Original paper

EFFECT OF ACUTE HEAT STRESS ON SOME HEMATOLOGICAL PARAMETERS, TRACE ELEMENTS AND MEAT QUALITY IN RABBITS

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Abstract

The aim of the present study was to investigate the effect of acute heat stress on some hematological parameters, trace elements and meat quality traits in rabbits. Twelve male rabbits at the age of 4 months were allocated into 2 groups: control and experimental. The experimental rabbits were exposed to intermittent solar radiation for 4 hours at 36 °C. The following parameters were determined: white blood cells (WBC) count, red blood cells (RBC) count, hematocrit, neutrophil and lymphocyte percentages, liver and meat content of chromium (Cr) and selenium (Se), pH of meat at 60 min and 24 h *post mortem*, meat color, myoglobin and water holding capacity (WHC). Exposure to heat increased neutrophil to lymphocyte ratio ($P<0.01$) and liver content of Cr ($P<0.01$), while WBC count, RBC count, hematocrit, liver Se, muscle Cr and Se, pH of meat at 24 h, meat color, myoglobin and WHC were not affected by the applied heat load. The experimental rabbits had significantly lower meat pH at 60 min after slaughter relative to the control rabbits ($P<0.05$).

It was suggested that experimental rabbits had sufficient muscle glycogen at the time of slaughter needed to produce the lactic acid that reduced the ultimate pH of post-mortem muscle within the range observed in the control rabbits.

Key words: *chromium, heat, hematocrit, lymphocytes, neutrophils, selenium*

Introduction

Rabbits are more susceptible to high than to low ambient temperatures. Recent studies on chickens and pigs have presented controversial results concerning metabolic effects of high ambient temperatures (Lin et al., 2006; Hicks et al., 1998). The research papers about the effect of acute thermal stress in rabbits are scarce and incomplete (Amici et al., 2000). Heat is known to cause oxidative stress, which leads to generation of free radicals. Reactive oxygen species can initiate lipid peroxidation and cause cellular damage to tissues (Aitken et al., 1989).

The effect of thermal stress on the quality of rabbit meat has been studied less intensively as compared with transportation stress, feed ratios and other biological and zootechnical factors (Zotte, 2002).

The available literature provides little data about the content of Cr and Se in tissues and blood. Cromium is known to increase liver glycogen and glycogen synthetase (Samanta et al., 2008) and acts as an antagonist to the glycolitic effect of stress hormones. Selenium is an essential component of the body's antioxidant system.

The aim of this study was to investigate the effect of heat stress on some hematological parameters, liver and muscle content of Cr and Se and meat quality in farm rabbits.

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. Rabbits were reared in an enclosed building under summer conditions with variable natural temperatures within the range of 24 to 27 °C. They 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 of 36 °C without any access to food and water. Blood samples were collected by ear venepuncture before exposure to heat (basal level) and 2 hr later, when heat exposure was interrupted for 15 min for the second blood sampling. The following hematological parameters were determined: white blood cell (WBC) count, hematocrit, neutrophil to lymphocite ratio (N:L), and red blood cell (RBC) count. Total erythrocyte and leukocyte counts were determined by manual haemacytometer chamber count. Haematocrit was measured by the microhaematocrit method. Peripheral blood leukocytes were counted on smears. The smears were stained using May-Grunvald and Gisma stains (Lucas and Jamros, 1961). 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 trace elements – Cr (Chromium) and Se (Selenium). Trace elements were measured by Inductively coupled plasma mass spectroscopy method – ICP-MS, using Agilent 7500 cx.

The following physicochemical characteristics of m. longisimus dorsi were determined: pH measured at 60 min and 24 h post mortem by pH-meter, color-measured by Specol 11 at 525 nm, water holding capacity (Grau and Hamm, 1952), and myoglobin (Hornsey et al., 1956).

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

Results and discussion

White blood cells (WBC) count in the experimental rabbits remained unchanged after exposure to heat (Table 1). Exposure to heat has been reported to decrease WBC count in laying hens (Mashaly et al., 2004), cocks (Nathan et al., 1976) and growing female rabbits (Ondruska et al., 2011). Glucocorticoids seem to play a certain role in the maintenance of leukocyte counts (Deutsch et al., 2007). Exposure to acute heat stress, unlike other stressors, results in a quick decline of glucocorticoids level following an initial nonspecific increase in the adrenal response (Gudev et al., 2004). The unaffected WBC count in our study is in agreement with the reported lack of change in WBC of growing and adult male rabbits exposed to 36 °C for 4 weeks (Ondruska et al., 2011).

The experimental rabbits in our study had higher neutrophil to lymphocyte ratio (N: L) after exposure to heat relative to that in the control rabbits (Table 1).

It has long been known that the increase in neutrophil to lymphocyte ratio is due to glucocorticoids-induced release of polymorphonuclear leukocytes from bone marrow, delayed apoptosis, and reduced egress of polymorphonuclear leukocytes into tissue (Nakagawa et al., 1997). Neutrophil to lymphocyte ratio is widely used as an indirect marker of stress-elicited increase in adrenal activity.

Table 1. *Effects of acute heat stress on the hematology of New Zealand rabbit*

HEMATOLOGICAL INDEX	n	CONTROL	EXPERIMENTAL
Erythrocyte no. ($X10^6/mm^3$)	6	5.94 ± 0.046	5.980 ± 0.058
Hematocrit (vol %)	6	39.74 ± 2.913	39.791 ± 3.648
Leukocyte no. ($X10^3mm^3$)	6	6507 ± 113.389	6475 ± 147.478
Neutrophil percentages	6	20.535 ± 4.753	37.541 ± 9.315**
Lymphocyte percentages	6	74.464 ± 4.753	55.791 ± 8.263***

Data are presented as means±standard deviation.
Statistically significant differences are indicated: ** P< 0.01; ***P<0.001 significantly different from the corresponding control value

Heat-induced increase in N:L ratio (from 0.276 in the control to 0.673 in the experimental rabbits) in the present study is not consistent with the reported lack of change in N:L ratio in growing female rabbits exposed to heat (36 °C) for 4 weeks (Ondruska et al., 2011).

This discrepancy could be explained with the different patterns of cortisol dynamics during acute and chronic exposure to heat and its effect on N:L ratio.

Red blood cells count in the experimental rabbits was not influenced by the applied heat load. This data is consistent with the reported slight fluctuation of erythrocyte count in rabbits exposed to heat for 4 weeks (Ondruska et al., 2011). In addition, exposure to heat had no effect on hematocrit value (Table 1). On the contrary, exposure of growing rabbits to 36 °C for 4 weeks was reported to induce a decrease in hematocrit value (Ondruska et al., 2011). The reported decline in hematocrit value was attributed to reduction of cellular oxygen as a requirement for reducing endogenous heat production in order to compensate for the elevated environmental heat. Heat has been reported to decrease corticosterone and ACTH levels in rats (Wang et al., 2009).

Consequently, the observed discrepancy in hematocrit values between our study and that reported by Ondruska et al. (2011) was probably due to the fact that hematocrit value in our study was measured under acute heat exposure, while in their experiment it was registered at the end of 4 weeks long exposure to heat.

Liver content of chromium was significantly higher (P<0.01) in the experimental rabbits relative to that in the control rabbits (Figure 1).

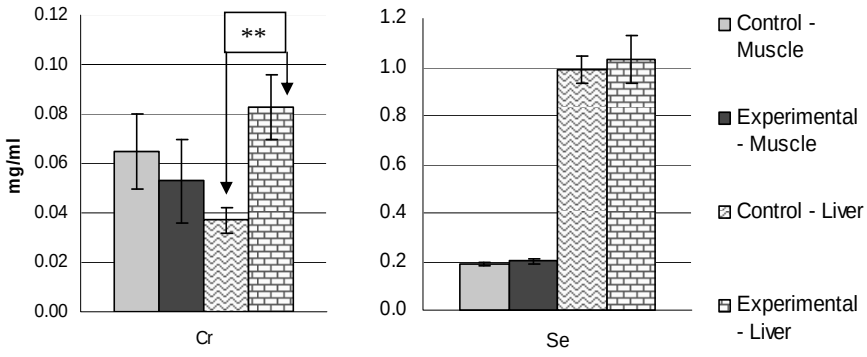

Figure 1. *Effect of heat exposure on selenium and chromium concentration (mg/kg) in rabbit's muscle and liver tissue*
** P<0.01

Chromium is known to potentiate the effect of insulin by facilitating insulin binding to receptors at the cell surface (Pechova and Pavlata, 2007). The demand of chromium is growing as a result of stress factors. High blood glucose concentration stimulates mobilization of tissue Cr and its irreversible loss through urine.

Supplemental chromium increased liver glycogen level and yielded less glucose in the blood under the influence of the catabolic effects of cortisol in broiler chickens kept at 35-36 °C (Samanta et al., 2008). Basal urinary excretion of Cr is related to maximum O_2 consumption (Anderson et al., 1988). Consequently, we expected higher rate of chromium excretion and a decline in liver and muscle Cr stores in the heat stressed rabbits, because of the increased N:L ratio. Contrary to our expectation liver content of Cr was twofold higher in the experimental than in the control rabbits (Figure 1). Muscle content of Cr showed a trend towards lower level in the experimental relative to the control rabbits ($P>0.05$). The lack of significant change in muscle concentration of Cr supports the view that muscle stores of Cr were sufficient to potentiate the effect of insulin thus reverting, at least partly, stress-induced glycogen decomposition during the heat stress episode. Our view is supported by the reported improvement of performance and water holding capacity of meat in pig given supplemental chromium picolinate (Chang Xui, 2009).

Exposure to heat had no effect on both muscle and liver content of selenium (Figure 1). Heat stress is known to increase the level of free radicals (Al-Zahrani et al., 2011). Selenium yeast supplementation improved resistance to oxidative stress in chickens subjected to heat stress (Mahmoud and Edens, 2005).

The unchanged muscle and liver content of Se under the conditions of our study suggests that the experimental rabbits were not Se deficient. It is worth to note that the liver Se was almost 5 times higher than in the muscle.

Muscle pH at 60 min post-mortem was significantly lower in the experimental rabbits in relation to the control rabbits (Table 2). This finding is consistent with the reported acceleration of post-mortem pH decrease in heat-stressed broilers due to the initial high muscle temperature (Wang et al., 2009). The increased post-mortem rate of pH decline in the experimental rabbits in comparison with control rabbits at 60 min was probably due either to pre-mortem increase of glucocorticoid-induced glycogenolysis and build up of lactic acid or to heat-induced alteration in glycolytic activity (Sandercock et al., 2001). On the other hand, it is widely accepted that the ultimate pH of meat (at 24h) is closely related with muscle glycogen concentration at the time of slaughter (Warriss et al., 1989).

Table 2. *Effects of heat exposure on meat quality traits in rabbits*

ITEMS	n	CONTROL	EXPERIMENTAL (EXPOSED TO HEAT)
pH, 60 min	6	7.218 ± 0.080	6.592 ± 0.382 *
pH 24 h	6	5.635 ± 0.079	5.754 ± 0.122
Color, 525 nm/R	6	30.804 ± 1.286	32.362 ± 1.150
Water-holding capacity	6	36.512 ± 1.9	35.588 ± 0.952

Data are presented as means± standard deviation
* $P<0.05$, significantly different from corresponding control level

Muscle pH values at 24 h were similar in both groups although the pH value in the experimental rabbits tended to be higher (Table 2). Our data suggest that experimental rabbits

lactic acid and ensure satisfactory pH at 24 h post-mortem. The control rabbits showed a trend toward better meat quality (P>0.05) as judged by the other meat quality parameters: color, water holding capacity and myoglobin (Table 2).

Conclusion

Exposure of rabbits to intermittent heat stress for 4 h resulted in an increase in N:L ratio and liver content of Cr. It caused higher rate of meat pH decline at 60 min after slaughter. There were no changes in RBC count, WBC count, hematocrit, liver Se, muscle Cr and Se, meat pH at 24h, meat color, myoglobin and water holding capacity.

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