Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits

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ABSTRACT: This study was conducted to investigate the effect of heat stress (i.e., elevated ambient temperature – Ta; 36 °C ± 3 °C) on growth performance, mortality rate, and on some haematological and biochemical parameters in different categories of gender and age of New Zealand White (NZW) rabbits. Animals were divided into two main groups (control and treatment), in each group there were 56 rabbits: adult females (n = 20), adult males (n = 4), growing females (n = 16), and growing males (n = 16). Results revealed that total and daily feed intake, feed conversion ratio, and total and daily gain in body weight for growing NZW rabbits were affected negatively by elevated Ta. Decreases in feed intake led to less protein biosyntheses and less fat deposition, which led to lower body weight gain. These observations were made in growing and adult rabbits of both genders. Analysis showed that red blood cell (RBC) counts showed alterations. Packed cell volume (PCV) (in adult females and males), white blood cell (WBC) counts (in growing females), lymphocytes (in growing males), monocytes (in growing females and adult males), basophils (in growing females and growing and adult males) were significantly (P < 0.05) decreased, and total proteins (TP) (in adult females), glucose (Glu) (in adult females), and calcium (Ca^{2+}) (in growing males and females) were significantly (P < 0.01) lower in the experimental group. Furthermore, elevated Ta increased the mortality rate (MR) in both age groups. The mortality rate was 30.36% for growing and adult rabbits of the experimental group, compared with 7.14% for the control group, and was 25% for adult compared with 34.38% for growing experimental rabbits. Exposure of NZW rabbits of both ages and genders to elevated ambient temperature (36°C ± 3 °C), negatively affected their internal homeostasis which was reflected in their growth rate and various physiological signs.

Keywords: rabbits; heat stress; growth performance; mortality rate; physiology; biochemistry

The breeding of rabbits is expanding; this is mainly attributable to the rabbit's high rate of reproduction, high genetic selection potential, rapid growth rate, early maturity, efficient feed utilization, high quality nutritious meat, and limited competition with humans for similar foods (Habeeb et al., 1996; Ayyat and Marai, 1997; Marai et al., 1999). However, the most obvious limitation to rabbit production in regions with a hot climate is the susceptibility of this species to heat stress. Heat stress (HS) is defined as a stress inflicted by a wide range of environmental conditions that induce a state of physiological strain within an animal's body, and means that animals are not able to regulate their heat homeostasis passively. It mainly occurs when animals are exposed to high ambient temperatures, high humidity, low wind speed, and high direct and indirect solar radiation (Willmer et al., 2000).

Rabbits, as a homoeothermic animal, can regulate the heat input and output of their bodies using physical, morphological, biochemical, and behavioural processes to maintain a constant body temperature (Marai and Habeeb, 1994). The thermo-neutral zone (TNZ) temperature in rabbits is around 18–21 °C (Marai and Habeeb, 1994; Habeeb et al., 1998). Thus, when rabbits are exposed to elevated ambient temperatures (Ta) imbalances are induced in their body temperature (Habeeb et al., 1999; Marai et al., 1996), which adversely affect their growth and reproductive traits (Habeeb et al., 1996; Marai et al., 2001, 2002; Okab and El-Banna, 2003; Okab et al., 2008). Further, disturbances in feed intake, feed utilization, water metabolism, blood parameters, enzymatic reactions, hormonal secretions, in addition to protein, energy and mineral imbalances had been also reported to be disrupted in heat-stressed rabbits (Habeeb et al., 1996; Marai and El-Kelawy, 1999, 2006; Cazabon et al., 2000; Okab and El-Banna, 2003; El-Banna et al., 2005; Burnett et al., 2006; Okab et al., 2008). In this study, the effect of heat stress (i.e., elevated Ta; 36 ± 3 °C) upon growth performance, mortality rate, and upon some select haematological and biochemical parameters was investigated in different categories of New Zealand White (NZW) rabbits of different ages and genders.

MATERIAL AND METHODS

The study was conducted on a total of 112 New Zealand White (NZW) rabbits, over the course of four weeks, at the Department of Small Farm Animals, Animal Production Research Centre Nitra, Slovak Republic.

Animals were divided into two main groups, each group of 56 rabbits contained: adult females (n = 20), adult males (n = 4), growing females (n = 16), and growing males (n = 16). The growing rabbits (1.89 ± 0.23 kg) were two months old, while adult rabbits (4.9 ± 0.7 kg) were two years old. The first group was housed in a partially air conditioned rabbitry designed for production of meat rabbits and maintained at Ta of 18 ± 2.5 °C and served as the control (C) group. The second group was housed in a rabbitry and exposed to cyclic ambient temperature with an elevated Ta of 36 ± 3 °C from 6:00 to 18:00. Then, the temperature was lowered to the temperature in the rabbitry of control group from 18:00 to 6:00. Ambient temperature (Ta) was recorded daily. Ventilator fans were timed to operate for 15 min at 2 h intervals. Rabbits in each group were individually housed in universal galvanized wire cages and feed and water were offered *ad libitum*. Rabbits were fed a commercial pelleted mixture containing 18% crude protein, 14% crude fiber, 2% fat and 10.89 MJ/kg ME.

The growth performance of growing rabbits was recorded as, initial and final live body weight, daily and total body weight gain, feed conversion ratio and daily feed intake. Blood samples were collected on a weekly basis, in the morning, from the marginal auricular vein of each animal, into heparinized tubes. Whole blood samples were analyzed shortly after collection for haematological parameters. Red blood cell (RBC) counts, packed cell volume (PCV), white blood cell (WBC) counts, and differential WBC's were determined using (Abacus Junior Vet, automatic haematological analyzer, Diatron, Austria). Thereafter, plasma samples were obtained by centrifugation of blood at G force 2721.6 g for 20 min, and stored at –20 °C until analysis. Biochemical parameters of blood plasma were measured using the Automatic Biochemical Analyzer, Microlab 300 (Merck, Germany), with kits from same manufacturer. The following parameters were analyzed; total protein (TP), glucose (Glu, as an energy profile), cholesterol (Chole, as a lipid profile) and calcium (Ca^{2+}) in rabbit plasma samples. Moreover, the effect of elevated Ta on mortality rates (MR) of growing and adult rabbits was calculated by dividing the number of dead animals by the initial number of live animals.

Statistical analysis for all measured parameters was performed using the general linear model (GLM) produced by the Statistical Analysis Systems Institute (SAS, 2009). Completely randomized design (CRD) was used to analyze the study data. Significant differences among means of haematological and biochemical parameters (Tables 1 and 2) were evaluated using Scheffe's test ($P \le 0.05$), growth parameters (Table 3) using the t-test ($P \le 0.001$) and mortality rates (Table 4) using the χ^2 test ($P \le 0.001$).

RESULTS AND DISCUSSION

The results presented in Table 3 show that daily feed intake decreased from 158.92 g to 67.28 g (42.4%) when NZW rabbits were exposed to el-

Table 1. Select haematological and biochemical parameters measured in the blood of female NZW rabbits subjected to heat stress (36 °C ± 3 °C) for one month

Parameters	Groups			
	control		experimental	
	growing	adult	growing	adult
Haematology				
RBC's (× 10^6/mm^3)	5.65 ± 0.38[b]	6.71 ± 0.67[a]	5.38 ± 0.46[b]	5.93 ± 0.76[b]
PCV (%)	35.0 ± 1.76[b]	41.0 ± 3.19[a]	34.0 ± 2.16[b]	36.0 ± 3.57[b]
WBC's (× 10^3/mm3)	12.42 ± 3.96[a]	14.78 ± 3.28[a]	7.38 ± 3.11[b]	12.72 ± 4.24[a]
Neutrophils (%)	37.1 ± 8.72	35.5 ± 6.21	35.5 ± 7.31	34.7 ± 7.82
Lymphocytes (%)	50.9 ± 9.45	52.4 ± 7.89	54.8 ± 7.94	55.1 ± 9.11
Monocytes (%)	7.87 ± 1.91[a]	4.79 ± 1.26[b]	5.77 ± 1.32[b]	4.95 ± 2.25[b]
Eosinophils (%)	0.28 ± 0.58	1.62 ± 1.99	0.67 ± 0.65	0.41 ± 1.98
Basophils (%)	3.9 ± 1.13[a]	5.7 ± 2.61[a]	3.0 ± 2.29[b]	5.0 ± 2.10[a]
Biochemistry				
Total protein (g/l)	57.51 ± 6.99[c]	63.32 ± 5.84[a]	57.34 ± 4.88[c]	61.64 ± 5.22[b]
Glucose (mmol/l)	6.65 ± 1.69[c]	6.32 ± 1.01[b]	7.61 ± 1.73[a]	6.08 ± 1.01[d]
Cholesterol (mmol/l)	2.26 ± 1.03[b]	2.32 ± 0.77[b]	3.36 ± 1.27[a]	3.19 ± 0.93[a]
Ca^{2+} (mmol/l)	3.62 ± 0.38[b]	3.64 ± 0.33[ab]	3.41 ± 0.38[c]	3.67 ± 0.34[a]

[a,b,c,d]means within rows with different superscripts are significantly different ($P < 0.05$)

evated Ta. Various authors have reported a depression in feed consumption in rabbits (Marai et al., 1994, 2006; Marai and Habeeb, 1994, 1998; Okab and El-Banna, 2003; Okab et al., 2008). Marai and Habeeb (1998) reported that high environmental temperatures stimulate peripheral thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus causing a decrease in rabbit feed intake. In addition, Marai et al. (1994) found that the decreases in DMI in summer were 46.9% on Day 60, 65% on Day 90, and 44.4% on Day 120 of age compared with winter values.

Furthermore, total and daily weight gains in growing NZW rabbits were also suppressed as a result of elevated Ta (Table 3). Total body weight gain was larger (749.20 ± 14.42 g) in the control group than that in the heat-stressed group (527.90 ± 10.06 g). The reduction in daily gain was due to a drastic decrease in rabbit total feed intake (1412.88 vs. 3337.32 g), and in the feed conversion ratio (2.68 ± 0.05 vs. 4.45 ± 0.09%) compared with the control group, which might have led to less protein biosynthesis and less fat deposition (Ayyat and Marai, 1997; Marai and El-Kelawy, 1999a; Marai et al., 2001, 2004; Okab and El-Banna, 2003; Okab et al., 2008; Ogunjimi et al., 2008).

Elevated Ta affected significantly the measured haematological parameters. Tables 1 and 2 show significant ($P < 0.05$) decreases in RBC and PCV (in adult females and males), WBC (in growing females), lymphocytes (in growing males), monocytes (in growing females and adult males), basophils (in growing females and growing and adult males) of the heat-stressed group. On the other hand, significant ($P < 0.05$) increases were found in numbers of neutrophils (in growing and adult males) in the heat-stressed rabbits compared to the control group. WBC (in growing and adult males), neutrophils and lymphocytes (in growing and adult females), and eosinophils (in all groups) were unaffected by elevated Ta. These results are in agreement with the findings of Seley (1960) who reported that heat stress in mammals decreased the level of ACTH, which might then result in decreases in RBC counts, PCV, and Hb concentration. In addition, the depression of PCV during the hot season was also reported to be related to a reduction in cellular oxygen, a requirement for reducing metabolic heat production in order to compensate for the elevated environmental heat load (Okab and El-Banna, 2003; Okab et al., 2008).

Table 2. Select haematological and biochemical parameters measured in the blood of male NZW rabbits subjected to heat stress (36 °C ± 3 °C) for one month

Parameters	Groups			
	control		experimental	
	growing	adult	growing	adult
Hematology				
RBC's (× 10^6/mm^3)	5.80 ± 0.20[b]	6.50 ± 0.62[a]	5.80 ± 0.25[b]	5.50 ± 0.47[b]
PCV (%)	38.0 ± 2.51[b]	41.0 ± 3.29[a]	36.0 ± 2.13[b]	35.0 ± 1.65[b]
WBC's (× 10^3/mm^3)	11.6 ± 2.73	11.7 ± 1.89	9.70 ± 2.27	12.1 ± 2.59
Neutrophils (%)	24.3 ± 13.93[b]	37.5 ± 11.99[a]	46.0 ± 13.02[a]	26.3 ± 12.36[b]
Lymphocytes (%)	56.7 ± 3.67[a]	61.4 ± 14.05[a]	40.1 ± 9.30[b]	66.6 ± 15.98[a]
Monocytes (%)	6.20 ± 1.49[a]	4.60 ± 1.97[a]	5.60 ± 2.07[a]	3.40 ± 1.92[b]
Eosinophils (%)	0.96 ± 1.95	0.62 ± 0.40	1.94 ± 1.07	0.69 ± 1.85
Basophils (%)	5.3 ± 1.34[a]	4.70 ± 1.46[ab]	3.8 ± 1.46[b]	3.50 ± 0.92[b]
Biochemistry				
Total Protein (g/l)	56.1 ± 5.83[b]	67.5 ± 3.12[a]	57.7 ± 11.70[b]	67.3 ± 7.47[a]
Glucose (mmol/l)	6.53 ± 1.51[b]	5.79 ± 0.89[c]	7.17 ± 1.74[a]	5.81 ± 0.93[c]
Cholesterol (mmol/l)	2.36 ± 0.94[b]	1.47 ± 0.38[c]	3.61 ± 1.90[a]	2.38 ± 1.11[b]
Ca^{2+} (mmol/l)	3.49 ± 0.37[b]	3.64 ± 0.25[a]	3.37 ± 0.34[bc]	3.62 ± 0.21[a]

[a,b,c]means within rows with different superscripts are significantly different ($P < 0.05$)

Observed lymphocyte values were higher in adults compared to growing rabbits of both genders. Such an effect could be mainly attributed to elevated Ta, which could cause an increase in blood viscosity leading to allergic effects and induction of WBC production in adult animals (Lee et al., 1976). The lower numbers of Lymphocytes in young rabbits could be explained as a manifestation of impaired defensive reactions of their body at this age (Ayoub et al., 2007). In general, haematological changes seem to play an important role in adjusting the rabbit's physiology during elevated Ta.

Biochemical analysis of blood plasma indicated that elevated Ta caused some changes in various blood plasma parameters in growing as well as in adult rabbits (Tables 1 and 2). Differences in measured parameters were found to be larger in growing individuals, which might be due to the instability of regulatory mechanisms for growth and metabolism in growing rabbits (Habeeb et al., 1993).

Total protein (TP) levels in the plasma of heat-stressed NZW rabbits was significantly ($P < 0.05$) lower only in adult females. However, a comparison of both groups showed that TP levels were actually lower in the heat-stressed group (Tables 1 and 2). The decline in plasma TP with rising Ta seems to be due to a dilution of plasma TP caused by the increase in water consumed (Okab et al., 2008), and/or it could be due to increases in protein utilization and amino acid transamination in the heat-stressed rabbits (Habeeb et al., 1993). Moreover, increases in serum cortisol concentrations during elevated Ta exposure might inhibit protein synthesis in tissues and may promote protein catabolism (Ayyat and Marai, 1997; Okab and El-Banna, 2003). In addition, plasma TP was decreased significantly during the summer season compared to the winter (Habeeb et al. 1993; Ayyat and Marai, 1997) and spring seasons (Okab and El-Banna, 2003). Significant changes in the concentration of plasma TP either due to species differences or seasonal variations have been thought to be related to changes in albumin or globulin concentrations (El-Masry and Marai, 1991; Okab and El-Banna, 2003; Okab et al., 2008).

The overall mean of plasma glucose (Glu) levels was significantly ($P < 0.01$) influenced by elevated Ta in NZW rabbits. Glucose values were decreased significantly ($P < 0.01$) only in adult females of the heat-stressed rabbits, and significantly ($P < 0.01$) increased in growing males and females of the heat-stressed rabbits compared to their counterparts in the control group (Tables 1 and 2). The increase in plasma glucose

Table 3. Growth performance of growing NZW rabbits subjected to heat stress (36 °C ± 3 °C)

Measurements	Groups	
	control (*n* = 32)	experimental (*n* = 32)
Body weight (g)		
Final	2637 ± 244.4^{a}	2174 ± 158.9^{b}
Total gain	749 ± 14.4^{a}	528 ± 10.1^{b}
Daily gain	36 ± 0.7^{a}	25 ± 0.5^{b}
Feed intake		
Total (g/day)	3337 ± 97^{a}	1413 ± 38.8^{b}
Daily (g)	159 ± 4.6^{a}	67 ± 1.9^{b}
Feed conversion ratio (%)	4.5 ± 0.1^{a}	2.7 ± 0.1^{b}

^{a,b}means within rows with different superscripts are significantly different *t*-test, $t_{0.001} > 3.291$

levels in growing rabbits might be due to a decrease in glucose utilization in order to preserve energy during their stressed condition (Habeeb et al., 1997). The decrease in glucose levels in the heat-stressed adult rabbits could be due to increases in glucose utilization during muscular movements required for high respiratory activity (Habeeb et al., 1992; Okab et al., 2008), or due to increases in corticosteroid concentrations (Habeeb et al., 1997). Nevertheless, other researchers have demonstrated that decreases in energy metabolism (gluconeogenesis and glycogenolysis) during heat exposure correlated with decreases in plasma insulin and thyroxin concentrations (Herbein et al., 1985; Habeeb et al., 1996).

Calcium (Ca^{2+}) data showed a similar tendency to total proteins results, that is, elevated Ta resulted in lower values of plasma Ca^{2+} in the heat-stressed rabbits compared to the control group. significantly ($P < 0.01$) lower Ca^{2+} concentrations were found only in growing males and females subjected to elevated Ta compared to the control group (Tables 1 and 2). These lower values coincided with environmental temperature rises and seemed to be due to a dilution of plasma volume caused by an increase in water consumption, or due to the drastic decrease in total feed intake of the heat-stressed rabbits (Okab et al., 2008).

Plasma cholesterol concentrations increased significantly ($P < 0.01$) in both age and gender categories of NZW rabbit, with exposure to elevated Ta (Tables 1 and 2). This might be due to an increased activity of HMG-CoA reductase – the limiting enzyme in cholesterol synthesis Akira (1992). These results are in concordance with those of Salem et al. (1998) who reported that plasma cholesterol concentrations were significantly higher during the summer compared to the winter, which could reflect total lipid content in the heat-stressed rabbits. However, some studies have reported falls in cholesterol concentrations due to increases in total body water resulting from exposure to elevated Ta (Marai et al., 1995; Habeeb et al., 1996).

Finally, the mortality rate (MR) was 30.36% in growing and adult rabbits exposed to elevated Ta

Table 4. Mortality rate (MR) in growing and adult (male and female) NZW rabbits subjected to heat stress (36 °C ± 3 °C)

Age	Groups					
	control			experimental		
	total number	number of mortalities	mortality rate (%)	total number	number of mortalities	mortality rate (%)
Adult	24	2	8.33^{a}	24	6	25.00^{b}
Growing	32	2	6.25^{a}	32	11	34.38^{b}
Total	56	4	7.14^{a}	56	17	30.36^{b}

^{a,b}means within rows with different superscripts are significantly different $\chi^2_{0.001} > 10.83$

compared to 7.14% in the control group (Table 4). In addition, MR in the heat-stressed growing rabbits was higher (34.38%) than in adult rabbits (25%). Thus, it is evident that elevated Ta increased the MR in rabbits of both ages and genders. Rabbits are very sensitive to heat stress since they have few functional sweat glands which means they have difficulties in eliminating excess body heat when the environmental temperature is high (Marai et al., 2002). The thermo-neutral zone in rabbits is around 18–21 °C (Habeeb et al., 1998). Exposing rabbits to elevated Ta has been reported to cause disturbances in their thermoregulatory system (Marai and Habeeb, 1994; Marai et al., 2002). Such disturbances led to various impairments of their physiological mechanisms. Adult rabbits were better able to respond to hyperthermic conditions and to adapt themselves to conditions of decreased ventilation and evaporation, compared with young rabbits which responded more sensitively to elevate Ta (Ayyat and Marai, 1997; Shehata et al., 1998). Habeeb et al. (1997) reported that MR from birth up to weaning was significantly increased in response to a Ta which increased from 19.5 °C in January to 34.8 °C in July. In addition, the same authors estimated MR in adult rabbits in summer to be 18%, while no mortality was recorded during winter.

CONCLUSIONS

The present study shows that elevated Ta (36 ± 3 °C) has an obvious influence on various physiological parameters of NZW rabbits of different ages and genders. Heat stress disrupts the growth performance of growing rabbits, alters some haematological and biochemical characteristics, and increases the mortality rate of both male and female, growing and adult NZW rabbits.

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