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STUDY OF THE METABOLIC PROFILE OF RABBITS IN RELATION TO TWO DIFFERENT ENVIRONMENTAL TEMPERATURES

CHIERICATO G.M. *, RAVAROTTO Licia **, RIZZI Chiara *.

* Dipartimento di Scienze Zootecniche,
Universita di Padova, Via Gradenigo, 6 - 35131 PADOVA - Italy

** Istituto Zooprofilattico Sperimentale delle Venezie,
Via G. Orus, 2 - 35131 PADOVA - Italy

SUMMARY : The trial was conducted on 75 male rabbits (50 days old) of a commercial cross bred type in order to study the effects of temperature on the metabolic profile of rabbits. The animals were housed in two different rooms at 12°C (LT) and 30°C (HT). Relative humidity was about 65 % and the natural photoperiod was characterized by 16L:8D. At the end of the experiment (106 days of age) a blood sample was taken from each rabbit after 2 hours' fasting. The LT animals showed higher (P<0.01) daily weight gain (36.7 *vs* 25.7 g), feed intake (173 *vs* 110 g) and feed conversion ratio (4.73 *vs* 4.37 g/g). LT rabbits had higher plasma levels of haematocrit (0.40 *vs* 0.38 l/l, P<0.05), glucose (7.7 *vs* 6.8 mmol/l, P<0.01), total proteins (65.1 *vs* 58.6 g/l, P<0.01), albumin (48.2 *vs* 42.9 g/l, P<0.01), urea nitrogen (6.71 *vs* 5.44 mmol/l, P<0.01), Ca (3.69 *vs* 3.51 mmol/l, P<0.01), inorganic P (2.21 *vs* 1.90 mmol/l, P<0.01) and Mg (1.00 *vs* 0.90 mmol/l, P<0.01). HT animals had higher plasma concentrations of cholesterol (2.56 *vs* 1.53 mmol/l, P<0.01), creatinine (143 *vs* 106 µmol/l, P<0.01), AST (26 *vs* 18 U/l, P<0.05), ALT (36 *vs* 29 U/l, P<0.05) and Cl (108 *vs* 106 mmol/l, P<0.05). In conclusion, our results indicate that environmental temperature has a significant effect on the biochemical profile of rabbits.

RÉSUMÉ : Etude du métabolisme des lapins soumis à deux températures ambiantes différentes.
L'expérience a été conduite avec 75 lapins mâles âgés de 50 jours, de type hybride du commerce, afin d'étudier les effets de la température sur leur métabolisme. Les animaux étaient logés dans deux pièces différentes l'une à 12°C (LT), l'autre à 30°C (HT). L'humidité relative était d'environ 65 % et la photopériode naturelle était définie par 16L:8D. A la fin de l'expérience (106 jours d'âge) un échantillon de sang a été prélevé sur chaque lapin après une période de 2 heures de jeûne. Les animaux LT avaient un gain de poids journalier plus élevé (36.7 vs *25.7 g, P<0.01), une consommation plus forte (173* vs *110 g, P<0.01) et un indice de consommation plus élévé (4.73* vs *4.37 g/g, P<0.01). Les lapins LT avaient des taux plasmatiques supérieurs pour l'hématocrite (0.40* vs *0.38 l/l, P<0.05), le glucose (7.7* vs *6.8 mmol/l, P<0.01), les protéines totales (65.1* vs *58.6 g/l, P<0.01), l'albumine (48.2* vs *42.9 g/l, P<0.01), l'azote uréique (6.71* vs *5.44 mmol/l, P<0.01), le Ca (3.69* vs *3.51 mmol/l, P<0.01), le P inorganique (2.21* vs *1.90 mmol/l, P<0.01) et le Mg (1.00* vs *0.90 mmol/l, P<0.01). Les lapins HT avaient des concentrations plasmatiques plus élevées de cholestérol (2.56* vs *1.53 mmol/l, P<0.01), créatinine (143* vs *106 µmol/l, P<0.01), AST (26* vs *18 U/l, P<0.05), ALT (36* vs *29 U/l, P<0.05) et Cl (108* vs *106 mmol/l, P<0.05). En conclusion, nos résultats montrent que la température ambiante a un effet significatif sur le profil biochimique des lapins.*

INTRODUCTION

An analysis of existing literature indicates that the metabolic profile in rabbits is considerably affected by feeding (PRUSIEWICZ–WITASZEK *et al.*, 1976 ; CHIERICATO, 1984 ; CHIERICATO *et al.*, 1984 ; CHIERICATO *et al.*, 1985 ; CORTI *et al.*, 1988 ; CHIERICATO *et al.*, 1990), genotype (ZUCCHI *et al.*, 1984 ; ZUCCHI *et al.*, 1992), sex (LOZSA *et al.*, 1974 ; ZUCCHI *et al.*, 1992), age (GASCON *et al.*, 1989 ; CHIERICATO *et al.*, 1992b) and the health of the animals (CLARK *et al.*, 1982 ; EKPENYONG *et al.*, 1986 ; GASCON and VERDE, 1985). There is a substantial lack of works on the effect of environmental temperature on the biochemical profile. Given the technological importance of this rearing factor, the objective of the present study is to contribute to the knowledge of the effects of rearing temperatures, above and below the thermal neutrality values on the haematological profile of growing rabbits.

MATERIAL AND METHODS

The research was carried out on 75 male rabbits (50 days–old) belonging to a hybrid commercial genotype, and weighing an average of 1168 ± 107 g.

Table 1 : Productive performances of the rabbits.

		HT	LT	Significance	EMS (1)
Animals	No	48	27		
Initial live weight	g	1176	1161	**	11456
Final live weight	g	2624	3237	**	62903
Daily live weight gain	g	25.7	36.7	**	20.24
Metabolic live weight	kg	1.62	1.80	**	0.0087
Daily intake	g	110.3	173.0	**	304.94
Feed efficiency	g/g	4.37	4.73	**	0.2024

** : P<0.01 ; (1) Error mean square (73 D.F.)

The rabbits were divided into two different rooms and subjected to different thermic treatments over an 8-week period. Twenty-seven rabbits were kept at an average temperature of 12°C (LT treatment), whereas 48 animals were reared at 30°C (HT treatment). While the lower temperatures were obtained in the natural environment, the higher were produced by a suitable environmental conditioning system. The photoperiod was natural and consisted of 16 hours of light and 8 hours of dark per day.

Environmental temperature and relative humidity values were constantly monitored by a thermohygrograph, whereas light intensity was monitored by a luxmeter on a weekly basis. Ammonia concentrations inside the rooms were checked every 7 days using Drager kits. The animals were reared in single cages on three staggered shelves, without hindering caecotrophy, and fed *ad libitum* with pelleted commercial food.

The food was subjected to chemical analysis (MARTILLOTTI *et al.*, 1987) ; its digestible energy was estimated on the basis of analytic data and using the equation of PARIGI-BINI and DALLE RIVE (1977). Macroelement concentrations were determined by following the standard methodology (MARTILLOTTI *et al.*, 1987).

The feed intake and state of health of each rabbit were checked daily, whereas live weight gain measurements were conducted weekly.

At the end of the experiment, all the animals were subjected to blood sampling after being kept without food for two hours. Blood samples of 10 cc were taken by intracardiac puncture, collected in lithium eparine tubes, and centrifugated for 15' at 3000 rpm. The plasma thus obtained was kept at -20°C until chemical analysis was performed using a DuPont (R.A. 1000) automatic analyser by Technicon and kits provided by the same firm (TECHNICON, 1988).

After controlling variance homogeneity, all the data were subjected to statistical analysis using the model I and the HARVEY (1990) package, according to the following pattern :

$$Y_{ij} = \mu + T_i + e_{ij}$$

where :
Y_{ij} = experimental data
μ = overall mean
T_i = fixed effect of i^{th}-temperature (i = 1, 2)
e_{ij} = residual random effect

RESULTS AND DISCUSSION

Rearing conditions and feeding characteristics

Room temperatures were uniform for each experimental treatment and equal to 30 ± 1.3°C for the HT group and 12 ± 1.4°C for the LT group. Relative humidity ranged from 60 to 70 %. Light intensity was between 10 and 45 lux, on cloudy and fine days respectively, which were roughly equal during rearing. Ammonia concentrations (5-10 ppm) remained within suitable levels for intensive rabbit rearing employing intensive feeding programs (ROCA CASANOVAS et al., 1980).

The rabbits were fed a commercial-type pellet characterized (on dry matter basis) by a value of 18 % crude protein, 11.7 MJ/kg digestible energy, 15.3 % of crude fibre, 38.3 % NDF and 18.9 % ADF.

Table 2 : Daily feed intake per unit of metabolic weight.

		HT	LT	Significance	EMS (1)
Animals	No	48	27		
Daily intake of :					
Dry matter	$g/P^{0.75}$	61.38	85.81	**	43.5035
Digestible energy	$kJ/P^{0.75}$	802	1120	**	7385
Crude protein	$g/P^{0.75}$	10.99	15.36	**	1.3933
Ether extract	"	3.66	5.09	**	0.1667
Crude fibre	"	9.33	13.03	**	0.9892
N-free extract	"	31.79	44.46	**	11.8321
Ash	"	5.61	7.85	**	0.3938
NDF	"	23.46	32.85	**	6.8037
ADF	"	11.63	16.26	**	1.5772
Calcium	"	0.88	1.05	**	0.0090
Phosphorus	"	0.37	0.52	**	0.0016
Magnesium	"	0.18	0.24	**	0.0006
Sodium	"	1.46	2.13	**	0.0272
Potassium	"	1.08	1.51	**	0.0010

** : P<0.01 ; (1) Error mean square (73 D.F.)

Productive performance of the animals

Table 1 shows the performance of the rabbits during the trial. As can be seen, the higher temperature had a noticeable effect on live weight gain, since the rabbits of the HT group presented values of 25.7 g, thus significantly (P<0.01) lower than those of the LT animals (36.7 g). As expected, an average final body weight of 3237 g was achieved in the LT group which was 23.6 % higher (P<0.01) than the weight of the group reared in heated conditions (2624 g). These results agree with those obtained in other experiments (STEPHAN, 1981 ; LEBAS and OUHAYOUN, 1987 ; SIMPLICIO *et al*, 1988 ; CHIERICATO *et al.*, 1992a).

Daily feed intake values were also significantly different between the animals belonging to HT and LT groups (110.3 *vs* 173.0, P<0.01). In other trials, a similar negative effect on feed intake was found at high temperatures (STEPHAN, 1981 ; LEBAS and OUHAYOUN, 1987 ; SIMPLICIO *et al.*, 1988 ; CHIERICATO et al., 1992a).

Feed efficiency showed a similar trend and was less favourable for the rabbits kept at low temperatures (4.73 *vs* 4.37 g/g, P<0.01). This effect can be ascribed to increased energy requirement due to thermogenesis and a predictable more intense adipogenesis, given the higher final live weight value of the rabbits belonging to the LT group. In previous research works carried out in similar environmental conditions, we notice a higher perivisceral fat synthesis in the rabbits maintained at the lower temperatures (CHIERICATO *et al.*, 1992a). This worsening of feed efficiency in LT rabbits is in line with results of other studies (STEPHAN, 1981 ; CHIERICATO *et al.*, 1992a).

Considering feed intake per unit of metabolic weight (Table 2), we saw how the former significantly (P<0.01) changed in relation to the two levels of environmental temperatures adopted, and decreased for rabbits in the HT group. This consideration is valid for all the nutrients considered and in particular, for the intake of dry matter (85.81 *vs* 61.38 $g/P^{0.75}$), digestible energy (1120 *vs* 802 $kJ/P^{0.75}$), crude protein (15.36 *vs* 10.99 $g/P^{0.75}$) and ADF (16.26 *vs* 11.63 $g/P^{0.75}$). The effects observed on feed intake confirm the findings of previous experiments (STEPHAN, 1981 ; LEBAS and OUHAYOUN, 1987 ; SIMPLICIO *et al*, 1988 ; CHIERICATO *et al.*, 1992a).

Metabolic profile

Table 3 shows the plasma levels of some metabolites. As can be seen, high rearing temperatures noticeably influenced the blood parameters examined.

The haematocrit values differed between the two experimental groups, to the extent that the LT rabbits showed significantly higher values than those of the rabbits reared at 30°C (0.41 *vs* 0.38 l/l, P<0.05).

Table 3 : Plasma concentration on some blood constituents.

		HT	LT	Significance	EMS (1)
Animals	*No*	*48*	*27*		
Haematocrit	l/l	0.38	0.41	*	0.0013
Glucose	mmol/l	6.8	7.7	**	0.78
Cholesterol	mmol/l	2.56	1.53	**	0.5406
Triglycerides	mmol/l	1.91	2.08		0.7036
Total proteins	g/l	58.6	65.1	**	16.81
Albumin	g/l	42.9	48.2	**	9.78
Globulins	g/l	15.8	16.9		8.72
Urea nitrogen	mmol/l	5.44	6.71	**	0.6696
Total bilirubin	μmol/l	1.34	1.13		0.3087
Uric acid	μmol/l	4.86	6.23		4.1463
Creatinine	μmol/l	143	106	**	469

* P<0.05 ; ** P<0.01 ; (1) Error mean square (73 D.F.)

Decreases in hematocrit were found also in other monogastrics kept at high temperature (VO *et al.*, 1978 ; DONKOH, 1989). Other studies on rabbits report that this parameter could be influenced by tylosine phosphate (RADU–TUDORAKE *et al*, 1981a) and by the presence of starch in the diet (ABDELHAMID, 1990). In our previous experience with male rabbits we noticed that a significant reduction of haematocrit was caused by feeding restriction (CHIERICATO *et al.*, 1985).

With regard to glucose levels, the rabbits kept at 30°C presented significantly lower values than those reared at 12°C (6.8 *vs* 7.7 mmol/l, P<0.01). In a study carried out on female rabbits reared in thermic stress conditions, TRAMMEL *et al.* (1989) observed negative effects on glucose levels. Another factor capable of modifying plasma concentrations of this metabolite is the type of lipids in the diet (ABDELHAMID, 1990). Glucose significantly decreases as the nutritive level is reduced (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1984) and with increasing age (CHIERICATO *et al.*, 1992b).

Cholesterol plasma levels presented an opposite trend with respect to that observed above, being higher in rabbits of the HT group (2.56 *vs* 1.53 mmol/l, P<0.01). A similar trend was observed by CHRISTON (1986) in pigs. Given the scarcity of existing literature concerning the effects of temperature on the examined metabolite in rabbits, it can be mentioned that cholesterol levels are affected positively by low protein diets and nutritive level (CORTI *et al.*, 1988 ; CHIERICATO *et al.*, 1990) and the presence of zeolite in the diet (NIZZA *et al.*, 1987) and negatively by age (CHIERICATO *et al.*, 1992b).

Triglyceride plasma contents were not significantly different, exhibiting values of 1.91 and 2.08 mmol/l in HT and LT rabbits, respectively. Nutritive level (CHIERICATO *et al*, 1990), time of blood sampling (CHIERICATO *et al*, 1990) and age (CHIERICATO *et al.*, 1992b) are factors capable of modifying triglyceride levels in rabbits. Copper sulphate added to diet (FEKETE *et al.*, 1988) and the different diet calcium rate (DOUGHERTY and IACONO, 1979) did not appear to influence this metabolite.

High temperature negatively influenced total protein plasma concentrations that reached values significantly (P<0.01) lower in HT rabbits than those of LT treatments (58.6 *vs* 65.1 g/l). Chickens reared in heat stress conditions exhibited a similar pattern (WARD and PETERSON, 1973 ; DONKOH, 1989). Factors causing variations of total proteins plasma levels in rabbits are age (CHIERICATO *et al.*, 1992b) and diarrhoea diseases (GASCON and VERDE, 1985 ; EKPENYONG, 1986).

Albumin and globulin fractions decreased in conditions of heat stress, but the difference was statistically significant only for the albumin contents (42.9 *vs* 48.2 g/l, P<0.01). Relevant effects on albumin and globulin plasma concentrations are due to the feeding level (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1984 ; CHIERICATO *et al.*, 1985 ; CHIERICATO *et al.*, 1990) and to lysine and methionine added to the diet (PRUSIEWICZ-WITASZEK *et al.*, 1976).

As concerns other blood variables of protein metabolism, urea nitrogen levels displayed the same trend as observed above, being lower at higher temperatures (6.71 *vs* 5.44 mmol/l, P<0.01). A similar trend was observed in swine (PARKER *et al.*, 1980).

In rabbits, urea nitrogen plasma concentrations are affected by circadian rhythms (ROSI *et al.*, 1986), diarrhoea (GASCON and VERDE, 1985 ; EKPENYONG, 1986), dietary protein content (CORTI *et al.*, 1988), caecotrophy (GISBERTI *et al.*, 1982) and feeding plans (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1984 ; CHIERICATO *et al.*, 1985 ; CHIERICATO *et al.*, 1990).

Total bilirubin concentrations did not differ between experimental treatments and averaged 1.24 µmol/l. In rabbits, this metabolite changes in relation to aflatoxicosis (CLARK *et al.*, 1982).

Uric acid plasma levels, although they presented an increase with temperature values under thermic neutrality, did not show significant differences, averaging 5.55 µmol/l. There are no indications to confirm the results obtained in this trial for rabbit uric acid levels. This parameter shows a similar trend also in other monogastrics : it increases in subjects kept at low temperatures due to a higher protein catabolism (WARD and PETERSON, 1973). Works conducted on rabbits pointed out significant variations of uric acid plasma levels in relation with caecotrophy (GISBERTI *et al.*, 1982) and protein content of the diet (CORTI *et al.*, 1984), but not in presence of diarrhoea disease (GASCON and VERDE, 1985).

The creatinine content appeared to be lower in the rabbits belonging to the LT group, as compared to those subjected to thermal stress (106 *vs* 143 µmol/l, P<0.01). This result is in agreement with that reported by BROUCEK *et al.* (1986) on other farm animals. Higher nutritive level (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1990) and absence of caecotrophy (GISBERTI *et al.*, 1982) induced a significant reduction of creatinine content.

Enzymatic profile

The different environmental temperatures modified some plasma enzyme activities.

The highest AST (Aspartate amino transferase) levels (Table 4) were observed in the rabbits kept at 30°C (26 *vs* 18 U/l, P<0.05). A similar trend was observed for ALT (Alanine amino transferase) values, which were 36 and 29 U/l (P<0.05) for the rabbits reared at high and low temperatures, respectively. Examination of existing literature regarding these two transaminases does not provide any information on the effect of environmental temperature on rabbits. An increase in transaminase activities was recorded in poultry under heat stress conditions (POLONIS, 1983 ; MANNING *et al.*, 1990). According to indications provided by other authors working on rabbits, these two enzymes are influenced by dietary lysine and methionine (PRUSIEWICZ–WITASZEK et al., 1976) and health conditions (CLARK *et al.*, 1982), whereas only ALT is affected by age (CHIERICATO *et al.*, 1992b).

Gamma–GT (gamma Glutamyl Transpeptidase)

Table 4 : Plasma concentration of some enzymes and mineral elements.

		HT	LT	Significance	EMS (1)
Animals	No	48	27		
Enzymes					
AST	U/l	26	18	*	73
ALT	U/l	36	29	*	87
Gamma–GT	U/l	9	10		20
LD	U/l	85	78		801
Mineral elements					
Calcium	mmol/l	3.51	3.69	**	0.0282
Phosphorus	mmol/l	1.90	2.21	**	0.0392
Magnesium	mmol/l	0.90	1.00	**	0.0102
Sodium	mmol/l	159	161		43
Potassium	mmol/l	5.23	5.92		2.2605
Chlorine	mmol/l	108	106	*	11

* : P<0.05 ; ** : P<0.01 ; (1) Error mean square (73 D.F.)

activity appeared constant, presenting values of 9 and 10 U/l, in the groups reared at 30°C and 12°C, respectively. No studies have been performed on the effect of environmental temperature on gamma-GT activity, which appears rather to be influenced by the protein content of the diet (CORTI *et al.*, 1988) and copper sulphate (FEKETE *et al.*, 1988).

Likewise, the LD (Lactate dehydrogenase) plasma level did not present significant differences, and exhibited concentrations equal to 85 and 78 U/l in subjects belonging to the HT and LT groups, respectively. Existing literature contains information on the effects of temperature on the LD activity for other monogastrics : in poultry (WARD and PETERSON, 1973), high environmental temperature appeared to increase plasma LD. In rabbits, this enzyme has been studied in relation to the genotype (ZUCCHI *et al*, 1984 ; CHIERICATO *et al.*, 1985) and the nutritive level adopted (CHIERICATO *et al.*, 1985). Results however indicate that the genotype and nutritive level have no influence.

Mineral profile

In regard to mineral profile (Table 4), variations in plasma levels of some mineral elements can be observed at different environmental temperatures.

Plasma levels of calcium (3.51 *vs* 3.69 mmol/l, $P<0.01$), inorganic phosphorus (1.90 *vs* 2.21 mmol/l, $P<0.01$) and magnesium (0.90 *vs* 1.00 mmol/l, $P<0.01$) were lower in HT rabbits than those of LT ones. In previous works no indications of the influence of heat stress on the minerals examined appeared. It is worth remembering that a similar trend was found in trials carried out on poultry (EDENS, 1978 ; BOGIN et al., 1983). In rabbits, calcium and phosphorus levels appear to be affected by circadian rhythms (ROSI *et al.*, 1986) and by feed intake (CHIERICATO *et al.*, 1984 ; CHIERICATO *et al.*, 1985), whereas the magnesium plasma level is affected by dietary calcium (DOUGHERTY and IACONO, 1979) and crude fibre rate (NIZZA *et al.*, 1987).

Sodium concentrations did not differ between rabbits belonging to the HT an LT groups and averaged 160 mmol/l. Likewise, potassium levels were similar in both groups with concentrations equal to 5.23 and 5.92 mmol/l. Sodium concentrations significantly increased with antibiotics administration (RADU-TUDORACHE *et al.*, 1981b), whereas potassium was lower in rabbits given low fibre diets (NIZZA *et al.*, 1987).

Chlorine plasma levels are significantly ($P<0.05$) lower in the LT group (106 *vs* 108 mmol/l, $P<0.05$). This result confirms that found in broilers (WARD and PETERSON, 1973). Chlorine plasma concentrations tend to be negatively modified by low crude fibre diet (NIZZA *et al.*, 1987) and positively by chronic toxicosis (RADU-TUDORACHE *et al*, 1981b).

CONCLUSIONS

The results of this research permit certain conclusions to be drawn on the effects that environmental temperature exerts on the productive performance and haematochemical profile of rabbits.

Lower thermal levels induced a marked variation on the productive performance of the animals which, due to an increased request for thermogenesis and a presumably greater adipogenesis, presented higher feed intakes, body daily gain and final live weight and a more unfavourable utilization of the feed.

As to metabolic profile, the rabbits showed a different physiological response at the two temperatures studied.

With regard to energetic metabolism, in particular we noticed an increase of glucose and triglyceride levels in the low temperature group, whereas the cholesterol level showed an opposite trend.

Protein metabolism is affected by low temperatures and generally presents an increase in total proteins, albumin and urea nitrogen, whereas creatinine levels exhibit an opposite trend.

The effect of environmental temperatures appears less evident on enzyme activity : we observed an increase, restricted however within normal AST and ALT ranges, in the HT group rabbits.

The environmental thermal conditions exerted a relevant and significant effect on some mineral plasma levels, such as calcium, phosphorus and magnesium which were higher in the animals reared at the lower temperatures.

The scarcity of research on the effects that environmental conditions can exert on the metabolite profile of growing rabbits, does not allow comparison with results of other authors. The data observed in this experiment can be profitably compared to studies we carried out in other trials in which the animals were treated with different feeding programs (CHIERICATO, 1984 ; CHIERICATO *et al.*, 1984 ; CHIERICATO *et al.*, 1985 ; CHIERICATO *et al.*, 1990). In light of these results, we can hypothesize that temperature effects can be ascribed primarily to itd influence on feed intakes.

This experience suggests the appropriateness of studying the effects of lower temperature differences than those used in this work. Among other questions worthy of discussion in future, the study of the response of rabbits belonging to different genotypes in relation to their possible interaction with environmental temperatures would be of interest.

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