SOME METABOLIC AND IMMUNOLOGICAL PARAMETERS IN RABBITS AS AFFECTED BY PROLONGED THERMAL STRESS

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Abstract - To study the effects of prolonged (24 days) heat stress on some metabolic and immunological parameters two groups of 8 New Zealand White rabbits, of 2.8 Kg of body weight and 11 weeks of age, were kept in individual cages, with feed and water available *ad libitum*, and were submitted to different environmental conditions. Group 1 was located in a climatic chamber at 33.5 ± 0.5 °C (r.h. 65 ± 5 %), group 2 (control) at 18 0 ± 0.5 °C. At days 0,1,6,12, and 24, the following parameters were measured: body weight, rectal temperature, feed intake, Vitamin A and Vitamin E, SH-groups, thiobarbituric acid-reactive substances (TBA-RS), total (peroxil) Radical-trapping Antioxidant Parameter (TRAP), total plasma proteins and immunoglobulins (Ig).
Feed consumption of animals exposed to 33.5 °C was strongly reduced in the first day (13.6 *vs* 161.6 g/day); a gradual increase till the end of the trial was then observed (98.8 *vs* 177.3 g/day). Rectal temperature rapidly increased and remained stable and higher than in the control groups all through the trial ($P<0.01$). The major changes in the measured parameters of the heat stressed animals were a significant increase of the plasmatic level of Vitamin E at days 6, 12, 24 ($P<0.05$), and a significant reduction of the plasmatic concentration of SH-groups and TRAP ($P<0.05$). For the immunological parameters both total proteins and Ig showed a significant decrease in the stressed animals.

INTRODUCTION

The interest for thermal stress in animal production has been increasing in the past years since they influence both animal welfare and the yield and quality of products. Furthermore this problem becomes pressing in those areas having particularly hot climates or seasons where thermal stress represents a considerable limiting factor in animal production.
Some variations were observed in metabolic parameters following stress conditions in rabbits by SIMPLICIO *et al.* (1988) but no indicators have yet been identified apt to define the degree of stress in productive installations. A decrease of Vitamin C plasma concentration in rabbits stressed at 30 ± 2°C for a week was observed by Verde and PIQUER (1986). A reduction of blood bicarbonates seems to take place in rabbits bred in thermal stress conditions as indicated by the results of BONSEMBIANTE *et al.* (1989) who found better growth performance in rabbits following addition of bicarbonates in their diets. Metabolic changes could be related to relevant respiratory panting that is the main response to match the increasing body temperature (FINZI *et al.*, 1986). There is no evidence on the effect of thermal stress on immune function of rabbits. The present study has been performed to investigate the variations of functional, metabolic and immunological parameters induced by prolonged thermal stress in this species.

MATERIAL AND METHODS

Eighteen New Zealand White rabbits (NZW) of 2810±77 g body weight were placed in climatic chambers in single cages provided with feed and water *ad libitum*. The rabbits were divided into two groups of eight animals each. The first group underwent heat stress by exposing the animals to an environmental temperature of 33.5±0.5°C (r.h. 62 ± 5%). This temperature is very close to the maximum limits of rabbits tolerance (MATHERON and MARTIAL, 1981; CASTELLO, 1984) even though easily verified in breeding of the Mediterranean area in the summer season (COSTANTINI and PANELLA, 1983). The control group was kept at an environmental temperature of 18.0±0.5°C (r.h. 62 ± 5%) corresponding to thermoneutral zone of this species (CASTELLO and ROCHA, 1980; CHEEKE *et al.*, 1982), an was fed *ad libitum*. A commercial balanced feed (161 g/kg crude protein, 153 g/kg crude fibre, 11.317 Mj/kg of digestible energy) was utilised for the experiment, and its consumption was daily registered.
Following a week of adaptation in the individual cages at a temperature of 18.0 °C., a group of animals was exposed to the stress temperature. On days 0, 1, 6, 12, 24 at 08.00 h rectal temperature was measured individually as well as body weight. At the same schedule blood samples were collected on EDTA-Na2 (1 µ

g/ml) through the ear vein. The plasma obtained by centrifugation (1500g x 30', at 4°C) was subdivided in aliquots and frozen at -20°C until analysed.
Vitamins A and E were determined by HPLC on lipid extracts of 0.1 ml plasma samples on reverse phase column according to the method described by BIERI *et al.* (1979). For Vitamin E determination a fluorimetric detector was used with excitation and emission wavelength at 292 nm and 340 nm respectively. Vitamin A was measured with spectrophotometric detection at 325 nm.
Thiobarbituric acid-reactive substance was measured as described by YAGI (1982) using malondialdehyde as standard. Standard malondialdehyde was prepared by acid hydrolysis of 1.1.3.3.-tetramethoxy-propane, and 0.1 g/l butylated hydroxytoluene (BHT) was added to TBA reagent. plasma TBA-RS values were expressed as malondialdehyde concentration.
Plasma SH-groups were measured on 0.1 ml samples by the spectrophotometric method described by ELLMAN (1959) after reaction with 5,5'-dithiobis (2-nitrobenzoic acid; DTNB).
Total (peroxil) Radical-trapping Antioxidant Parameter (TRAP) was measured on plasma by subjecting it to controlled peroxidation using the thermal decomposition of 2,2'-azobis(2-amidinopropane hydrochloride; ABAP), as described by WAYNER *et al.* (1985). The oxygen consumption was measured by a Clark oxygen electrode (YSI) with a Gilson 5/6 oxigraph on 0.1 ml plasma in 1.8 ml 5 mmol Na-phosphate buffer pH 8.0 containing 9 g/l NaCl and 10 mmol ABAP at 41° C.
Total proteins and total Ig serum levels were determined by nephelometry (Beckman Analytical, Milan, Italy) according to the recommendations of the manufacturer.
The data were submitted to analysis of variance and comparisons between the means were evaluated with the Students' t-test (SAS, 1993). Correlations were performed utilising the Pearson test (SAS, 1993), and the significance was declared in the text as $P < 0.05$.

RESULTS AND DISCUSSION

Rabbits exposed to 33.5°C showed a significant increase of rectal temperature ($P<0.01$) in comparison with the group kept at 18.0°C. The increase was constant throughout the entire period of exposure (Table 1). In the first test day, the food intake of stressed rabbits was reduced to less than a tenth in comparison to control group (13.6 ± 8.9 *v.* 167.1 ± 25.1 g/day; $P<0.01$). This value was about a third on the 6th day (54.2 ± 17.7 *vs* 139.4 ±31.7 g/day; $P<0.01$) and from the 12th day up to the end of experiment the food intake of stressed animals increased up to about the half of the control group, constantly maintaining significant differences (Table 1).
In Table 1, it may be also observed that the control group presented a normal growth rate passing from 2805 g to 3090 g, whereas the stressed animals presented a weight reduction with a tendency to stabilise during the experimental period.

Table 1 : Rectal temperature, feed intake and live body weight of rabbits exposed to 33.5 or 18.0°C environmental temperature (mean SE± s.e.)

	Feed intake (g/d)			Body weight (g)			Rectal temperature (°C)		
Temperature	33.5°C	18.0°C	s.e.	33.5°C	18.0°C	s.e.	33.5°C	18.0°C	s.e.
Day									
0	161.6	166.9	2.4	2815	2805	67.7	39.0	38.8	0.10
1	13.6 ^{B}	167.1 ^{A}	6.2	2680 ^{b}	2805 ^{a}	68.1	40.5 ^{A}	39.1 ^{B}	0.14
6	54.2 ^{B}	139.4 ^{A}	9.4	2672 ^{b}	2927 ^{a}	81.7	40.5 ^{A}	39.1 ^{B}	0.21
12	79.5 ^{B}	162.2 ^{A}	7.3	2612 ^{b}	3050 ^{a}	91.4	40.7 ^{A}	38.9 ^{B}	0.19
24	98.8 ^{B}	177.3 ^{A}	8.6	2726 ^{b}	3090 ^{a}	82.5	40.7 ^{A}	39.0 ^{B}	0.21

^{ABab} Different superscript letters on the same row indicate significant differences: capital $P<0.01$; small $P<0.05$.

In Table 2 concentration of vitamin A, vitamin E and SH-groups of the experimental groups are listed. Vitamin A tended to decrease in the stressed group at days 1, 6 and 12 of the experimental period. This difference was not significant at day 24. Vitamin E plasma levels were higher in the stressed group in comparison to control group at day 6, 12 and 24. No differences were registered at day 1. A significant decrease in the SH-groups values was observed in the stressed animals from the 6th through the 24th day in comparison to control group.

Table 2 : Plasma concentration of Vitamin A, Vitamin E and SH-groups in rabbits exposed to 33.5 or 18.0°C environmental temperature (mean ± s.e.)

	Vitamin A (ng/ml)			Vitamin E (µg/ml)			SH-groups (nmol/ml)		
Temperature	33·5 °C	18·0 °C	s.e.	33·5 °C	18·0 °C	s.e.	33·5 °C	18·0° C	s.e.
Day									
0	599.4	624.1	18.9	2.03	1.98	0.12	301.4	324.5	9.8
1	502.4 [b]	639.5 a	31.1	1.52	1.31	0.11	308.6	316.6	22.5
6	534.9 [b]	645.5 a	45.8	3.53 a	2.26 b	0.64	220.8 [b]	349.5 a	7.2
12	545.9 [b]	619.3 a	44.3	3.43 a	2.27 b	0.33	198.1 [b]	317.0 a	14.2
24	553.4 [b]	595.4 b	47.3	2.20 a	1.78 b	0.17	200.9 [b]	313.8 a	9.9

[ab] Different superscript letters on the same row indicate significant differences P<0.05.

Plasma concentration of TBA-RS did not constantly show significant variations among the groups and within the experimental period (Table 3). In the case of TRAP a decrease of the antioxidant potential of plasma was observed beginning from the 6th up to 24th day in the stressed group in comparison to control group at 18.0 °C (Table 3).

Table 3 : Plasma concentration of TBA-RS (nmol malondialdehyde / ml) and plasma total antioxidant capability in rabbits exposed to 33.5 or 18.0°C environmental temperature (mean ± s.e.).

	TRAP (µM)			TBA-RS (nmol/ml)		
Temperature	33.5 °C	18.0 °C	s.e.	33.5 °C	18.0 °C	s.e.
Day						
0	559.3	539.8	14.6	0.57	0.61	0.03
1	574.0	553.8	23.1	0.60	0.59	0.11
6	381.0 [b]	505.2 [a]	17.8	0.69 [a]	0.58 [b]	0.04
12	370.0 [b]	549.3 [a]	18.1	0.62	0.65	0.02
24	361.6 [b]	551.4 [a]	18.7	0.56 b	0.67 [a]	0.04

[ab] Different superscript letters on the same row indicate significant differences P<0.05.

Total proteins serum concentration showed a decrease in heat stressed rabbits on days 6 and 12. No differences were registered on day 24 (Table 4). A similar trend was also observed for Ig serum concentration nevertheless stressed group on day 24 had a mean value higher than control group.

Table 4 : Serum level of total proteins and immunoglobulins in rabbits exposed to 33.5 or 18.0°C environmental temperature (mean ± s.e.).

	Total protein (g/100 ml)			Immunoglobulins (g/100 ml)		
Temperature	33.5 °C	18.0 °C	s.e.	33.5 °C	18.0 °C	s.e.
Day						
0	6.06	6.19	0.28	0.51	0.46	0.06
6	4.51 [b]	5.88 [a]	0.20	0.29 [b]	0.48 [a]	0.08
12	4.99 [b]	5.92 [a]	0.19	0.24 [b]	0.38 [a]	0.06
24	5.53 [b]	6.25 [a]	0.25	0.68 [a]	0.42 [b]	0.05

[ab] Different superscript letters on the same row indicate significant differences P<0.05.

The level of metabolites and of substances connected with the oxidative/anti-oxidative equilibrium in plasma of animals, which have undergone stress, can indicate changes in the regulation at molecular level and in the ability of adaptation following periods of stress.

Impaired growth rate in heat stressed animals observed in this trial seems strictly in relationship to reduced food intake; this reduction was observed at the beginning of the stress and it remained constant through all the experimental period even though at a lower level. However the dramatic food intake reduction of the early days could have caused a severe non-thermal stress that is not easily distinguishable from heat stress.

Regarding the antioxidant defence system, a significant decrease of the TRAP and Vitamin A was observed with a simultaneous increase in the concentration of Vitamin E in the stressed group in comparison to control group.

Since the greatest contribution to the plasma TRAP was due to uric acid and to SH-groups, whereas vitamin A and E contributed in a minor way, it can be supposed that the decrease of SH-groups must be the main responsible factor in reduced defence capability (WAYNER *et al.*, 1987).

A positive correlation was observed between rectal temperature and plasma concentration of Vitamin E, which could indicate a mobilisation of this vitamin away from structure of organs and tissues in which it is normally

found with a consequent decrease in the concentration of Vitamin E in them. This hypothesis could be verified experimentally, even though it is difficult to understand the cause of such mobilisation.
The trend of TBA-RS values is not very clear, probably because of the scarce accuracy of the method which cannot be considered a sure index of the state of oxidation.
The decrease of total protein and Ig levels have to be considered as an important biological event indicating a defect of the immune function in rabbits undergoing heat stress. The recovery observed at day 24 could represent an indicator of adaptation to stress that suggest further studies on this argument.

CONCLUSIONS

On the basis of the significant changes obtained in the parameters of the anti oxidative and immune systems, possible indicators of thermal stress in rabbit breeding can be identified in these systems. Nevertheless the scarce knowledge on the variations induced by a dramatic feed intake reduction suggest to continue the study on the effect of reduced feed intake as stress factor.

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Riassunto - Se ha estudiado el efecto del estrés termico crónico sobre algunos parámetros metabólicos e imunologicos del conejo. Un grupo de 8 conejos NZB de kg 2.8 de peso vivo ha sido expuesto a una temperatura ambiental de 33.5 ± 0.5 °C por 24 dias, mientras otro grupo se ha quedado como control a 18 ± 0.5 °C. Se ha medido el peso vivo, la tempeatura rectal, la ingestión de pienso, vitaminas A y E, grupos-SH, substancias reactivas al ácido thiobarbiturico, la capacidad antioxidante del plasma (TRAP), proteínas e imunoglobulinas del plasma.

Las modificaciones más evidentes inducidas por el estrés termico fueron un incremento significativo del nivel plasmático de la vitamina E y una reducción de la concentración de los grupos-SH y del TRAP. Los parámetros imunológicos también sufrieron una significativa diminución.