WORLD RABBIT SCIENCE 2000, VOL. 8 (1), 11-16.

SHORT TERM ACUTE HEAT STRESS IN RABBITS: FUNCTIONAL, METABOLIC AND IMMUNOLOGICAL EFFECTS

AMICI A.[1], FRANCI O.[2], MASTROIACONO P.[3], MERENDINO N.[2], NARDINI M.[4], TOMASSI G.[2]

[1]Istituto di Zootecnia, Università della Tuscia, Via De Lellis, 01100 VITERBO, Italy
[2]Laboratorio di Immunologia e Nutrizione, Università della Tuscia, VITERBO, Italy
[3]Istituto di Fisiologia Generale, Università "La Sapienza", ROMA, Italy
[4]Istituto Nazionale della Nutrizione, ROMA, Italy

ABSTRACT: Sixteen male New Zealand White rabbits (NZW) accustomed to an environmental temperature of 18.0 ± 0.5 °C , and relative humidity of 45 ± 4% were placed in a climatic chamber for 1 hour at 42 °C with r.h. maintained at 45 ± 4%. Feed consumption, rectal temperature and body weight were registered individually for five days before and five days after the heat stress. Blood samples were collected before the exposition to the stress temperature, and at 0.5 h, 6 h, 30 h and 54 h after the end of exposure to stress. Plasma and peripheral blood mononuclear cells (PBMC) were prepared. Glucose, urea, total cholesterol and triglycerides concentration, GOT and GPT activities were measured on plasma samples together with vitamin C, vitamin E, SH-groups and TRAP levels. PBMC proliferative response and immunoglobulin synthesis were measured through [^{3}H]-thymidine incorporation and competitive Elisa assay, respectively. The results showed that exposure to high temperature induced a feed intake reduction lasting 5 days. Concerning plasma metabolic parameters, glucose decreased throughout the entire observation period, cholesterol decreased at 36 hours, triglycerides and urea increased at 0·5 hours after stress. Transaminases activity, Vitamin C, sulphydril groups and total radical-trapping antioxidant capability increased in the first hours following stress. Immune cell proliferation and immunoglobulins synthesis were reduced at 0·5 hours after submission to stress.

RÉSUMÉ : Conséquences fonctionnelles, métaboliques et immunologiques d'un stress termique bref chez le lapin.
Seize lapins mâles Néo-Zélandais Blancs accoutumés à une température environnementale du 18,0 ± 0,5°C, et à une humidité relative du 45 ± 4%, ont été placés dans une chambre climatique pendant 1 heure à 42 °C. La consommation d'aliment, la température rectal et le poids corporel ont été enregistrés individuellement pendant cinq jours avant et cinq jours après l'exposition à la chaleur. Des échantillons de sang ont été collectés avant l'exposition à l'environnement chaud puis à 0,5 h, 6 h, 30 h et 54 h après. Le plasma et les globules blancs mononuclés du sang périphérique (PBMC) ont été préparés. Le glucose, l'urée, la concentration en cholestérol totale et en triglycérides, les GOT et GPT ont été mesurés sur des échantillons de plasma ainsi que la vitamine C, la vitamine E, les groupements SH et les niveaux de TRAP. La réponse proliferative des PBMC et la synthèse d'immunoglobuline ont été mesurées respectivement à travers de l'incorporation de thymidine triée et du test Elisa. Les résultats ont montré que l'exposition à la haute température induit une réduction de prise d'aliment durant 5 jours. Pour ce qui concerne des paramètres métaboliques de plasma, le glucose a diminué dans tout la période, le cholestérol diminués à 36 heures, les triglycérides et l'urée accrue à 0,5 heure après l'exposition à la haute température. L'activité de transaminases, la vitamine C, les groupes sulphydril et la capacité antioxydante total ont augmenté dans les premières heures suivant l'exposition à la chaleur. La prolifération de globules blancs et la synthèse d'immunoglobulines ont été réduites à 0,5 heures après la soumission à la chaleur.

INTRODUCTION

Negative effects of prolonged thermal stress on functional parameters (PRUD'HON, 1976 ; EBERHART, 1980 ; CHIERICATO *et al.*, 1992 ; PACI *et al.*, 1993 ; FINZI *et al.*, 1994), on immune system response (FRANCI *et al.*, 1996) and on oxidative-antioxidative status (AMICI *et al.*, 1995) in rabbits have been investigated in recent years. These studies roughly resemble field stress conditions and consider the long term reaction of the organism to chronic heat stress conditions, including habituation and adaptation (ZULKIFLI and SIEGLE, 1995). However, there is no evidence that acute thermal stress induces functional, metabolic and cellular changes similar to those produced by chronic heat stress, and there is scarce knowledge on the mechanisms by which the alteration at cellular level influences, in particular, the response of the immune system, and by which mechanism the animal organism counteracts the changes of functional and metabolic parameters in the time following stress.

For example, as far as the immune response is concerned, one experiment involving bovine lymphocytes (stressed *in vitro)* showed a reduction in proliferative response (KAMWANJA *et al.*, 1994). However *in vivo* experiments in humans undergoing acute hyperthermia did not showed significant differences in lymphocyte proliferative response (KAPPEL *et al.*, 1991).

Concerning metabolic parameters, hyperthermia in rabbits has been demonstrated to increase the levels of glucose, urea and lactate, and also GOT, GPT and CPK activities (ABDELATIF and MODAWI, 1994). It is also unclear if the thermal stress can be associated with the oxidative stress as suggested by LOVEN (1988), since glutathione and taurine do not protect the development of bovine embryos against heat shock effect (EALY *et al.*, 1995), and a similar effect has been obtained with

Table 1 : Rectal temperature (°C) and daily feed intake (g) of sixteen rabbits before, during, and after exposition (one hour) at 42.0°C environmental temperature (mean ± s.d.).

Sampling time	Rectal temperature (°C)		Feed intake (g)	
	Mean	s.d.	Mean	s.d.
Before 5 d	38.9	0.5	200	18
4 d	39.0	0.3	195	24
3 d	38.8	0.3	211	28
2 d	39.1	0.4	185	32
1 d	39.0	0.3	203	47
Stress 20'	40.6***	0.4	--	--
50'	41.6***	0.6	--	--
After 0·5 h	40.6***	0·7	--	--
2 h	39.0	0.2	--	--
4 h	39.1	0.5	--	--
6 h	39.1	0.3	--	--
1 d	39.1	0.6	160	58
2 d	39.2	0.3	177	70
3 d	38.8	0.3	175	50
4 d	39.1	0.3	167	51
5 d	39.0	0.2	185	48

Significance of the difference in comparison to time 1d before heat exposure: *** P<0.001.

glutathione or thioredoxin in cultures of bovine lymphocytes (KAMWANJA *et al.*, 1994).

All these results do not clarify the comprehensive effect of acute thermal stress in animal organisms, the possible relationship between various responses observed in different biological parameters, and their duration following stress. In order to investigate these aspects, rabbits were submitted to short term acute thermal stress to determine the direct effect on the cells of the immune system and to evaluate the functional, metabolic and antioxidative changes at various times following stress.

MATERIAL AND METHODS

Sixteen male New Zealand White rabbits (**NZW**) of 2916±162 g body weight were placed in single cages provided with feed and water *ad libitum* at an environmental temperature of 18.0 ± 0.5 °C, and relative humidity of 45 ± 4%. These environmental conditions correspond to the thermo-neutral zone of this species (CASTELLO and ROCHA, 1980). After 10 days of adaptation, the animals were placed in a climatic chamber located in the same ambient, for one hour at 42°C with r.h. maintained at 45 ± 4%. This temperature is very close to the maximum limit of rabbits tolerance (MATHERON and MARTIAL, 1981 ; CASTELLO, 1984) but the survival of the animals was 100 % under these conditions.

A commercial balanced feed (175 g/kg crude protein, 151 g/kg crude fibre, 11.3 Mj/kg DM of digestible energy) was utilised for the experiment. Daily feed consumption was individually registered for five days before and five days after the stress since the exposure to heat stress is reported to reduce food intake (PRUD'HON, 1976 ; PACI *et al.*, 1993 ; FINZI *et al.*, 1994). At the same schedule, rectal temperature was also individually measured.

Blood samples were collected (on EDTA-Na) through the ear vein, before the exposition to the stress temperature, and at 0.5 h, 6 h, 30 h and 54 h after submission to stress. The plasma obtained by centrifugation (400g x 30', at 4°C) was subdivided into aliquots and frozen at -80°C until analysed.

Peripheral blood mononuclear cells (**PBMC**) were isolated from blood samples collected before and 0.5 h after the end of thermal stress, to measure cell proliferation and immunoglobulins (Ig) synthesis *in vitro*.

The following parameters were determined from plasma samples: glucose, urea, total cholesterol, triglycerides, glutamate oxalacetate transaminase (**GOT**: EC 2.6.1.1) and glutamate pyruvate transaminase (**GPT**: 2.6.1.2) activities, and some components with antioxidant activity (vitamin C, vitamin E and SH-groups). The total (peroxyl) radical-trapping antioxidant capability of plasma (**TRAP**) as expression of the state of antioxidative defence was also measured.

Glucose, proteins, total cholesterol, triglycerides, GOT and GPT activities were determined with

Table 2 : Plasma concentration of glucose, cholesterol, triglycerides and urea in sixteen rabbits exposed (one hour) at 42.0°C environmental temperature (mean ± s.d.).

Sampling time	Glucose (mg/dl)		Cholesterol (mg/dl)		Triglycerides (mg/dl)		Urea (mg/dl)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Control	126	3.5	55	32.5	67	41	30	4.0
0·5 h	106**	3.3	44	27.4	109*	43	48**	5.1
6 h	116**	2.6	42	7.5	74	27	29	3.6
30 h	113**	9.6	36*	13.0	85	34	22**	6.6
54 h	115**	13.0	32**	9.5	81	14	22**	8.2

Significance of the difference in comparison to control time: * P<0.05 ; ** P<0.01.

Table 3 : Plasma concentration of GOT and GPT in sixteen rabbits exposed (one hour) to 42°C environmental temperature (mean ± s.d.).

	GOT, U/l		GPT, U/l	
Sampling time	Mean	s.d.	Mean	s.d.
Control	20	3.4	20	1.7
0·5 h	31**	4.5	27**	2.3
6 h	28**	9.5	20	3.0
30 h	22	3.9	18*	1.1
54 h	20	8.8	17**	1.1

Significance of the difference in comparison to control time: * P<0.05 ; ** P<0.01.

enzymatic colorimetric methods utilising diagnostic kits (Boheringer Biochemical) normally used in clinical tests. Vitamins E was 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). A fluorimetric detector was used with excitation and emission wavelength at 292 nm and 340 nm, respectively. Vitamin C was measured by HPLC on 0·1 ml plasma samples according to the method of FARBER *et al.*, (1983). 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).

TRAP was measured from 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.

PBMC was obtained by centrifugation over Histopaque 1083 (Sigma Chemical) as described by LOW and HANSEN (1988) and resuspended at a concentration of 2 x 10^6 cells/mL in complete culture medium (RPMI-1640, GIBCO BRL, Life Technologies B.V., Breda, The Netherlands) enriched with 10% heat-inactivated fetal calf serum (**FCS**, HyClone, Logan, UT), 2 mM L-glutamine (GIBCO BRL), and 2% (vol/vol) antibiotics (10,000 U/mL penicillin, 10 µg/mL streptomycin, Bio-Whittaker, Walkersville, MA. The cells were cultured in the presence or absence of pokeweed mitogen (PWM 1:500, GIBCO BRL) at 37°C in a 5% CO_2 incubator for five days to determine DNA and antibody synthesis.

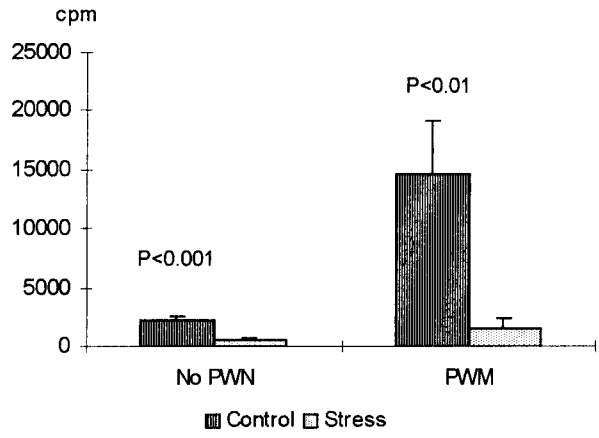
To perform PBMC proliferation assay, triplicate cultures were made in 96-well tissue culture plates (Costar, Cambridge, MA). Each well contained 1.5 x 10^5 PBMC in 200 µl of complete culture medium. The proliferation response to antigens was determined after four days of culture adding [^{3}H]-thymidine (**[^{3}H]-TdR**, Specific radioactivity 5 Ci/mmol, Amersham International plc., Buckinghamshire, UK) to each culture well (0.5 µCi). After 18 hours, the cultures were harvested on filters using a semi-automatic cell harvester (Skatron Instrument Ltd, Suffolk, UK) and [^{3}H]-TdR incorporation was measured in a liquid scintillation counter (Wallac, Turku, Finland). The results were expressed as counts per minute (cpm).

The PWM-stimulated cells in complete culture medium were collected after 8 days of culture and centrifuged at 10,000 x g for 15 min, and the supernatant was stored at -20 °C for analysis. The immunoglobulins (Ig) released in culture media were determined by competitive ELISA assay. The supernatants, diluted 1:1 with PBS containing 5% bovine serum albumin (BSA), were added to plates (3912 Falcon Labware, Becton Dickinson Co., Oxnard, California) absorbed with anti-rabbit sheep antibodies (Sigma Chemical). The secondary antibody was anti-rabbit sheep Ig-peroxidase conjugate (1:500; Sigma Chemical) and the substrate was o-phenilendiaminedihydrochloride (Sigma Chemical).

Table 4 : Plasma concentration of vitamin E, vitamin C, SH-groups and total antioxidant capability (TRAP) in sixteen rabbits exposed (one hour) at 42.0°C environmental temperature (mean ± s.d.).

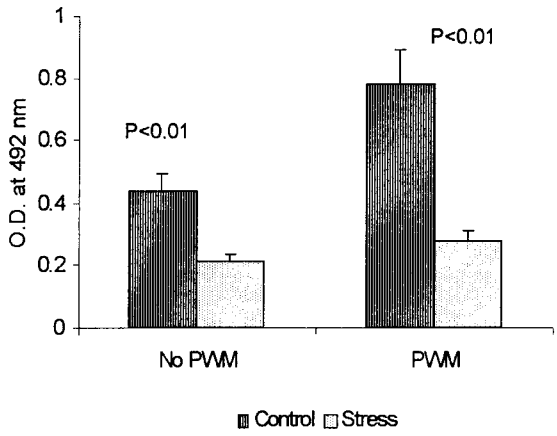
	Vitamin E µg/l		Vitamin C mg/l		SH-groups µmol/l		TRAP µmol/l	
Sampling time	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Control	2.2	0·6	5.2	1.8	408	54	568	100
0·5 h	1.8	0·6	6.5**	2.0	455**	38	706**	97
6 h	2.1	0·9	5.9	1.2	409	40	613*	59
30 h	2.3	0·9	5.2	1.6	386*	35	587	46
54 h	2.2	0·9	6.5**	2.0	404	41	606	56

Significance of the difference in comparison to control time: * P<0.05 ; ** P<0.01.



P<0.01 ; P<0.001: Significance of the difference between stressed and control rabbits within mitogen treatment.

Figure 1 : DNA synthesis ([^{3}H]TdR incorporation) from cultures of PBMC (Pokeweed mitogen stimulated: PWM, or non stimulated) isolated from rabbits 0.5 h after the end of one hour thermal stress (open bars) or control animals (solid bars).



P<0.01 ; P<0.001: Significance of the difference between stressed and control rabbits within mitogen treatment.

Figure 2 : Immunoglobulins synthesis (ELISA assay ; OD 492 nm) from cultures of PBMC (Pokeweed mitogen stimulated: PWM, or non stimulated) isolated from rabbits 0.5 h after the end of one hour thermal stress (open bars) or control animals (solid bars).

Optical density (OD) was measured at 492 nm using an automatic ELISA reader (Bio-Rad, Hertfordshire, UK).

This experiment was performed in compliance with the Italian law on procedures for experimental animals. All procedures to minimise pain and discomfort were adopted during the operative and post-operative period.

The data were submitted to analysis of variance according to a repeated measures design. Comparisons between the means were evaluated using orthogonal contrasts (SAS, 1993).

RESULTS

Rectal temperature (Table 1) varied around normal values during the adaptation period and showed a dramatic and significant increase (about 2.5°C; P<0.001) as a consequence of the increased ambient temperature. After the stress the temperature rapidly decreased and reached normal values within two hours.

The average feed ingestion registered before stress was about 6.8% of LW. In the 24 hours following the stress, a slight decrease of feed intake was registered (about 20%) and a complete recovery of feed ingestion was obtained only five days after stress (Table 1), although because of the small number of subjects, did not there was no significant difference.

A significant decrease, in comparison to control time, was observed for plasma concentration of glucose (Table 2) at 0.5, 6, 30 and 54 hours after stress (P<0.01) and for cholesterol at 30 (P<0.05) and 54 hours (P<0.01). Urea concentration showed an increase at 0.5 hours (P<0.05) and a decrease at 30 and 54 hours (P<0.01). No significant changes were observed for triglycerides except for an increase at 0.5 hours (P<0.05).

Liver function indicators (Table 3) revealed an increase at 0.5 and 6 hours (P<0.01) for GOT. GPT showed an increase at 0.5 hours (P<0.01) followed by a decrease at 30 hours (P<0.05) and at 54 hours (P<0.01).

Regarding the antioxidative response (Table 4), an increase of TRAP was registered at 0.5 hours (P<0.01) and 6 hours (P<0.05), and for SH-groups only at 0.5 hours (P<0.01), both parameters returning to normal values in the subsequent hours after stress. No relevant changes were observed for vitamin E, and only an increase at 0.5 hours was observed for vitamin C (Table 4).

The levels of DNA and immunoglobulins synthesis in PBMC of rabbits at 0.5 hours after thermal stress, expressed as cpm [^{3}H]-TdR and optical density (OD) at 492 nm, are reported in Figure 1 and 2. The results indicate an immunosuppressive effect of the acute stress, particularly evident in presence of mitogen.

DISCUSSION

Heat stress as other types of stress of chemical or psychosocial origin has been generally associated with detrimental effects on physiological equilibrium of the

animal organism and various systems (nervous, endocrine and immune) have been implicated with specific responses and reciprocal regulatory influences (KHANSARI *et al.*, 1990). Our results show that immune cell-mediated function was strongly compromised by the acute heat stress exposure, with possible reductions in the resistance of rabbits to infectious agents in these extreme environmental conditions. However, the operating mechanism in this effect is unclear. Altered composition of blood mononuclear cells of humans or the decrease in the receptor number on the immune cells surface have been reported (MEHDI *et al.*, 1977 ; KAPPEL *et al.*, 1991).

Among the antioxidative parameters, the TRAP value increases in the first moments after the stress due essentially to the higher concentration of SH-groups. However, we cannot exclude that this phenomenon is the consequence of the release of albumin or glutathione (VENDEMIALE *et al.*, 1989), very rich in SH-groups, from the liver since the increase of transaminases level in plasma at the same time, indicate an hepatic cellular damage as found in humans in case of acute ethanol ingestion (VENDEMIALE *et al.*, 1989).

As far as the general metabolism is concerned , the immediate decrease of glucose and delayed decrease of cholesterol were the main changes registered after thermal stress. The decrease of these parameters has been observed in rabbits undergoing prolonged heat environmental conditions (CHIERICATO *et al.*, 1994 ; MARAY *et al.*, 1994 ; AMICI and MERENDINO, 1996 ; AMICI *et al.*, 1998), but also in rabbits submitted to restricted feeding level (CHIERICATO, 1984 ; AMICI *et al.*, 1995). Contrasting results were registered by EL-MASRY *et al.* (1994) in adult males undergoing continuous heat conditions (35±3°C, 46±8% R.H.), involving increases of both glucose and cholesterol. To explain these contrasting results, the role of reduced feed intake and direct effect of hot conditions on thyroid activity can be hypothesised. In effect, thyroxine decrease may reduce metabolic heat production under hot environmental conditions as observed by SANO (1983) in sheep.

As a consequence of the hot environment, no relevant changes were detected for triglycerides at 6, 30 and 54 hours after the stress as also observed in other studies (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1994 ; MARAY *et al.*, 1994 ; AMICI *et al.*, 1995), perhaps mainly due to the individual variability and scarce number of subjects utilised. The significant but transient increase of tryglicerides observed 30 hours after the end of the stress is not supported by other observations in rabbits but it can be explained with an increased mobilisation of lipid reserves as a consequence of the intense heat dissipation as reported by PEREZ *et al.* (1996) in draught stressed horses. Concerning urea concentration, in agreement with our data, a decrease was often observed during long term heat exposure (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1994) but no data are available to support the transient increase observed immediately after stress.

The net increase observed immediately after stress for GOT and GPT could be related to the net and transient increase in cortisol levels following stress. The slight decrease of GPT at 30 and 54 hours, is supported by previous studies that recorded contrasting results on these parameters. CHIERICATO *et al.* (1994) suggested that hot environment induces a decrease of both parameters, but an opposite trend was observed by EL-MASRY *et al.* (1994).

The functional parameters examined (rectal temperature, and feed intake) showed that while the acute hyperthermia lasted no more than two hours, the effect on appetite is very long to be completely recovered (more than five days), suggesting that the acute stress has lasting consequences at the level of the central nervous system from whom activity the amount of feed intake is dependent. This effect is obviously very important from the breeders point of view, especially because the phenomena of acute hyperthermia exposure in rabbits are frequent, particularly in hot environments.

The immediate detrimental effect of acute thermal stress seems to act at the level of immune and nervous system function, while metabolic changes are more difficult to evaluate in terms of physiological significance and duration, even though they are rapidly recovered to normal levels. Therefore, immunological functions represent the most sensitive and immediate response to acute thermal stress and the relationship between immune response and antioxidative system is not negligible. It has been demonstrated that impaired immune activity *in vitro* can be stimulated by some antioxidant substances (FRANCI *et al.*, 1997). Interesting results could be derived from *in vivo* experiments which investigate the utilisation of antioxidants in stressed animals.

Acknowledgements : Research supported by the National Research Council of Italy Special Project RAISA, sub-project no. 3.

Received : September 6th, 1999
Accepted : January 28th, 2000

REFERENCES

ABDELATIF A.M., MODAWI S.M., 1994. Effects of hyperthermia on blood constituents in the domestic rabbit (Oryctolagus *cuniculus*). *J. Thermal Biol.*, ***19*** *(6), 357-363.*

AMICI A., FINZI A., MASTROIACONO P., NARDINI M., TOMASSI G., 1995. Functional and metabolic changes in rabbit undergoing continuous heat stress for 24 days. *Anim. Sci., **61**, 399-405.*

AMICI A., MERENDINO N., 1996. Some metabolic and immunological parameters in rabbits as affected by prolonged thermal stress. *In: Proc. 6th World Rabbit Congr., Toulouse jul. 1996, vol. **2**, 147-150.*

AMICI A., CANGANELLA F., BEVILAQUA L., 1998. Effects of high ambient temperature in rabbits: metabolic changes, caecal fermentation and bacterial flora. *World Rabbit Science, 1998, **6** (3-4), 319-324*

BIERI J.G., TOLLIVER G.T., CATIGNANI L.G., 1979. Simultaneous determination of alpha tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin. Nutr., **32**, 2143-2149.*

CASTELLO J.A., 1984. Control ambiental en la crianza intensiva del conejo. *Cunicultura, **47**, 13-22.*

CASTELLO J.A., ROCHA T., 1980. Factores de confort de los conejos. In: *Tratado de Cunicultura. (ed Real Escuela Oficial y Superior de Avicultura), **2**, 431-438. Barcelona.*

CHIERICATO G.M. 1984. Effetto del livello alimentare su alcune variabili ematiche in giovani coniglie. *Zootecnica e Nutrizione Animale, **10**, 191-196.*

CHIERICATO G.M., BAILONI L., RIZZI C., 1992. The effect of the environmental temperature on the performance of growing rabbit. *In: Proc. 5th World Rabbit Congr., Corvallis jul. 1992, vol **B**, 723-731.*

CHIERICATO G.M.,RAVAROTTO L., RIZZI C., 1994. Study on the metabolic profile of rabbits in relation to two different environmental temperatures on the performance of growing rabbit. *World Rabbit Sci. **2** (4), 153-160.*

EALY A.D., HOWELL J.L., MONTEROSSO V.H., ARÉCHIGA C.F., HANSEN P.J., 1995. Developmental changes in sensitivity of bovine embryos to heat shock and use of antioxidants as thermoprotectants. *J. Anim. Sci., **73**, 1401-1407.*

EBERHART S., 1980. The influence of environmental temperatures on meat rabbits of different breeds. . *In: Proc. 2nd World Rabbit Congr., vol **1**, 399-409.*

ELLMAN G.L., 1959. Tissue sulphydril groups. *Arch. Biochem. Biophys., **82**, 70-77.*

EL-MASRY K.A., NASR A.S., KAMAL T.H., 1994. Influences of season and dietary supplementation with selenium and vitamin E or zinc on some blood constituents and semen quality of New Zealand White rabbit males. *World Rabbit Sci., **2** (3), 79-86.*

FARBER C.M., KANENGISER S., STAHL R., LIEBES L., SILBER L., 1983. A specific high-performance liquid chromatography assay for dehydroascorbic acid shows an increased content of CLL lymphocytes. *Anal. Biochem., **134**, 355-360.*

FINZI A., VALENTINI A, FILIPPI BALESTRA G., 1994. Approche de quelques indicateurs du stress chez le lapin. *Cuniculture, **118**, 189-193.*

FRANCI O., AMICI A., MARGARIT R., MERENDINO N., PICCOLELLA E., 1996. Influence of thermal and dietary stress on immune response of rabbits. *J. Anim. Sci., **74**, (7), 1523-1529.*

FRANCI O., RANFI F:, SCACCINI C., AMICI A., MERENDINO N., TOMASSI G., PICCOLELLA E., 1997. Differential effect of α-tocopherol and ascorbate on oxidative injury induced in immenue cells by thermal stress. *J. Biological Regulators and Homeostatic Agents, **10** (2), 54-59.*

KAMWANJA L.A., CHASE C.C. JR., GUTIERREZ J.A., GUERRIERO V. JR., OLSON T.A., HAMMOND A.C., HANSEN P.J., 1994. Responses of bovine lymphocytes to heat shock as modified by breed and antioxidant status. *J. Anim. Sci., **72**, 438-444.*

KHANSARI D.N., MURGO A.J., FAITH R.E., 1990. Effects of stress on immune system. *Immunology Today, **11** (5), 170-175.*

KAPPEL M., STADEAGER C., TVEDE N., GALBO H., KLARLUND PEDERSEN B., 1991. Effects of in vivo hyperthermia on natural killer cell activity, in vitro proliferative responses and blood mononuclear cell subpopulation. *Clinical and Experimental Immunology, **84**, 175-180.*

LOW B.G., HANSEN P.J., 1988. Immunosuppressive actions of steroids and prostaglandins secreted by the placenta and uterus of the cow and sheep. *Am. J. Repro. Immunology Microbiology, **18** (3), 71-75.*

LOVEN D.P., 1988. A role for reduced oxygen species in heat induced cell killing and the induction of thermotolerance. *Med. Hyphoteses, **26**, 39-45.*

MARAY I.F., EL-MASRY K.A., NASR A.S., 1994. Heat stress and its amelioration with nutritional, buffering, hormonal and physical techniques for New Zealand White rabbits maintained under hot summer conditions of Egypt. *In: Proc.1st International Conference on Rabbit Production in Hot Climates, CHIEAM, Cahiers Option Mediterranéennes **8**, 475-487.*

MATHERON G., MARTIAL S.P., 1981. L' élevage de lapin en ambiance chaude et humide : étude de quelque réponses zootecniques et physiologiques. *Thesis, Ecole National Supérieure de Agriculture, E.N.S.A., Rennes.*

MEHDI S.Q., NUSSEY S.S., SHINDELMAN J., KRISA J.P., 1977. The influence of lipid substitution of thyrotropin-receptor interactions in artificial vesicles. *Endocrinolog,y **101**, 1406-1412.*

PACI G., BAGLIACCA M., MARZONI M., LIPONI G.B., 1993. Consumo giornaliero e CUDa di conigli sottoposti a differenti temperature. *Annali della Facoltà di Medicina Veterinaria di Pisa, **XLVI**, 377-385.*

PEREZ R., VALENZUELA S., MERINO V., CABEZAS I., GARCIA M., BOU R., ORTIZ P., 1996. Energetic requirements and physiological adaptation of draught horses to ploughing work. *Anim. Sci., **63** (2), 343-351.*

PRUD'HON M., 1976. Comportament alimentaire du lapin soumis aux temperatures de 10, 20 et 30 °C. . *In: Proc. 1st World Rabbit Congr., 1996, **14**, 1-6.*

SANO H., TAKAHASHI K., AMBO K., TSUDA T., 1983. Turnover and oxidation rates of blood glucose and heat production in sheep exposed to heat. *J. Dairy Sci., **66**, 856-861.*

SAS. 1993. SAS User's Guide: Statistics (Version 6.08). *SAS Inst. Inc., Cary, NC, USA.*

VENDEMIALE G., ALTOMARE E., GRATTAGLIANO I., ALBANO O., 1989. Increased levels of glutathione and malondialdehyde after acute ethanol ingestion in humans. *J. Hepathology, **3**, 359-365.*

WAYNER D.D.M., BURTON G.W., INGOLD K.U., LOCKE S., 1985. Quantitative measurements of the total peroxyl radical-trapping antioxidant capability of human blood by controlled peroxidation. *FEBS Letters, **187**, 33-37.*

ZULKIFLI I., SIEGLE P.B. 1995. Is there a positive side to stress? *World's Poultry Sci. J., **51**, 63-67.*