

## THE EFFECT OF THE DIETARY ELECTROLYTE BALANCE ON THE PLASMA ENERGY, PROTEIN, MINERAL VARIABLES AND ENDOCRINE PROFILE OF PLURIPAROUS RABBIT DOES

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### ABSTRACT

In this research, the effect of the electrolyte balance (dEB, dEB=Na+K-Cl) on the plasma energy, protein, enzyme, mineral profile and on the endocrine response of rabbit does was studied. The animals, belonging to the Grimaud genotype, were at the third reproductive cycle. The rabbits were allocated to reproductive cages and divided into two groups to be fed two diets with different electrolyte balances (dEB1=27 meq/100g and dEB2=35 meq/100g). The diets were similar in ingredients, protein (CP=19.81% d. m.) and energy content (DE=2945 kcal/kg d. m.), whereas they had different sodium content (dEB1=0.21% d.m. and dEB2=0.38% d. m.). The animals were fed the two diets from first mating (20 weeks of age) until the litter weaning of the third reproductive cycle. From the mating of the third cycle the environmental temperature averaged 15°C and the relative humidity was about 79%. At about 39 weeks of age, five days after parturition, a blood sampling was performed on the does. Results pointed out that the two dEB levels did not significantly affect the body weight and feed intake of rabbit does. No significant dEB effect was observed on the main variables of plasma metabolic (glucose, triglycerides, cholesterol, total proteins, albumin and globulines), enzyme (aspartate-aminotransferase, alanine-aminotransferase,  $\gamma$ -glutamyl-transpeptidase), and mineral (Ca, P, K, Na, Cl) profile. The plasma concentration of 17 $\beta$ -oestradiol was similar between the two groups. Cortisol plasma level slightly increased in dEB2 rabbits. Also the triiodothyronine (T3) and thyroxine (T4) were unaffected by the dietary dEB level as well as the free fractions (FT3 and FT4). The results point out that a prolonged administration of a diet with an electrolyte balance of 35 meq/100 g does not affect the metabolic and mineral profile nor the oestradiol plasma level but tendentially increases the thyroid hormones of pluripare lactating rabbit does when they are in neutral thermal conditions.

**Key words:** rabbit, does, electrolyte balance, blood.

## INTRODUCTION

Literature reports give indications on the effect of the dietary electrolyte balance (dEB,  $dEB=Na+K-Cl$ ) on the productive performance and physiological status of some mammal animal species. Several research works on swine show physiological and nutritional effects of dEB in the diet (KEMME-KROONSBERG, 1992; DERSJANT *et al.*, 2002; BUDDE and CRENSHAW, 2003). In mice, a significant influence on the reproductive response of the animal was observed (MC BURNIE *et al.*, 1999).

Otherwise, little information on the effect of different levels of dietary dEB on the productive and reproductive performance in rabbit still exists (MATEOS and de BLAS, 1998; MC NITT *et al.*, 2000). A dietary dEB level of 35 meq/100g did not affect the reproductive performance of the rabbit does nor the growth response of the litter in comparison to a dEB of 25 meq/100g (CHIERICATO and RIZZI, 2003). Other authors (HABEEB *et al.*, 1997; MARAI *et al.*, 1999) studied the effect of different dEB content on the productive and physiological responses of the rabbit. Administration of water with high salinity induces variations on some plasma variables (HABEEB *et al.*, 1997). Furthermore, indications on the effects of dietary dEB on the endocrine status are numerically limited (HABEEB *et al.*, 1997; MARAI *et al.*, 1999).

The present work studied the effect of a dietary electrolyte balance ranging from 27 to 35 meq/100 g on the plasma metabolic and hormonal profile of pluripare does.

## MATERIAL AND METHODS

In this trial, 26 rabbit does (Grimaud genotype) were used. They were placed in single reproductive cages and divided into two groups (dEB1 and dEB2), to be fed *ad libitum* two diets with the same formulation (Table 1) and similar crude protein (19.81% d. m.) and energy content (Digestible energy=2945 kcal/kg d. m.), but with different electrolyte balances. The dEB1 diet was 27 meq/100 g and the dEB2 diet was 35 meq/100g. The dietary dEB was modified by adding sodium bicarbonate to the supplement.

**Table 1. Feed formulation (%).**

Alfalfa meal	21.00	Corn meal	7.00	Sodium chloride	1.50
Sunflower meal	20.00	Molasses	3.00	L-lisine	0.20
Wheat middlings	20.00	Wheat meal	2.66	DL-methionine	0.10
Wheat bran	12.00	Soybean	2.00	Supplement*	0.04
Beet pulps	7.50	Calcium carbonate	2.00		

\*Supplement per kg: vit. A 12000 U.I.; vit. D<sub>3</sub> 1500 U.I., vit. E 60 mg; vit. K<sub>2</sub> 1 mg; vit. B<sub>2</sub> 4.6 mg; vit. B<sub>6</sub> 2 mg; vit. PP 40 mg; vit. B<sub>12</sub> 0.02 mg; folic acid 5.5 mg; D-panthotenic acid 21 mg; Cu 10 mg; I 0.4 mg; Co 1 mg; Zn 80 mg; Fe 100 mg; Mn 11 mg; Se 0.10 mg.

The experimental diets were fed to the animals from first mating (20 weeks of age) until the third reproductive cycle, which lasted until litter weaning. After parturition all the litters were equalized at 8 kids. Throughout the third cycle the environmental

temperature averaged  $15\pm 1^{\circ}\text{C}$ , and the relative humidity was  $79\pm 10\%$ , whereas during the previous cycles the thermal-hygrometric conditions were  $26\pm 4^{\circ}\text{C}$  and  $74\pm 6\%$ , respectively. The photoperiod was 16L:8D, with a light intensity of 38 lux.

At the third cycle, at 39 weeks of age and about 5 days after parturition, the animals of each group were submitted to a blood sampling, early in the morning. The blood of each animal was collected in vacutainers containing lithium eparine (140 USP). The blood was centrifuged (3500 rpm at  $4^{\circ}\text{C}$  for 15') and the obtained plasma frozen at  $-20^{\circ}\text{C}$ , until assayed. The main variables of metabolic, enzyme and mineral profile were analyzed using automatized instruments, namely HITACHI 911 and kits (Roche BM). The procedures for assaying the biochemical profile and the plasma hormonal contents are reported in a previous research work (RIZZI *et al.*, 2004).

All the data were submitted to the analysis of the variance (SAS, 1990) following the model:  $Y_{ik} = \mu + D_i + e_{ik}$  where  $Y_{ik}$  = experimental data,  $\mu$  = overall mean,  $D_i$  = effect of the dietary electrolyte balance ( $i=1,2$ ),  $e_{ik}$  = effect of casual error.

## RESULTS AND DISCUSSION

The live body weight of the does after parturition at litter equalization, and the feed intake referring to the week after parturition until the blood sampling are in Table 2.

The rabbits of dEB2 group presented slightly higher weight and daily feed consumption in comparison to those of the dEB1 group, but the differences were not statistically significant. The intake of the main nutrients of the diet referred to units of metabolic weight did not differ between the groups, excepted ( $P < 0.01$ ) in sodium.

The dietary contents of crude protein, crude fibre, some minerals and digestible energy were able to meet the requirements of the animals (DE BLAS and MATEOS, 1998). Given the high quantity of drinking water consumed by the rabbit does during lactation demonstrates particularly important results in its chemical and physical properties. Analysis carried out on water samples gave values, in particular sodium (5.96 mg/l), potassium (2.58 mg/l) and chloride (12.47  $\mu\text{g/l}$ ) into the range accepted by literature (LEBAS *et al.*, 1998).

The main variables (Table 3) of energy metabolism (glucose, cholesterol, triglycerides) resulted unaffected by the dietary dEB. It was observed that cholesterol decreases during the second half of pregnancy and increases after parturition and in the first week of lactation (KRIESTEN and MURAWSKI, 1988; EL MAGHAWRY *et al.*, 2000).

Also total proteins, albumin and globulines plasma values resulted statistically similar between the groups. A significant increase in plasma total proteins occurs in the early stage of pregnancy and they decline in the later stages (EL MAGHAWRY *et al.*, 2000). Other authors stated that salt addition to drinking water (3000 mg/kg) induces a decrease in total protein plasma level in young rabbits reared under high environmental temperature (HABEEB *et al.*, 1997)

**Table 2. Body weight, feed intake, nutrients and energy intake.**

	dEB1	dEB2	Error Variance (24 d. f.)
Body weight (g)	4201	4281	123602
Feed intake (g/d)	336	355	725
<b>Intake of:</b>			
-crude protein (g/kg <sup>0.75</sup> )	19.63	20.41	2.3456
-crude fiber (g/kg <sup>0.75</sup> )	17.50	18.20	1.0923
-sodium (g/kg <sup>0.75</sup> )	0.25 <sup>B</sup>	0.46 <sup>A</sup>	0.0106
-potassium (g/kg <sup>0.75</sup> )	1.25	1.31	0.0124
-chloride (g/kg <sup>0.75</sup> )	0.41	0.41	0.0003
-digestible energy (MJ/kg <sup>0.75</sup> )	11.56	12.02	0.8067

A, B:P<0.01

**Table 3. Plasma levels of some metabolites.**

	dEB1	DEB2	Error Variance (24 d. f.)
Glucose (mmol/l)	6.97	7.14	0.2540
Cholesterol (mmol/l)	1.11	1.22	0.0450
Triglycerides (mmol/l)	0.67	0.72	0.1103
Total proteins (g/l)	54.72	54.73	29.1100
Albumin (g/l)	45.08	44.44	19.9899
Globulines (g/l)	9.63	10.29	3.0294

Table 4 shows plasma levels of some enzyme and minerals. The higher dietary dEB did not affect the enzyme activities studied nor the mineral concentrations. Indications from other researchers report that the plasma activities of AST and ALT increased significantly in rabbits in advanced pregnant status (EL MAGHAWRY *et al.*, 2000). Drinking tap water supplemented with high levels of NaCl (2000-5000 mg/kg) induced a rise of some blood electrolytes such as Na, K and Ca and P in growing rabbits particularly under hot summer conditions (HABEEB *et al.*, 1997).

**Table 4. Plasma levels of some enzymes and minerals.**

	dEB1	dEB2	Error variance (24 d. f.)
AST (U/l)	26.38	20.77	256.5120
ALT (U/l)	19.11	23.35	35.4123
?-GT (U/l)	4.68	4.50	5.0124
Calcium (mmol/l)	3.72	3.74	0.0254
Phosphorus (mmol/l)	1.37	1.46	0.0895
Sodium (mmol/l)	135	137	6.5023
Potassium (mmol/l)	5.28	5.13	0.1456
Chloride (mmol/l)	100	99	38.9543

The electrolyte balance of the diet exerted a limited influence on the hormonal response (Table 5) of the animals. The females which were fed a diet with a dEB level of 35 meq/100 g did not show any difference in plasma 17 $\beta$ -oestradiol when compared to the dEB1 does. It is worth remembering that plasma oestrogen levels increases during gestation and decreases before parturition (KRIESTEN and MURAWSKI, 1988). Also some management practices can influence the plasma oestrogen levels. UBILLA *et al.* (2000) observed a plasma oestradiol increase after keeping the does apart from their litter for a 48h prior to artificial insemination on day 11 of the lactation period.

Cortisol resulted higher ( $P<0.10$ ) in dEB2 animals in comparison to dEB1 does. Also cortisol plasma levels show variations during reproductive activity: it markedly increases at the end of the pregnancy and decreases before parturition (KRIESTEN and MURAWSKI, 1988). Furthermore, other authors observed a slight decrease of cortisol in pregnant rabbits fed on a basal diet containing chromium (SAHIN *et al.*, 1997).

As far as the thyroid activity is concerned, the plasma levels of T3 and T4 was not significantly influenced by the higher dietary dEB level. Also free T3 and T4 levels were similar between the groups. The T3 and T4 plasma levels in pregnant rabbits show a decrease in the first half of pregnancy and a rise near the end (EL MAGHAWRY *et al.*, 2000). In growing rabbits, drinking water with high level of NaCl (3000 ppm) decreased plasma T3 particularly under high environmental temperature (HABEEB *et al.*, 1997).

**Table 5. Plasma levels of 17 $\beta$ -oestradiol, cortisol and thyroid hormones.**

	dEB1	dEB2	Error Variance (24 d. f.)
17 $\beta$ -oestradiol (pg/ml)	34.97	32.72	184.1122
Cortisol ( $\mu$ g/dl)	0.32 <sup><math>\beta</math></sup>	0.47 <sup><math>a</math></sup>	0.0577
T3 (ng/dl)	168	163	618
T4 ( $\mu$ g/dl)	3.76	4.40	0.7072
FT3 (pg/ml)	4.81	5.53	0.9267
FT4 (ng/dl)	2.12	2.78	0.9221

a,  $\beta$ :  $P<0.10$

## CONCLUSIONS

The results obtained in this trial give first indications to the knowledge of the effects of dietary electrolyte balance on the response of reproductive female rabbits. On a physiological point of view a long-term feeding of two diets with different ratios among Na, K and Cl ranging from 27 to 35 meq/100g resulted tolerant by the animals. The higher dietary dEB did not affect significantly the metabolic, enzyme and mineral profile of lactating animals at third reproductive cycle. The endocrine status studied was slightly affected by increasing the dietary dEB.

These results are in agreement with the data obtained on the reproductive performance of the does (CHIERICATO and RIZZI, 2003) which presented similar reproductive

responses when fed diets with a dEB ranging from 26 to 35 meq/100g. Further investigations are needed to verify the effect of the dEB range tested in this trial in stress thermal-hygrometric conditions.

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