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PLASMA ENDOCRINE PROFILE OF RESTRICTED FEMALE RABBITS DURING PREPUBERTAL AGE

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ABSTRACT

A trial was conducted to study the plasma endocrine profile of restricted female Provisal rabbits during prepubertal age. The animals (98 days of age) were reared in individual cages: control group pellet food was fed *ad libitum* (C group) and the restricted group (R group) was fed 50% ad libitum. After 21 days of experimental feeding, blood samples were taken from each rabbit after two hours' fasting. Feeding restrictions negatively influenced ($P<0.01$) productive performance (body gain, food intake and feed efficiency). The restricted rabbits presented lower ($P<0.01$) T3 (0.73 vs 1.02 ng/dl), and higher ($P<0.01$) FT4 (4.11 vs 2.93 ng/ml) plasma concentrations. T4 (5.20 μg/dl) and FT3 (2.91 pg/ml) did not change. Plasma progesterone (0.27 ng/ml) did not significantly change and testosterone decreased ($P<0.01$) in R rabbits (0.10 vs 0.18 ng/ml). Feeding restrictions significantly ($P<0.05$) affected plasma cortisol level (13.25 vs 17.50 ng/ml).

INTRODUCTION

The effects of under nutrition on reproductive processes occur at the different steps of the animal life: puberty, ovulation, pregnancy. Indeed, under nutrition alters all regulatory levels of the reproductive function (hypothalamus-pituitary-gonad), though mechanisms may differ according to physiological stage and species.
Adaptation of animals to under nutrition results from numerous digestive, metabolic and endocrine changes, whose relative importance depends largely on the severity and the duration of the feed restriction.
Effects of feed restrictions in female rabbits have been studied mainly in terms of reproductive performance (FORTUN - LAMOTHE, 1998; XICCATO et al., 1999). There is little knowledge of the effect of feeding levels during the growing phase (MAERTENS, 1992), in particular on plasma metabolic profile (CHIERICATO et al., 1999) and on endocrine status of prepubertal rabbits. In this paper, we have examined the changes in circulating concentrations of thyroid, sexual and corticosteroid hormones in female rabbits, which were feed-restricted during prepubertal phase.

MATERIALS AND METHODS

The research was carried out on 36 female rabbits (98 days old). The animals were 4-way crossbred type (Provisal) and they weighed 2750 ± 150 g. During the experimental period, the rearing temperature was 21°C and the relative humidity averaged 80%. The photoperiod was 16L:8D, and the light intensity was 25 lux. Temperature and relative humidity were continuously recorded by a thermohygrograph (TIG-ITH, LSI) and light intensity was daily checked by a silicon sensor lux-meter (HD 8366 Delta Ohm).
The animals, reared in single cages, were fed a commercial pelleted feed; the control group (Ad libitum group, 18 rabbits) was fed *ad libitum*, the restricted group (Restricted group, 18 rabbits) received about 50% of *ad libitum* feeding intake. Water was freely administered to both groups. The commercial feed was submitted to chemical analysis according to the

official methods (AOAC, 1990). Starch, NDF and ADF content were also evaluated. The concentration of digestible energy was calculated using the equations of PARIGI BINI and DALLE RIVE (1977).
After 21 days of trial, at morning all the rabbits after a fasting of two hours were submitted to a blood sampling by the marginal vein of the ear. Each blood sample, collected in vacutainers with lithium heparin (140 USP), was immediately centrifuged (3500 rpm for 15 minutes) and the plasma frozen at - 20°C until assayed. Triiodothyronine (T3), thyroxin (T4), free triiodothyronin (FT3), free thyroxin (FT4), and cortisol plasma concentrations were evaluated by RIA using kits (ORTHO-Clinical Diagnostic, Jonhson & Jonhson). Testosterone was quantified in plasma by RIA as described by BERGER et al. (1976). The intra- and inter- assay coefficients of variations for each assay were within 8%. All measurements were made in duplicate and only the values included in the limits of each method were retained.
All the data were submitted to analysis of variance (SAS, 1990), using the following model:
$Y_{ik}=\mu+R_i+\varepsilon_{ik}$
where
Y_{ik}= experimental data
μ= overall mean
R_i= fixed effect of feeding level (i=1,2)
ε_{ik}= residual random effect

RESULTS AND DISCUSSION

In table 1 the formulation and the chemical composition of the commercial pellet given to the animals are presented. The food had chemical-nutritional characteristics capable to completely satisfy the requirements of growing rabbits.
The young females presented (Table 2) a different growth rate in relation to the daily feed intake. The feed restricted rabbits, which ingested about 48% of *ad libitum* quantity (80 vs 165 g/d, $P<0.01$) showed a negligible weight gain in comparison to subjects fed to appetite (0.62 vs 29.09 g/d, $P<0.01$). The absence of body gain emphasises that this experimental feeding restriction gave to the animals only the nutrient supply for the maintenance.
As consequence, restricted rabbits showed significantly lower final body weight in comparison to *ad libitum* group (2758 vs 3369 g, $P<0.01$). A comparison between the conversion indices results as being difficult, in that the data regarding the feed restricted animals is scarcely realistic and has not been reported in the table. It can only be noted that the animals fed to appetite presented, relative to the increased final live weight, particularly high final value (5.67 g/g).
As concerns the thyroid hormones, reported in table 3, T3 circulating levels significantly decreased in feed restricted animals (0.73 vs 1.02 ng/dl, $P<0.01$); the same trend was observed on plasma T4, even if at not significant level (5.61 vs 4.79 μg/dl).
The lack of indications regarding the rabbit species on this topic, make it difficult to compare our results to those of other authors. Our findings are in agreement with those of YAMBAYAMBA et al. (1996) and CHILLIARD et al. (1998), who observed a decrease in plasma levels of T3 and T4 in other zootechnical species submitted to under nutrition conditions. This response may be attributable to a limitation of excessive mobilisation of body fat and protein.
Feed restriction exerted a negative but not significant influence on FT3 concentrations (2.71 vs 3.11 pg/ml), whereas FT4 levels were lower in full-fed rabbits in comparison to the restricted animals (2.93 vs 4.11 ng/ml, $P<0.01$). It is well remember that also in other

mammals in under nutrition condition FT4 concentrations may be increased somewhat owing to decreased T4 carrier protein availability (SCHUSSLER, 1986).

Table 1. Percentage and chemical composition of the diet

Ingredient			Chemical composition [(b)]		
Dehydrated alfalfa meal	%	41.0	Dry matter	%	90.83
Corn meal	%	16.0	Crude protein	%	17.73
Wheat middlings	%	12.0	Ether extract	%	4.63
Dried beet pulp	%	8.0	Crude fiber	%	17.44
Roasted soybean seeds	%	5.0	Ash	%	8.49
Soybean meal	%	5.0	N- free extracts	%	51.71
Sunflower meal	%	4.0	NDF	%	21.71
Molasses (sugar beet)	%	4.0	ADF	%	15.15
Milk whey	%	3.0	Ca	%	1.21
Dibasic calcium phopshate	%	1.0	P	%	1.07
DL-methionine	%	0.1	Digestible energy	MJ/kg	9.59
Premix [(a)]	%	0.9			

[(a)]Premix provided per kilogram of complete diet: vit. A 7000 I.U., vit. D3 1000 I. U., Vit. E 50 mg, vit. K 1.60 mg, vit. B2 2.40 mg, vit. B6 2 mg, vit. PP 50 mg, vit. B12 0.01 mg, folic acid 3.20 mg, D-pantothenic acid 20 mg, Cu 20 mg, I 1mg, Co 1 mg, Fe 100 mg, Mn 10 mg, Zn 104 mg, Se 0.10 mg, Flavofosfolipol 2 mg.
[(b)]Dry matter basis

Table 2. Productive performance

		Ad libitum	**Restricted**	Error mean square*
Live body weight:				
- Initial	g	2758	2745	58254
- Final	g	3369[A]	2758 [B]	59120
Weight gain	g/d	29.09 [A]	0.62 [B]	41.7014
Feed intake	g/d	165 [A]	80 [B]	0.56
Feed efficiency	g/g	5.67	-	-

[A, B] : $P<0.01$
* 34 degrees of freedom

Table 4 presents the circulating levels of some sexual steroid and corticosteroid hormones. The level of feeding did not influence plasma progesterone (0.27 ng/ml). Other authors (BURNS et al., 1997) have not observed significant changes in progesterone plasma levels

studying the effect of energy restriction on other mammals in physiological conditions however different from ours.

Table 3. Thyroid hormones

		Ad libitum	**Restricted**	Error mean square*
T3	ng/dl	1.02 ^{A}	0.73 ^{B}	0.0312
T4	µg/dl	5.61	4.79	1.3252
FT3	pg/ml	3.11	2.71	0.1041
FT4	ng/ml	2.93 ^{B}	4.11 ^{A}	0.6260

$^{A, B}$: P<0.01
* 34 degrees of freedom

Testosterone significantly decreased in restricted female group in comparison to ad libitum group (0.10 vs 0.18 ng/ml, P<0.01).
Previous works reported that insufficient feed intake in male rabbits can induce a negative influence in plasma levels of sexual steroids (CHIERICATO, 1984)
Feeding level influenced cortisol circulating levels also, resulting in a significant difference between treatments (P<0.05), with lower values in underfed rabbits (13.25 vs 17.50 ng/ml). A negative effect on cortisol concentrations in feed restricted prepubertal animals was observed by BOOTH et al. (1994) on gilts and by STICKER et al. (1995) on horse mares in energy restriction conditions.

Table 4. Sexual steroid and corticosteroid hormones

		Ad libitum	**Restricted**	Error mean square*
Progesterone	ng/ml	0.33	0.21	0.0394
Testosterone	ng/ml	0.18 ^{A}	0.10^{B}	0.0041
Cortisol	ng/ml	17.50^{a}	13.25^{b}	8.5357

$^{a, b}$: P<0.05 ; $^{A, B}$: P<0.01
* 34 degrees of freedom

This work, alongside another previously published one, contributes to the knowledge of thyroid, steroid and corticosteroid plasmatic concentrations in prepubertal female rabbits.
In addition, the findings obtained in this trial allow some preliminary considerations on the effect of interaction between feeding level and endocrine status of female rabbits before starting their reproductive cycle.
A severe reduction of feed intake induces in the growing prepubertal rabbit a negative effect both on the growth rate both on the hormonal plasma profile. The animals in fact try to overcome the lower nutritional supply reducing in particular the energy utilisation of the diet by mean a mechanism involving mainly thyroid hormones and cortisol too. Present results show that under nutrition may alter productive and physiological functions of rabbits. In such physiological conditions the organism is not able to reach an adequate somatic development and to start her reproductive cycle. Further studies are needed to clarify the role of an adequate feeding level to allow the does to perform the best endocrine status.

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