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EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURE ON PLASMA TESTOSTERONE, CORTISOL, T3 AND T4 LEVELS IN THE GROWING RABBIT

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ABSTRACT

The study was carried out on 54 hybrid male rabbits, 50 days old, weighing 1159 ± 112 g. The rabbits were maintained for 57 days in individual cages with a commercial pellets diet and water ad libitum, housed in two areas characterized by high (H) and low (L) temperatures of 30 and 12°C respectively with 60-70% relative humidity and natural photoperiod of 8 h light and 16 h darkness. At the end of the trial three blood samples were taken from each animal every other day by intracardiac puncture. The group L rabbits were significantly affected ($P<0.01$) by the low ambient temperature and showed an average daily weight gain of 36.6 g/d, 43.5% higher than that of group H and a higher final live weight (3227 vs 2604 g, $P<0.01$). Also, the daily feed intake of dry matter per unit of metabolic weight (MBW) was significantly higher in the rabbits of group L (86.22 vs 59.94 g/$P^{0.75}$, $P<0.01$). Mean plasma testosterone (T), triidotyronine (T_3) and thyroxine (T_4) concentrations were all significantly influenced by envirnonmental temperatures ($P<0.01$). T and T_3 were higher (10.74 ng/ml, 164 ng/dl) in group L rabbits than in group H animals (1.54 ng/ml, 100 ng/dl) respectively. On the contrary, T_4 was significantly lower in group L (2.75 vs 3.86 µg/dl, $P<0.01$). The mean T_4/T_3 ratio was almost double in animals raised in the hot environment compared to those of group L (0.0396 vs 0.0167, $P<0.01$). The mean cortisol levels (about 1.1 µg/dl) were similar in both groups. A significant ($P<0.01$) positive correlation between T, T_3 and ADG and feed intake of digestible energy per unit of metabolic weight was found. T_4 and the T_4/T_3 ratio had opposite tendencies.

INTRODUCTION

Several studies have reported the influence of season and temperature on the secretion of hormones in farm animals. However, information on the hormonal response of rabbits to the ambient temperature are still rather limited and often contradictory.
Testosterone concentrations tend to be lower in the summer than in winter months (Moor and Yuonglai; 1975). Engel (1989) also, observed an increase in the weight of testicles and

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higher concentrations of plasma testosterone in half-wild European rabbits in the period between December and July. Carson and and Anam (1972) also found similar seasonal effects in New Zealand White rabbits. By contrast, Yan et al. (1985) did not find any significant androgen variations in Angora rabbits raised in environments with temperatures varying from 28 to over 30°C.

The effects of elevated ambient temperatures on plasma cortisol have been investigated by Trammel et al. (1988) in pregnant does raised at temperature of 16.8 and 32.2 °C. These authors observed decreasing cortisol levels in the group of does exposed to the higher temperature. Similar patterns were found in other monogastric (1, 15) and polygastric (10) species exposed to elevated ambient temperatures.

The effects of environmental temperature on plasma levels of triiodiotyronine (T_3) and thyroxine (T_4) have been studied in female rabbits at the University of Arkansas (17). According to this report the high ambient temperature decreased the plasma levels of T_4, whereas those of T_3 remained almost unvaried. Different results have been reported by other authors in rats (8), chickens (12) and ruminants (10, 14) subjected to heat stress or hot climate.

The present study was undertaken to characterize the changes in growing rabbits as regards testosterone, thyroid hormones and cortisol induced by different environmental temperatures, so continuing our previous research (4,5).

MATERIALS AND METHODS

The study was carried out on 54 male rabbits aged 50 days with initial live weight of 1159 ± 112 g. The rabbits, of the same commercial four-way hybrid, were raised during the pre and post-weaning period under the same rearing conditions and nutritional plan.

The rabbits, immediately upon arrival, were randomly housed in two different areas characterized by high (H) and low (L) temperatures of about 30 and 12°C respectively with 60-70% relative humidity.

Both the ambient temperature and humidity were constantly monitored and recorded by a thermohydrograph. The rabbits were exposed to a natural photoperiod of 8 h light and 16 h darkness.

The animals were then maintained for 57 days in individual farm-type cages with a commercial diet in pellets and water ad libitum. They were daily monitored for health conditions and their feed intake was also recorded on a daily basis, while their weight was controlled once a week.

At the end of trial period three blood samples were taken from each animal every other day by intracardiac puncture. The rabbits were always bled at the same time in the morning after a fasting period of 2 hours. Each blood sample, collected in test tubes containing 150 USP lithium heparin, was then immediately centrifuged for 15 minutes at 3000 rpm. The plasma samples thus obtained were frozen at -20 °C until assayed.

The plasma concentrations of testosterone - dihydrotestosterone(T), triiodiotyronine (T_3), thyroxine (T_4) and cortisol (C), were determined by radioimmunoassays with

commercial kits obtained from Amersham (UK) and Cambridge Medical Technology (USA). The intra- and interassay coefficients of variations for each assay was 6,1-12.5%.

The research protocol was planned according to a one-factor (temperature) two-level (high and low temperature) model. After ascertaining the existence of analogous variance patterns, the average values of the data relating to the three samples were subjected to a one-way statistical variance analysis (16) according to the "package" model as proposed by Harvey (1990). The correlations and regressions relating to certain field performances and the hormone levels in the plasma were also calculated.

RESULTS AND DISCUSSION

a) Animal performance during the growing period.

The overall animal performance observed during the growing period for each treatment group together with the daily intake of the main chemical components of the diet are summarized in Table 1.

The rabbits of group L were significantly affected ($P<0.01$) by the low ambient temperature and showed an average daily weight gain rate (ADG) of 36.6 g/d, 43.5% higher than that recorded for the rabbits of group H. Therefore, the final live weight of the animals raised in the cold environment was higher than those in group H (3227 vs 2604 g, $P<0.01$).

The low temperature induced a significant ($P<0.01$) increase in the dry matter daily feed intake which reached 86.22 g/$P^{0.75}$ in the rabbits of group L as opposed to only 59.94 g/$P^{0.75}$ observed in group H animals.

The environmental temperature, also significantly affected the feed conversion efficiency ($P<0,01$) which was 4.24 and 4.76 g/g for the rabbits of group H and L respectively. In the latter case, the considerable worsening of the feed efficiency ratio could be due to the energy needs for thermoregulatory purposes, as well as to a greater adipogenesis based on their greater final live weight.

Comparing the two groups, the group L animals had a higher daily feed intake of the chemical components of the diet of about 45% ($P<0.01$). Thus the intake of digestible energy per unit of metabolic body weight (DEI/MBW) was 1126 vs 778 kJ/$P^{0.75}$. Similarly, protein intake was 15.44 vs 10.66 g/$P^{0.75}$, N-free extracts 44.66 vs 30.84 g/$P^{0.75}$ and crude fibre 13.10 vs 9.05 g/$P^{0.75}$.

The above listed differences indicate that ambient temperature may have significant effects on the growth of farm animals by greatly modifying their feed intake levels .

It is not surprising, therefore, that the hormonal picture of the rabbits was also influenced to a considerable extent by the environmental conditions.

b) Testosterone-dihydrotestosterone.

Androgen concentrations (Table 2) were greatly influenced by treatment as they were significantly ($P<0.01$) lower (1.54 ng/ml) in group H rabbits than in group L animals (10.74

ng/ml).

These results, while in agreement with those reported by Moore and Yuonglai (1975), are higher than those described by Berger et al.(1982). However, this disparity of results may presumably be due to the different experimental conditions in evaluating the hormonal alterations during heat stress (prolonged seasonal heat effects versus short/mid-term temperature modifications in laboratory environmental chambers, using rabbits of different ages, sex and breeds).

The higher plasma androgen levels found in subjects raised at low temperatures (group L) are certainly related to the stimulating effect of the prolonged cold environment on the feed intake. The positive effect of diet on total androgens was in fact clearly demonstraded by Chiericato (1984) in growing rabbits raised at 18-20 °C and fed with different amounts of metabolizable energy.

The total androgen levels in the two groups of animals are also positively related to their different final live weight. These results confirm those of Berger et al. (1982) who also observed a positive correlation between the body weight, the testicle and seminal vesicle weight and testosterone levels in growing rabbits.

These positive relationships between feed intake of digestible energy (DEI/MBW), ADG and T plasma levels of the two groups of rabbits are clearly illustrated in Figure 1. Table 3 lists the correlation coefficients and the linear regressions equations relating total plasma androgens (T) to ADG and DEI/MBW.

Finally, a direct effect of the environmental temperature on androgens of rabbits cannot be ruled out, since seasonal depression of fertilty, associated with hot ambient temperatures, has been described in several mammallian species (17). The high ambient temperature may directly or indirectly inhibit the secretion of gonadal stimulating hormones, thus affecting the reproductive functionality of the rabbits as accounted for by the marked decrease of the T plasma levels observed in the young male rabbits under our experimental conditions or in adult female by the drop in the concentrations of the plasma estriol (17).

c) Triidotyronine, tyroxine and cortisol.

As shown in Table 2, the mean plasma levels of triidotyronine were considerably lower ($P<0.01$) in group H (100 ng/dl) than those in group L animals (164 ng/dl). On the contrary, the mean T_4 plasma levels were significantly higher in the same group (3.86 μg/dl vs 2.75 μg/dl, $P<0.01$). The mean T_4/T_3 ratio also was almost double in animals raised in the hot environment compared to those of group L (0.0396 vs 0.0167, $P<0.01$).

The T_3 levels are comparable to those obtained by Trammel et al. (1988) in mature does and by Fekete and Rudas (1988) in growing male rabbits. The T_4 levels were also within the range of those reported for the young rabbits (7), but were much higher than those found by Trammel et al. (1988).

Furthermore if it is not always easy to assess precisely the thyroid function on the basis of only T_3 and T_4 plasma concentrations these results are partly unexpected, because a reduction of the function in relation to high environmental

temperatures is a common and well documented finding. It should, however be remembered that T_3 and T_4 plasma levels, which are mainly regulated by TSH and by a feedback mechanism from the same hormones, are also modulated by many other factors (season, photoperiod, environmental temperature, feed intake, stress, etc.). The rabbits when raised for a long period at ambient temperature, outside their thermoneutral zone and above (or below) their critical temperature, maintain homeothermy by primarily reducing (or increasing) their food intake. Therefore, the lower T_3 plasma concentrations may well reflect the hormonal response of group H rabbits to prolonged heat exposure and to the marked decrease in feed intake. In fact, during prolonged heat exposure both anabolic and catabolic hormone secretions are depressed thus protecting the animals from excessive protein catabolism and energy loss.

Similar results were also obtained by Nelson and Zimmermann (1989) in chickens under similar experimental conditions. Other recent studies (13) on the same species, show that the increase in energy levels of feed rations was associated with a significant rise in T_3 levels and a drop in T_4 levels and consequently a reduction in the T_4/T_3 ratio.

The relationships between thyroid hormones, food intake and growth rate are listed in Table 3 and illustrated in Figure 1. The linear regression analyses of plasma T_3 levels showed a positive and highly significant correlation ($r=0.730$, $P<0.01$) both with the digestible energy intake per unit of metabolic weight ($r=0.697$, $P<0.01$) and the average daily weight gain. On the contrary there was a negative correlation between T_4/T_3 ratio and intake of DEI per unit of MBW ($r=-0.707$, $P<0.01$) and ADG ($r=-0.629$, $P<0.01$).

The mean cortisol levels (1.1 μg/dl) were very similar in both groups of rabbits irrespective of the environmental temperature at which they were raised. The cortisol levels found are in agreement with those reported by Trammel et al. (1988), but under our experimental conditions, we were unable to confirm any decrease of the cortisolemia in relation to high ambient temperatures, as described by this author.

The absence of any significant difference in the cortisol levels between the two groups, together with the observed values, very close to those found in untreated and unstressed animals, suggests that they had probably overcome the initial alarm reaction stage evoked by the ambient temperatures.

Verde and Piquer (1986) also found increasing concentrations of corticosteroids in growing rabbits kept at temperatures of about 30°C, only during the first days of exposure to heat. Thereafter they observed a progressive decline in corticosteroid levels down to basal values.

In conclusion the temperatures employed had their greatest impact in terms of a clear differentiation of the productive performance of the two groups of animals. Those raised at an ambient temperature of 12°C had, in fact, a growth rate and a food intake about 45% higher than those bred at about 30°C.

Under our experimental conditions the influence of temperature could be caused by the drastic and prolonged change in the nutritive levels influencing the rabbit growth rate and hormonal balance. So the rabbits which benefited from higher energy rations had considerably higher testosterone and T_3 plasma levels. A positive correlation between the plasma levels of both hormones and growth rate and the food intake was found.

T_4 and the T_4/T_3 ratio had opposite tendencies, whereas no differences were found with reference to cortisol.

The results obtained underline the importance of adequate feeding programmes for rabbits at the pre-puberty stage, particularly if bred at high temperatures to achieve an adequate hormonal and therefore sexual maturity.

These results provide us with indications for further in-depth experiments. In view of the current lack of available information, the most important is the extension of this type of research to other temperatures, sexes and genetic types of rabbits. In this framework the manipulation of feeding programmes appears to be particularly interesting since it could, at least under certain conditions, permit us to isolate the effects of temperature "per se" compared to the combined effect of changes in the intake of nutrients.

Another interesting line of research emerges from the results of this experiment: the verification of the hormonal response to environmental temperature over time. In seems particularly interesting to look to possible phenomena of homeostatic adjustment of the endocrinological response, with particular reference to those hormones more directly involved in stress reaction processes.

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Table 1: Average live weight, daily gain and feed efficiency

		H	L	Error mean square (1)
Animals	n	25	29	
Initial live weight	g	1161	1156	12680
Final live weight	"	2604^{A}	3227^{B}	49682
Daily live weight gain	"	25.5^{A}	36.6^{B}	17.3568
Feed efficiency	g/g	4.24^{A}	4.76^{B}	0.1882
Daily intake:				
- dry matter	g/P^{0,75}	59.54^{A}	86.22^{B}	30.9278
- digestible energy	KJ/P^{0,75}	778^{A}	1126^{B}	5256
- metabolizable energy	"	748^{A}	1083^{B}	4858
- crude protein(Nx6,25)	g/P^{0,75}	10.66^{A}	15.44^{B}	0.9906
- ether extract	"	3.54^{A}	5.13^{B}	0.7077
- N-Free extract	"	30.84^{A}	44.66^{B}	8.1689
- crude fibre	"	9.05^{A}	13.10	0.7077
- ADF	"	11.28^{A}	16.34^{B}	1.1206
- NDF	"	22.78^{A}	32.97	4.8820
- Ca	"	0.85^{A}	1.24^{B}	0.0067
- P	"	0.36^{A}	0.42^{B}	0.0011

(1) D.f. = 52; A,B; P<0,01

Table 2: Plasma T, C, T_4, T_3 and T_4/T_3 levels

		H	L	Error mean square (1)
Animals	n	25	29	
T	ng/ml	1.54^{A}	10.74^{B}	30.1362
C	µg/dl	1.10	1.09	0.7206
T_4	"	3.86^{B}	2.75^{A}	2.1894
T_3	ng/dl	100^{A}	164^{B}	700.7459
T_4/T_3		0.0396^{B}	0.0167^{A}	2.1894

(1) D.f.= 52; A,B; P<0,01

Table 3: Linear regression equations(1) relating plasma T (Y_1), T_3 (Y_2), T_4/T_3 (Y_3) to ADG (X_1) and DEI/MBW (X_2)

Linear regession equations	Residual S.D.	r
$Y_1 = -7.45 + 0,41\ X_1$	4.12	0.564**
$Y1 = -10.62 + 0.02\ X_2$	3.87	0.633**
$Y_2 = +9.79 + 4.01\ X_1$	28.15	0.697**
$Y_2 = -10.62 + 0.15\ X_2$	26.80	0.730**
$Y_3 = 68.18 - 1.18\ X_1$	10.63	- 0.629**
$Y_3 = 74.04 - 0,0498\ X_2$	9.61	- 0.707**

(1) Number of observations = 46; ** P<0,01

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Figure 1. Relationships among T, T_3, T_4/T_3 and ADG or DEI/MBW