start of the experiment and 1 h after heat exposure. Peripheral blood leukocytes were counted on smears. The smears were stained using May-Grunvald and Gisma stains (Lucas and Jambos, 1961). Four hundred leukocytes including neutrophils, eosinophils, basophils, lymphocytes and monocytes were counted microscopically on a slide.

At the end of the stress period the animals of both groups were slaughtered. Liver and muscle (*m.biceps femoris*) samples were taken and analyzed for the following trace elements: Na - sodium, K - potassium, Fe - iron, Zn - zinc, Cu - copper, Se - selenium, Cr – chromium. Two samples of each tissue were taken – for mineral analysis and dry matter determination. Liver and muscle samples were frozen in clean plastic bags until analyzed. Trace elements were processed 24 hours after the slaughter by nitric acid – hydrogen peroxide and microwave digestion. They were measured by Inductively coupled plasma mass spectroscopy method – ICP-MS, using Agilent 7500 cx. Dry matter was analyzed by drying the samples at 100°C for 4 hours.

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Results and Discussion

Liver samples moisture was significantly lower ($p<0.001$) in the rabbits exposed to direct solar radiation as compared to its control values. Muscle content of water decline slightly ($p>0.05$) in experimental rabbits relative to its control value (Figure 1).This data indicates higher level of dehydration in experimental rabbits. The adverse effect of heat and direct solar radiation was further supported by the higher heterophil to lymphocyte ratio (H:L) which is widely used as an indirect indicator of stress in many mammalian species. Heterophil to lymphocyte ratio increased from 0.275 in control to 0.672 in experimental rabbits. Heterophil and lymphocyte percentages in both groups are shown in Figure 2. Our results show that H:L ratio can be used as an indicator of stress in rabbits as well. Liver content of sodium increased significantly in experimental rabbits (Figure 3). The observed increase of Na could be attributed to increased levels of the adrenal hormones cortisol and aldosterone which are expected to be increased in the experimental rabbits as assessed by the increased H:L ratio in experimental rabbits. Cortisol is known to promote tubular reabsorption of sodium in exchange for potassium (Mills et al., 1960). Sodium to potassium ratio in the liver of heat exposed rabbits remained unchanged (0.21 in experimental group versus 0.20 in control group) in spite of the increased liver level of sodium thus indicating strict regulatory effect on Na:K ratio probably exerted by the adrenal

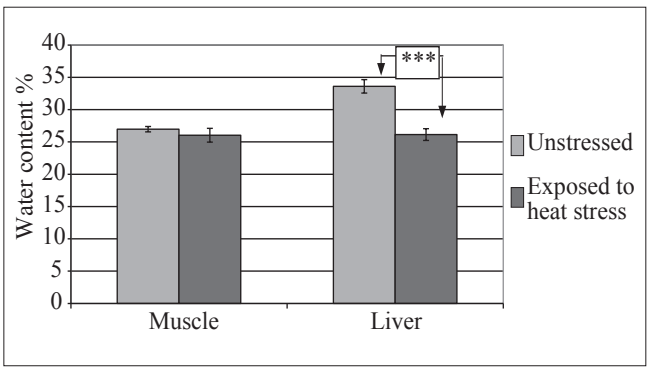

Fig. 1. Liver and muscle water percentage in unstressed and heat exposed rabbits
***p<0.001

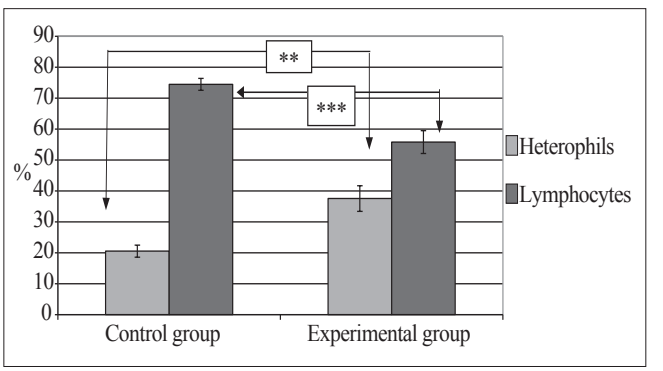

Fig. 2. Heterophil and lymphocyte percentages in peripheral blood of rabbits, before and after exposition to high ambient temperature
**p<0.01 ***p<0.001

mineralcorticoid aldosterone in conformity with the change of Na concentration.

Rabbit exposure to heat elevated liver content of Cr. Chromium is a structural component of a glucose-tolerance factor which potentiates the action of insulin (Mertz, 1994). Stress-induced increase in plasma glucose level leads to depletion of body chromium stores. It seems that chromium stores were not depleted under the conditions of our experiment. Exposure to high levels of chromium has been reported to enhance plasma lipid peroxidation (Kalahasthi et al., 2006; Anand, 2005). However chromium sufficiency within the normal range has been shown to improve cellular antioxidant capacity in isolated hepatocytes (Ueno et al., 1988). We are prone to assume that heat exposure in our case might have caused lipid peroxidation and loss of hepatic glutathione. Our assumption is in agreement with the reported liver lipid peroxidation and glutathione depletion in rat and guinea pig exposed to hot environment (Ando et al., 1994; Skibba et al., 1989). Furthermore, exposure to heat in our experiment was accompanied by food and water deprivation, which have been reported to increase both liver and kidney lipid peroxidation levels in rats (Giralt et al., 1993). Therefore, the increased Cr level in the liver of heat exposed rabbits could be considered as an attempt of the body defense to counteract heat-induced oxidative stress. An alternative explanation concerning the elevated Cr level in the liver of heat exposed rabbits is based on the proposed binding of Cr to transferrin and its movement in the form of Cr-tranferrin to insulin dependent cells in response to increased blood glucose level during stress (Vincent, 2000). It could be speculated that the release of hepatic Cr toward extrahepatic tissues was suspended as an effort aimed at reducing the process of lipid peroxidation.

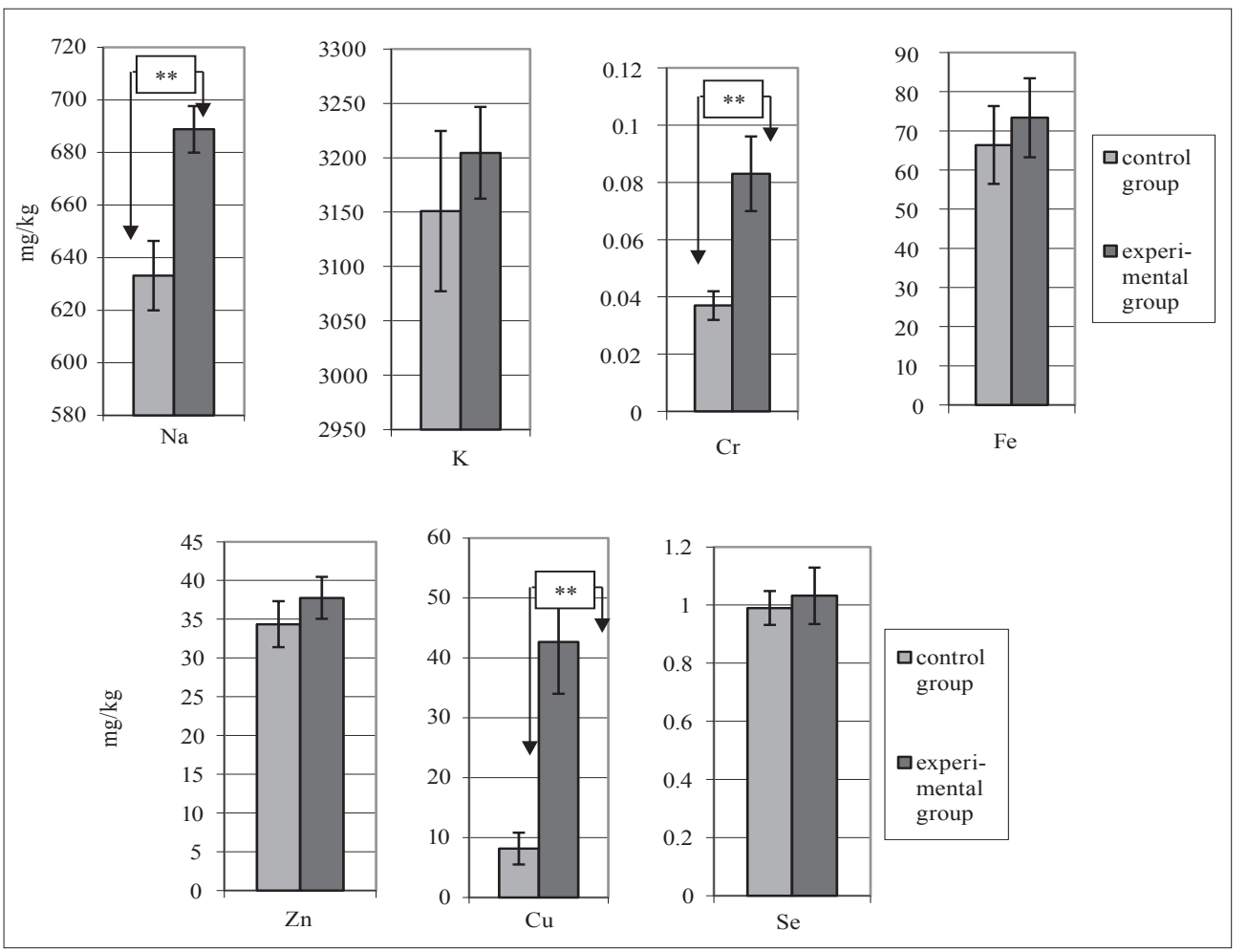

Fig. 3. Concentration of some trace elements (mg/kg) in rabbit's liver in control and experimental groups, **p<0.01

Copper concentration increased sharply in the liver of heat exposed rabbits. Copper retained by hepatocytes is mostly bound by metallothionein and certain cuproproteins. Binding of copper to metallothionein provides a temporary storage for cytoplasmic copper, preventing it from occurring as potential toxic, free ionic metal. The unbound copper may generate hydroxyl radicals which could result in tissue damage (Luza et al., 1996). It has been reported that stress of food and water deprivation with or without physical immobilization elevates liver metallothionein level. Lipid peroxidation promoter, Dimethyl sulfoxide was found to increase liver metallothionein (Hidalgo et al., 1988). Both stress stimuli (food and water deprivation) were present in our experimental design and suggest that the expected increase in lipid peroxidation in heat exposed rabbits, was accompanied by increase of liver metallothionein. Our assumption is further supported by the reported dramatic enhancement of metallothionein in liver, kidney and brain following exposure to X-irradiation (Shiraishi et al., 1986), immobilization stress (Hidalgo et al., 1990) and thermal injury (Ding et al., 2002). This data indicates that wide range of stress stimuli that generate free radicals are potent inducers of metallothionein. Metallothionein enhancement in response to oxidative stress is an important compensatory mechanism of natural defense system. Consequently, it could be assumed that heat exposed rabbits probably had higher liver level of metallothionein in response to copper-induced cytotoxicity. However, it seems that stress-induced increase of liver metallothionein was not able to ensure complete defense against free radicals, as indicated by the results reported in stress related experiments. The reduced effectiveness of metallothionein could be due to the fact that copper incorporation in the metallothionein require prior binding of copper to glutathione,

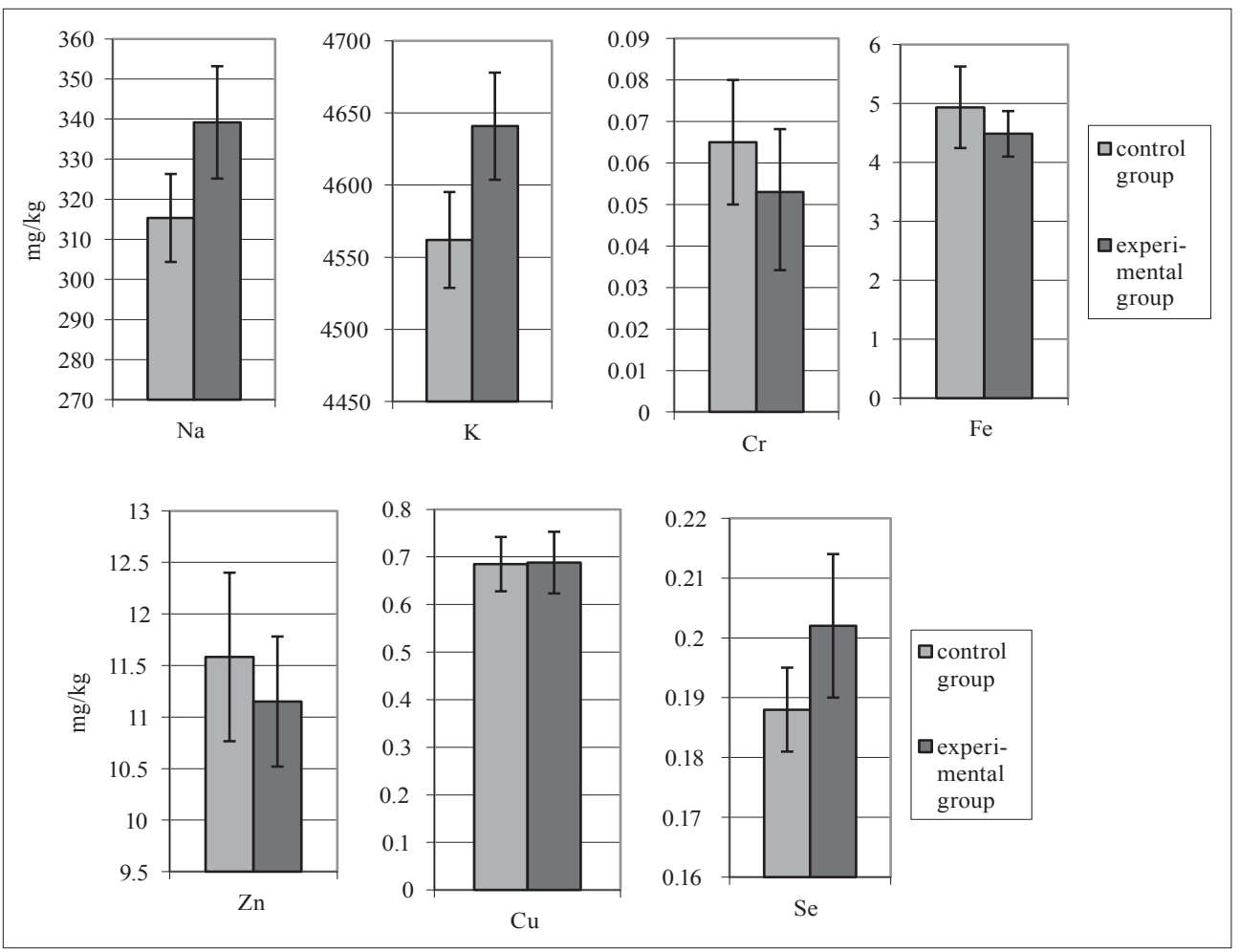

Fig. 4. Concentration of some trace elements (mg/kg) in rabbit's muscle in control and experimental groups

thus defining relation between copper metabolism and the intracellular availability of glutathione (Luza et al., 1996).

Hyperthermia has been found to induce temperature dependent loss of hepatic glutathione (Skibba et al., 1989). Therefore, heat exposure in our experiment might have decreased liver glutathione level, thus decreasing metallothinein ability to bind copper which ultimately led to increase in the portion of the unbound potentially toxic metal. Besides, hyperthermia has been reported to induce lipid peroxidation not only in liver homogenate but also in intracellular structures such as mitochondria and microsomes. The activity of hepatic mitochondrial electron transport system (cytochrome C-oxidase and cytochrome C-reductase) was simultaneously inhibited in hyperthermia (Ando et al., 1994). It is well known that any disturbance in the mitochondrial respiratory chain generates free radicals. This data gives further support to the assumption that hyperthermia induces higher liver content of unbound, free ionic copper.

Exposure to heat had no effect on muscle concentrations of the investigated trace elements (Figure 4). It is worth to note that Na, Fe, Zn, Cu and Se concentration were significantly higher ($p<0.001$) in liver than in muscle tissue of control rabbits. These results are consistent with the fact that metallothionein concentrations are highest in the liver. Muscle concentrations of K and Se, unlike the rest of the investigated trace elements, were higher ($p<0.001$) than the corresponding liver concentrations in control rabbits.

The normal range of the investigated trace elements were as follows: liver: Na – 581-666 mg/kg, K – 2907-3362 mg/kg, Cr – 0.024-0.052 mg/kg, Fe – 34.2-91.8 mg/kg, Zn – 26.9-41.9 mg/kg, Cu – 3.8-15.7 mg/kg, Se – 0.79-1.15mg/kg; muscle: Na – 285-350 mg/kg, K – 4439-4640 mg/kg, Cr – 0.025-0.108 mg/kg, Fe – 3.7-7.9 mg/kg, Zn – 8.6-13.6 mg/kg, Cu – 0.51-0.84 mg/kg, Se – 0.17-0.21 mg/kg. Exposure to heat did not have sizable effect on both muscle and liver Na:K ratio. However, muscle Na:K ratio was almost three times lower than that in the liver of control rabbits.

Conclusion

Exposure of rabbits to direct solar radiation (+35°C) accompanied by food and water deprivation for four hours caused significant ($p<0.001$) increase in liver content of Na, Cr and Cu but had no significant effect on muscle content of Na, K, Cr, Fe, Zn, Cu and Se. Unstressed rabbits had three times lower ratio of Na: K in muscle than in liver.

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Received March, 12, 2013; accepted for printing December, 2, 2013.