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EFFECTS OF HEAT STRESS AND AGE ON GROWTH PERFORMANCE AND ENDOCRINE STATUS OF MALE RABBIT

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ABSTRACT : The effect of environmental temperature and age on plasma hormonal concentrations of testosterone (T), dihydrotestosterone (DHT), cortisol (C), triiodothyronine (T3) and thyroxine (T4) were evaluated in 40 male Grimaud rabbits. Twenty animals were reared at 20°C (TNT = Thermic Neutrality Temperature) and the other 20 at 27°C (HST = Heat Stress Temperature). For both treatments relative humidity levels averaged 77 % and the photoperiod was 8D:16L with a light intensity of about 40 lux. The animals were fed *ad libitum* with a commercial pelleted feed. Blood samples were collected from each animal by intracardiac puncture at 71 and 85 days of age. No interaction effect between temperature and age was found. With regard to effect of temperature, the HST animals had a lower (P<0.01) final body weight (2325 *vs* 2555 g), daily gain (32.7 vs 38.1 g) and feed intake (106 *vs* 126 g/d). The HST treatment significantly (P<0.05) decreased plasma levels of T (1.01 *vs* 1.96 ng/ml), DHT (0.42 *vs* 0.73 ng/ml) and T + DHT (1.43 *vs* 2.66 ng/ml), but not the T/DHT ratio (2.69). TNT animals had higher (P<0.01) plasma levels of T3 (1.30 *vs* 1.01 ng/ml) and a lower (P<0.01) T4/T3 ratio (32.7 *vs* 41.3). The concentrations of C (18.9 ng/ml) and T4 (41.0 ng/ml) resulted unchanged. The effect of age in the older rabbits was a significantly higher (P<0.01) final body weight (2698 vs 2181 g) daily intake (123 vs 109 g) and feed/gain ratio (3.44 vs 3.12 g/g). No significant difference was observed in plasma concentrations of T (1.48 ng/ml), DHT (0.58 ng/ml), T/DHT (2.69) and T + DHT (2.05 ng/ml) in 71 and 85 days old rabbits. Age had no effect on C levels, which averaged 19.0 ng/ml. In blood samples of younger and older rabbits there was no difference in T3 (1.16 ng/ml), T4 (41.0 ng/ml) values and T4/T3 ratio (37.0). Correlations and linear regression equations were also calculated for daily gain (DG), digestible energy intake per unit of metabolic body weight (DEI/MBW) and each hormone plasma level. Only the thyroid hormones showed significant correlations : T3 was positively (P<0.01) correlated with DG (r = 0.624) and DEI/MBW (r = 0.749), whereas the T4/T3 ratio was negatively influenced (P<0.01) by DG (r = – 0.563) and DEI/MBW (r = – 0.654).

RÉSUMÉ : Effets du stress de chaleur et de l'âge sur la croissance et le statut endocrinien des lapins mâles.
Cette étude réalisée sur 40 lapins Grimaud mâles a pour but d'évaluer l'effet de la température ambiante et de l'âge sur les concentrations plasmatiques des hormones suivantes : Testostérone (T), Dihydrotestostérone, (DHT), Cortisol (C), Triiodothyronine (T3), Thyroxine (T4). Vingt mâles ont été maintenus à 20°C (TNT = Temperature de Neutralité Thermique) et les vingt autres à 27°C (HST = Température de Stress de Chaleur). Pour les deux lots l'humidité relative moyenne était de 77 %, la photopériode était de 8D:16L avec une intensité lumineuse d'environ 40 lux. Les animaux ont été nourris ad libitum *avec un aliment granulé du commerce. Sur chaque animal, des prélèvements de sang ont été effectués par ponction intracardiaque à l'âge de 71 et 85 jours. Aucun effet d'interaction entre l'âge et la température n'a été observé. Pour ce qui concerne l'effet de la température, on a enregistré pour les animaux HST un poids vif final (2325 vs 2555 g), un gain de poids journalier (32.7 vs 38.1 g) et une consommation (106 vs 126 g/jour) inférieurs (P<0.01). Dans le lot HST, les concentrations plasmatiques de T (1.01 vs 1.96 ng/ml), DHT (0.42 vs 0.73 ng/ml) et T + DHT (1.43 vs 2.66 ng/ml) ont diminué significativement (P<0.01), mais pas le rapport T/DHT (2.69). Les animaux TNT ont présenté des concentrations plasmatiques plus élevées de T3 (1.30 vs 1.01 ng/ml ; P<0.01)) et un rapport T4/T3 moins élevé (32.7 vs 41.3 ; P<0.01). Les concentrations plasmatiques de C (18.9 ng/ml) et de T4 (41.0 ng/ml) n'ont pas changé. L'effet de l'âge s'est manifesté chez les lapins les plus vieux par une augmentation significative (P<0.01) du poids vif final (2698 vs 2181 g), de la consommation journalière (123 vs 109 g) et de l'indice de consommation (3.44 vs 3.12 g/j). Aucune différence significative n'a été décelée dans les concentrations plasmatiques de T (1.48 ng/ml), DHT (0.58 ng/ml) T/DHT (2,69) et T + DHT (2.05 ng/ml) pour les lapins âgés de 71 ou de 85 jours. Le taux de C moyen (19.0 ng/ml) n'est pas affecté par l'âge. Les concentrations sanguines de T3 (1.16 ng/ml), T4 (41.0 ng/ml) et du rapport T4/T3 (37.0) sont les mêmes qu'il s'agisse des lapins de 71 ou 85 jours. Les relations existant entre le gain journalier (DG) l'énergie digestible ingérée par unité de poids métabolique (DEI/MBW) et la concentration plasmatique de chaque hormone ont fait l'objet de calculs de corrélations et d'équations de régression linéaire. Seules les hormones thyroïdiennes montrent un correlation significative : T3 est positivement (P<0.01) corrélée avec DG (r = 0.624) et DEI/MBW (r = 0.749), tandis que le rapport T4/T3 est négativement influencé (P<0.01) par DG (r = – 0.563) et DEI/MBW (r = – 0.654).*

INTRODUCTION

The environmental temperature can influence notably the hormone profile of the rabbit by conditioning the productive performance of the animals. In a previous experiments it was observed that high temperature levels significantly altered the performance of growing rabbit and their plasma androgen and thyroid hormone profiles (BOITI *et al.*, 1992).

Age is also an important factor affecting the endocrine status of the animals. BERGER *et al.* (1976, 1979) observed a positive pattern of plasma testosterone in rabbits from birth to 90 days and changes in the testosterone/dihydrotestosterone ratio in maturing subjects.

Since in the available literature there is not sufficient information about the effect of temperature and age and their possible interactions, we carried out a study using Grimaud rabbits to obtain more knowledge.

MATERIALS AND METHODS

Animals and rearing conditions

In this trial 40 Grimaud males rabbits, 35 days old and weighing 934 ± 57 g , were used. Upon arrival, the rabbits were randomly housed in two different rooms, one at 20°C (Thermic Neutrality Temperature, TNT) and the other at 27°C (Heat Stress Temperature, HST) with a mean relative humidity level ranging between 70 and 86 %. During the trial the rabbits were reared individually in Californian battery cages without hindering caecotrophy. Commercial pelleted feed (DE = 10.57 MJ/kg as feed basis ; CP = 17.18 % d.m. ; CF = 15.48 % d.m.) and water were administered *ad libitum*. The feed was submitted to chemical analysis according to the official methods (MARTILOTTI *et al.*, 1987). Starch, NDF and ADF content were also evaluated (BLAKENEJ and MUTTON, 1980 ; HILLIARD and DAJNARD, 1984). The concentrations of gross, digestible and metabolizable energy were calculated using the equations or PARIGI-BINI and DALLE RIVE (1977).

A heating system provided the high temperature levels in the HST room and the air diffusion inside the two environments was natural. Temperature and the relative humidity were continuously recorded by a thermohygrograph (TIG-I TH, L.S.I.). The photoperiod was 16 hours of light and 8 hours of darkness for both treatments. The daily controls of light intensity (about 40 lux) were detected by a silicon sensor luxmeter HD 8366 (Delta Ohm). Ammonia concentrations, measured by Dräger pump and kits (CIGR, 1994) every two days, averaged 11 ppm.

Performance controls and blood sampling procedures

The live weight of the rabbits was recorded on arrival and then every week while health control and feed intakes were checked on a daily basis.

At 71 and 85 days of age the rabbits were submitted to a blood sampling by intracardiac puncture at the same time in the morning after a fasting period of three hours. Each blood sample, collected in tubes containing 150 USP lithium heparin, was immediately centrifuged for 15 minutes at 3000 rpm and the plasma frozen at -20°C until assayed. Testosterone (T), dihydrotestosterone (DHT) were quantified in plasma by RIA as described by BERGER *et al.* (1976). Triiodothyronine (T3), thyroxine (T4) and cortisol (C) plasma levels were evaluated using specific and standardized RIA procedures based on kits (Cambridge Medical Technology, USA) which have been validated for use in rabbits. The intra- and inter- assay coefficients of variations for each assay were within 6.1 - 12.5 %. All measurements were made in duplicate and only the values included in the limits of each method were retained.

Statistics

All the data were submitted to variance analysis, using the model I and the package of HARVEY (1990) as follows :

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + \in_{ijk}$$

Where :

Y_{ijk} = experimental data
μ = overall mean
T_i = fixed effect of i^{th} environmental temperature (i = 1, 2)
A_j = fixed effect of j^{th} age (j = 1, 2)
TA_{ij} = effect of interaction
$\in_{ijk}$ = residual random effect

Correlations and regression equations relating to some productive performance (daily gain, DG, and digestible energy intake per unit of metabolic weight, DEI/MBW) and the hormonal plasma concentrations were also calculated.

RESULTS AND DISCUSSION

As no interaction effect between temperature and age was statistically significant, the tables report only the main effects of the treatments.

Table 1 : Productive performance

		Temperature		Age		Error variance *
		TNT	HST	71 days	85 days	
Animals	*n*	*20*	*20*	*20*	*20*	
Initial body weight	g	936	931	917	950	3280
Final body weight	g	2555^b	2325^a	2181^a	2698^b	36393
Metabolic body weight	kg	1.52^b	1.44^a	1.39^a	1.57^b	0.0049
Weight gain	g/d	38.1^b	32.7^a	35.1	35.7	20.89
Feed intake	g/d	126^b	106^a	109^a	123^b	192
Feed/gain ratio	g/g	3.31	3.25	3.12^a	3.44^b	0.0478

a, b : Means within treatment with different superscript are significantly different (P<0.01) ; * : 36 DF.

The absence of interaction effects also indicates that heat stress affects similarly performance and the hormonal profile for both ages of rabbits considered.

Productive performance

Table 1 gives the growth performance of the rabbits during the productive cycle examined. The evaluation of these data is important because of the influence of the body development on variation of hormonal profile of the animals. The two ages considered are relating to two different meat production cycles ; moreover at these ages considerable variations may be occurred on endocrine status (BERGER *et al.*, 1976 ; BERGER *et al.*, 1979 ; BERGER *et al.*, 1982 ; CHIERICATO, 1984).

With reference to temperature effects (Table 1), the values are very different for animals reared at the two thermic levels. The subjects kept at 27°C had final body weights, metabolic body weights, daily weight gains and feed intakes significantly (P<0.01) lower than those reared in neutral thermic conditions. The feed/gain ratio was similar for the two experimental groups.

The age of animals significantly (P<0.01) affected the final body weight, the metabolic body weight and the daily feed intake. The daily live weight gain was similar for both age groups of rabbits. The feed/gain ratio also differed significantly (P<0.01) in the two ages, reaching the highest values in the 85 day subjects with respect to the 71 day-old rabbits. The worsening of feed/gain ratio with increasing age is well known and it has already been observed in other trials conducted on rabbits (CHIERICATO *et al.*, 1992 ; PARIGI-BINI *et al.*, 1992).

Considering the feed intake per unit of metabolic weight (Table 2), the environmental temperature markedly affected nutrient intakes of TNT subjects which ingested significantly more (P<0.01) dry matter, digestible energy, crude protein and starch

Table 2 : Intake of the main nutrients.

		Temperature		Age		Error variance *
		TNT	HST	71 days	85 days	
Animals	*n*	*20*	*20*	*20*	*20*	
Daily intake of :						
Dry matter	g/MBW	74.0^b	65.5^a	69.8	69.7	35.34
Digestible energy	kJ/MBW	782^b	692^a	738	736	3954
Metab. energy	kJ/MBW	755^b	668^a	712	711	3679
Crude protein	g/MBW	12.7^b	11.3^a	12.0	12.0	1.04
Ether extract	"	2.57^b	2.27^a	2.42	2.42	0.0427
Crude fiber	"	11.5^b	10.1^a	10.8	10.8	0.85
N-free extract	"	41.7^b	37.0^a	39.4	39.3	11.26
Starch	"	13.4^b	11.9^a	12.6	12.6	1.16
Ash	"	5.55^b	4.91^a	5.24	5.23	0.1985
NDF	"	21.2^b	18.7^a	20.0	19.9	2.89
ADF	"	14.9^b	13.2^a	14.1	14.1	1.44

a, b : means within treatment with different superscript are significantly different (P<0.01) ; * : 36 D.F.

Table 3 : Plasma level of some hormones (means ± standard deviations)

		Temperature		Age	
		TNT	HST	71 days	85 days
Animals	*n*	*20*	*20*	*20*	*20*
T	ng/ml	$1.96^{b} \pm 0.66$	$1.01^{a} \pm 0.38$	1.77 ± 0.62	1.19 ± 0.50
DHT	ng/ml	$0.73^{b} \pm 0.31$	$0.42^{a} \pm 0.16$	0.64 ± 0.28	0.52 ± 0.22
T/DHT		3.09 ± 0.61	2.28 ± 0.27	3.10 ± 0.64	2.27 ± 0.26
T + DHT	ng/ml	$2.66^{b} \pm 0.86$	$1.43^{a} \pm 0.48$	2.41 ± 0.75	1.68 ± 0.62
C	ng/ml	19.8 ± 8.04	18.0 ± 6.13	20.6 ± 8.91	17.3 ± 4.37
T3	ng/ml	$1.30^{B} \pm 0.19$	$1.01^{A} \pm 0.22$	1.17 ± 0.24	1.14 ± 0.26
T4	ng/ml	41.7 ± 5.60	40.3 ± 5.10	42.3 ± 5.80	39.7 ± 4.70
T4/T3		$32.7^{A} \pm 5.09$	$41.3^{B} \pm 8.14$	37.6 ± 8.90	36.4 ± 7.01

a, b, A, B : Means within treatment with different superscript are significantly different (P<0.05), (P<0.01).

than the HST animals.

On the contrary, there were no differences for any of the nutrients considered in relation to the age of the animals.

Androgen hormones

Considering the effect of ambient temperature, the HST group subjects (Table 3) had lower androgen levels than the TNT group. This decrease was significant (P<0.05) for T, DHT and T + DHT plasma concentrations, but not for the T/DHT ratio. These data agree with our previous findings for other genotypes of rabbit (BOITI *et al.*, 1992).

The androgen plasma levels were not significantly affected by age (Table 3). However, all values were numerically lower in the 85 day-old rabbits. These findings disagree with those reported by BERGER *et al.* (1979, 1982) and CHIERICATO (1984), who observed a significant increase of T, DHT and T/DHT plasma concentrations from about 80 days of age. However, given the important role of the nutritive level on the steroid plasma concentrations (CHIERICATO, 1984 ; BOITI *et al.*, 1992) these discrepancies must be considered in relation to the feeding plan adopted during the productive cycle of the rabbits. Probably, as a consequence of the higher intensive feeding plan adopted, the rabbits reached sexual maturity earlier.

The results of the present trial are also comparable to those obtained by other authors, even if a strict comparison is difficult especially for testosterone, given the well known pulsatile pattern and age-related secretions of these hormones (MOOR and YOUNGLAY, 1975 ; ROWE *et al.*, 1976). The values found are within the range reported in the literature (MOOR and YOUNGLAY, 1975 ; BERGER *et al.*, 1976 ; ROWE *et al.*, 1976 ; CHIERICATO, 1984 ; BOITI *et al.*, 1992).

Corticosteroid hormone

The plasma cortisol levels (Table 3) were significantly affected either by temperature or by age. The lack of effect of environmental temperature, while confirming our previous results (BOITI *et al.*, 1992), is not in accordance with the findings of TRAMMEL *et al.* (1989) who found decreased cortisol levels in heat stressed rabbits, reared, however, at temperatures different from ours and for a more limited period of time. In the present trial, probably, the rabbits had already overcome the initial alarm reaction stage induced by the high environmental temperature. This consideration on corticosteroid hormones is also confirmed by VERDE and PIQUER (1986). In the literature the effect of age on cortisol plasma levels is not well documented, but according to MORERA *et al.* (1991) cortisol plasma concentrations decrease in older rabbits. The number of studies in literature regarding the effects of temperature and age on cortisol plasma levels also appears to be limited for other farm monogastric species. In swine (MC CAULEY and HARTMANN, 1985 ; KIRKWOOD *et al.*, 1988) there is an increase of plasma cortisol in relation to low environmental temperature (RAFAI *et al.*, 1988). The cortisol values presented in Table 3, are comparable to the data obtained in previous trials conducted in Arkansas (TRAMMEL *et al.*, 1989) and in Italy (BOITI *et al.*, 1992).

Thyroid hormones

The heat stress induced a significant decrease in T3 plasma levels (Table 3). T4 concentrations presented a similar pattern but were not statistically significant. The T4/T3 ratio was significantly (P<0.01) lower in TNT than in HST rabbits.

The plasma concentrations of thyroid hormones were not significantly different in young rabbits compared to older subjects. The T4/T3 ratio did not differ significantly between the two ages.

The number of researches on plasma level rabbit thyroid hormones in relation to rearing temperature and age is very limited. Our findings differ from those of a previous study on the temperature effect (BOITI *et al.*, 1992) probably due to the different temperature level range tested in the experiments. Since the results reported in literature are not homogeneous (TRAMMEL *et al.*, 1989 ; MORERA *et al.*, 1991) further studies are needed with similar environmental conditions to be comparable.

Among the monogastrics, the farm species most studied in relation to the effect of environmental temperature and age on the thyroid hormone profile is poultry. As regards the effect of temperature, a decrease in T3 and an increase in T4 and in T4/T3 ratio were observed in chickens reared in increasing temperature conditions (SINURAT *et al.*, 1988). T3 plasma levels decrease and T4 increase with increasing age in chicks (KUHN *et al.*, 1982 ; LEENSTRA *et al.*, 1991). The thyroid hormone plasma values of the present research are in accordance withe those reported by other authors (TRAMMEL *et al.*, 1989 ; FEKETE and RUDAS, 1988 ; MORERA *et al.*, 1991).

The hormonal concentrations were correlated with productive performance. In Table 4 only the significant correlations and the relative linear regression equations are summarized.

The significant correlations only involved the thyroid hormones : DEI/MBW was positively correlated with T3 ($r = 0.749$, $P<0.01$) and negatively with the T4/T3 ratio ($r = 0.654$, $P<0.01$). The correlation between DEI/MBW and the hormonal plasma levels in Table 4 shows a direct relationship with T3 ($r = 0.624$, $P<0.01$) and a negative correlation with T4/T3 ratio ($r = -0.563$, $P<0.01$). These results agree with previous findings (BOITI *et al.*, 1992). Figure 1 illustrates the relationships between DG, DEI/MBW and the hormonal concentrations studied. They are similar to those obtained in a recent work (BOITI *et al.*, 1992), characterized, however, by experimental conditions rather different from those considered in this trial.

Table 4 : Linear regression equations (1) relating plasma T3 (y1), T4/T3 (y2), and DG (x1) and DEI/MBW (x2)

Linear regression equation	Residual Standard Error	r
y1 = 0.069 + 0.031 x1	0.2028	0.624**
y1 = - 0.715 + 0.003 x2	0.1719	0.749**
y2 = 68.009 - 0.877 x1	6.8131	- 0.563**
y2 = 88.740 - 0.070 x2	6.2355	- 0.654**

(1) Number of observations : 40 ; ** = P<0.01.

CONCLUSIONS

This experimental work adds knowledge to the study of the hormonal profile in relation to environmental and physiological conditions in the rabbit.

The absence of significant interaction effect between the rearing temperature and age indicates similarity of physiological and growth reaction to heat stress in the rabbits of both ages considered.

As regards the effects of temperature, almost all the growth performance were affected. In the heat stressed rabbits live weight, daily weight gain and feed intake were significantly lower with respect to the subjects kept in neutral thermic conditions.

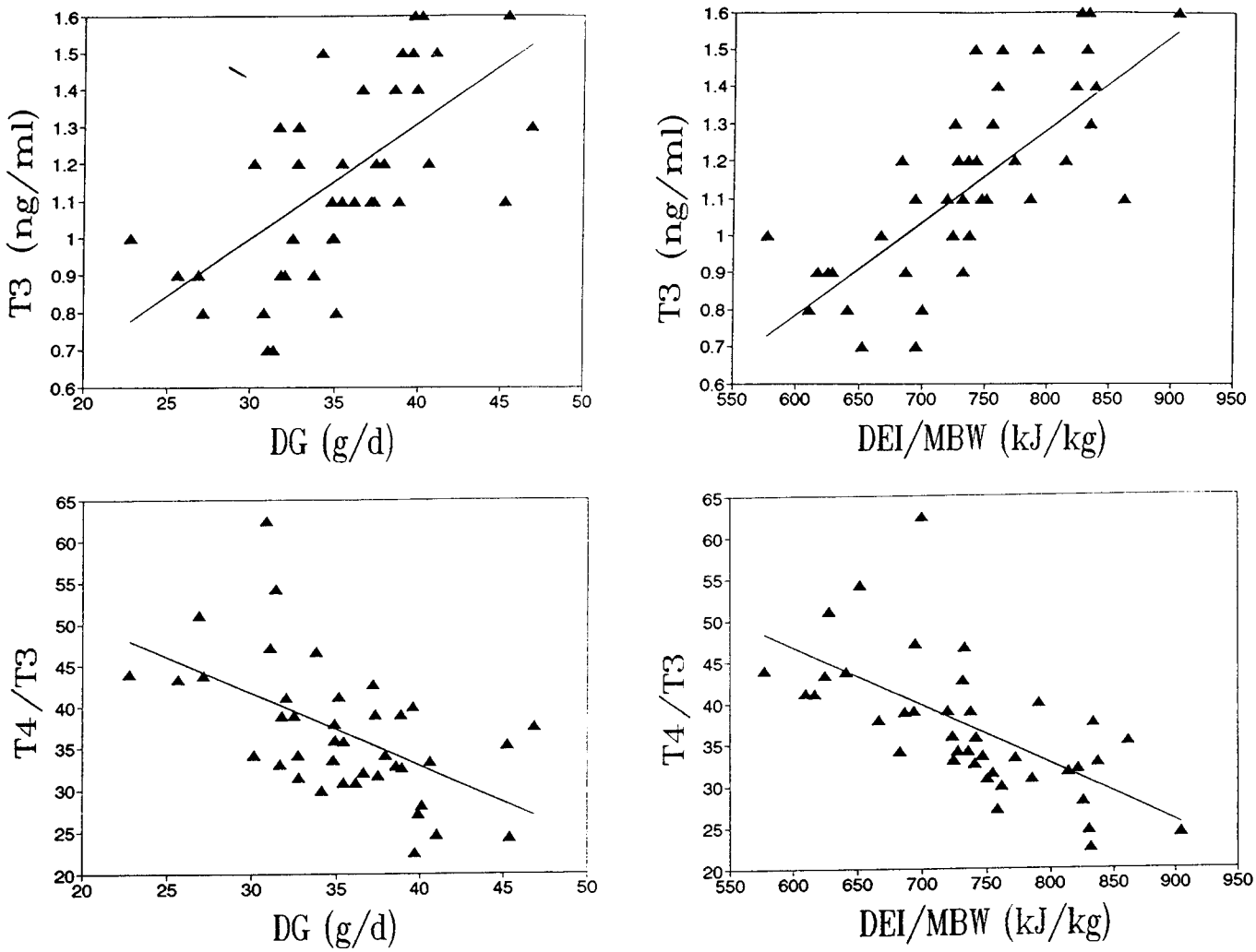
The effect of age on the productive performance resulted in higher live weight, daily feed intake and a consequent worsening of feed/gain ratio in older rabbits. The growth rate did not differ in the two ages.

With reference to hormone profile, the effect of temperature was considerable for androgen hormones, since T, DHT and T + DHT plasma levels were significantly lower in the animals kept at 27°C. These findings, together with the indications of a previous trial (BOITI *et al.*, 1992), show there are a relationships between androgen hormones, the nutritive level of the diet and the rearing temperature conditions that could be studied further, using a wider range of temperature levels. Cortisol plasma levels were not influence by the temperatures adopted. The environmental conditions also affected sensibly T3 concentrations which decreased with heat stress temperature, and the T4/T3 ratio which was significantly higher with the 27°c treatment. These results are in agreement with those reported in an other trial (BOITI *et al.*, 1992).

Only age had a tendency to influence all the plasma values, probably due to the limited time interval studied. This consideration regards all the variables studied : T, DHT and their ratio and sum, C, T3, T4 and T4/T3 ratio. These findings, supported by those of previous research (CHIERICATO, 1984) suggests it would be opportune to study the effect of feeding plans and their possible interactions with age on the androgen plasma concentrations in rabbits.

The plasma concentration of some hormones resulted correlate with some performances, particularly DG and DEI/MBW. Significant correlations were recorded for thyroid hormones : T3 was positively correlated with DG and DEI/MBW, while a negative relationship existed between T4/T3 ratio and these performance traits.

Figure 1 : Relationships between T3, T4/T3 ratio and DG or DEI/MBW.



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