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## A STUDY ON THE REPRODUCTIVE PERFORMANCE AND PHYSIOLOGICAL RESPONSE OF RABBIT BUCKS FED ON DIETS WITH TWO DIFFERENT MINERAL CONTENTS

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### ABSTRACT

The reproductive performance and the plasma biochemical profile of rabbit bucks relating to two dietary electrolyte balances (dEB, dEB=Na+K-Cl) were investigated. Two pelleted diets were fed the male rabbits from the first mating (50 weeks of age) until the weaning of the litters of the third reproductive cycle. The diets were similar in composition and were isoproteic (crude protein=19.81% d. m.) and isoenergetic (digestible energy=2945 kcal/kg d. m.); the sodium content was 0.21 and 0.38% d. m. and the electrolyte balance was 27 meq/100g and 35 meq/100 g in the dEB1 and dEB2 diets, respectively. The higher sodium level in the diet was obtained by increasing the mineral content in the supplement of the feed. Matings occurred with natural insemination. The trial started in the summer period under a hot climate but the third cycle presented neutral thermal hygrometric conditions (temperature=15°C and relative humidity=79%). The photoperiod was 16L:8D. At the third cycle, at 70 weeks of age, the bucks were submitted to a blood sampling early in the morning. At the third reproductive cycle, the dEB2 diet did not significantly affect the body weight and the feed intake of the rabbits. The two experimental groups presented a similar number of kids born and born alive and number of kids/mating. Plasma glucose, triglycerides, cholesterol, total proteins, albumin and globulines resulted unaffected by the diets. The plasma enzyme activities (aspartate-aminotransferase, alanine-aminotransferase,  $\gamma$ -glutamyl-transpeptidase) and the mineral contents of calcium, phosphorus, potassium, sodium and chloride were similar between the groups. No significant difference was observed for plasma cortisol, even if the higher dEB level induced a tendential decrease in dEB1 group. T3 (triiodothyronine), T4 (thyroxine) and FT3 (free T3) were similar between the groups. FT4 (freeT4) slightly increased in the dEB2 rabbits. Results indicate that using daily rations with an electrolyte balance ranging from 27 to 35 meq/100 g continuously for three reproductive cycles did not exert any effect on the reproductive performance of the bucks nor on the biochemical profile.

**Key words:** rabbit, bucks, electrolyte balance, reproductive performance, blood.

## INTRODUCTION

The more recent knowledge of nutritional requirements for reproductive and growing rabbits (de BLAS AND MATEOS, 1998) permits to formulate adequate pelleted feed able to achieve good productive and reproductive performance.

The more favourable daily intake of the main macroelements calcium and phosphorus are reported in literature (MATEOS and de BLAS, 1998). Otherwise, the indications in sodium, potassium and chloride content of the daily ration are often referred to practical experience rather than to scientific reports, and no experimental result is available on the more adequate values of the dietary electrolyte balance (dEB,  $dEB=Na+K-Cl$ , meq/100 g) (MATEOS and de BLAS, 1998). It is worth remembering that the concentration of these three elements in the ration can widely vary relating to different mineral compositions of raw materials depending generally on the agronomic practices and on the environmental conditions where they grow.

Previous experiments carried out on monogastric species report significant effects of the dietary dEB on the performance and on the physiological status of sows (DERSJANT *et al.*, 2002; BUDDE and CRENSHAW, 2003) and chickens (JOHNSON and KARUNAJEEVA, 1985; OVIEDO-RONDON, 2001; BETANCOURT and ROMIERO, 2002) particularly under thermal stress conditions.

Literature gives few and no exhaustive indications on the effect of the dEB of the diet both for growing and reproductive rabbits (HABEEB *et al.*, 1997; MARAI *et al.*, 1999; BONSEMBIANTE *et al.*, 2004). Generally the dEB of rabbit pellet is 25 meq/100 g but more research is needed to know the effect of higher dEB levels (MATEOS and de BLAS, 1998; MC NITT *et al.*, 2000).

Recent results report that high dietary dEB level (35 meq/100 g) is tolerated by rabbit does: the treatment did not affect the reproductive performance of the animals nor the growth response of their litters (CHIERICATO and RIZZI, 2003).

The aim of this research work was to study the reproductive performance, the metabolic and the endocrine status of rabbit bucks being long-term fed on two diets with different electrolyte balances ranging from 27 to 35 meq/100 g.

## MATERIAL AND METHODS

The trial used 19 rabbit bucks of Grimaud genotype. The animals were divided into two groups (dEB1=9 animals and dEB2=10 animals); the dietary treatment started at first mating when the rabbits were 50 weeks old and lasted at the litter weaning of the third reproductive cycle.

Throughout the experimental period, two diets (dEB1 and dEB2 diets) were fed the animals adopting a slight restriction. The diets were similar in formulation and energy (DE=2945 kcal/kg d.m.), crude protein (19.81% d.m.), crude fiber (15.08% d.m.) and

mineral content, except the sodium content which averaged 0.21 and 0.38% d. m. in dEB1 and dEB2 diet, respectively. The electrolyte balance in dEB1 diet was 27 meq/100 g and in the dEB2 diet was 35 meq/100g.

Natural insemination was used. Each buck mated 5 to 7 does, once a week. The does were mated 12 days after parturition. Throughout the third cycle the environmental temperature was  $15.3 \pm 1.3^\circ\text{C}$  and the relative humidity was  $78.7 \pm 9.7\%$ . The photoperiod was 16L:8D with a light intensity of 38 lux.

At the third reproductive cycle (70 weeks of age), the animals from each group were submitted to a blood sampling, early in the morning. Each blood sample was collected in vacutainers containing lithium eparine (140 USP). The blood was centrifuged (3500 rpm at  $4^\circ\text{C}$  per 15') and the plasma was stored at  $-20^\circ\text{C}$  until the analysis. These were performed using automatized instruments HITACHI 911 (Roche BM) and kits (Roche BM) by means of the following methods: glucose (enzymatic UV, HK), cholesterol (CHOD, PAP), triglycerides (GPO, PAP), total protein (biuret), albumin (bromocresol), alanine aminotransferase (ALT) and aspartate-aminotransferase (AST) (IFCC,  $37^\circ\text{C}$ ),  $\gamma$ -glutamyl-transpeptidase ( $\gamma$ -GT) ( $\gamma$ -glutamyl-3C-p-N-anilide,  $37^\circ\text{C}$ ), calcium and phosphorus (o-cresolptalein), potassium, sodium and chloride (ISE).

The plasma hormonal levels of  $17\beta$ -oestradiol, cortisol and triiodothyronine (T3), tiroxine (T4), free T3 (FT3), free T4 (FT4) were evaluated by RIA using ICN Pharmaceuticals kits (INC, Costa Mesa, USA). All measurements were made in duplicate and only the values included in the limits of each methods were retained.

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

## RESULTS AND DISCUSSION

The experimental rations were similar to the commercial diets and based on similar parts of alfalfa meal (21%), sunflower meal (20%) and wheat middlings (20%), on wheat bran (12%), beet pulps (7.5%) and corn meal (7%). Also molasses, wheat and soybean as well as mineral integrators and vitamin supplement were present in the diet. The contents of all the nutrients in the two pellets were able to meet the nutritional requirements of the animals according to their physiological status (de BLAS and MATEOS, 1998).

The analyses on the chemical composition of drinking water revealed in particular concentrations of sodium, potassium and chloride in the range of tolerable values reported in literature (LEBAS *et al.*, 1998).

The productive performances are summarized in table 1.

**Table 1. Body weight, feed intake, nutrients and energy intake.**

	dEB1	dEB2	Error Variance (17 d. f.)
Body weight (g)	5253	5532	310642
Feed intake (g/d)	150	148	131
<b>Intake of:</b>			
-crude protein (g/kg <sup>0.75</sup> )	7.40	7.01	3.0035
-crude fiber (g/kg <sup>0.75</sup> )	6.60	6.25	1.0043
-sodium (g/kg <sup>0.75</sup> )	0.10 <sup>b</sup>	0.16 <sup>a</sup>	0.7344
-potassium (g/kg <sup>0.75</sup> )	0.47	0.45	0.0099
-chloride (g/kg <sup>0.75</sup> )	0.16	0.14	0.0050
-digestible energy (MJ/kg <sup>0.75</sup> )	4.36	4.13	0.7344

a, b: P<0.05

The live body weight, although tendentially higher in the dEB2 rabbits, and the feed intake, recorded at 70 weeks of age, were not significantly different between the groups. The consumption per unit of metabolic weight of each nutrients resulted similar in dEB1 dEB2 animals, except for sodium (P<0.05).

**Table 2. Reproductive performance.**

	dEB1	dEB2	Error Variance (17 d.f.)
Kids born (n)	37.04	32.14	250.3110
Kids born/mating (n)	9.80	9.89	2.2745
Kids born alive (n)	35.45	30.78	232.1074
Kids born alive/mating (n)	9.32	9.47	2.0037

As the reproductive data of the third cycle are concerned (Table 2), the number of kids born and born alive for each buck was neither significantly different between the two experimental groups nor when calculated on the number of matings. The fertility rate was 92.1 and 92.0%, in dEB1 and dEB2 groups, respectively.

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

	dEB1	dEB2	Error Variance (17 d.f.)
Glucose (mmol/l)	7.20	6.83	0.4412
Cholesterol (mmol/l)	1.38	1.43	0.0798
Triglycerides (mmol/l)	1.79	1.14	0.4656
Total proteins (g/l)	64.19	62.09	13.2036
Albumin (g/l)	51.41	48.20	21.6015
Globulines (g/l)	12.78	13.89	9.1203

The dietary dEB did not significantly affect some of the variables (Table 3) involved in energy (glucose, cholesterol, triglycerides) and protein (total proteins, albumins and globulines) metabolism. Adding natural clay (5%) to the diet of heat-stressed male rabbits induced a decrease of albumin and globulines (MARAI *et al.* 1999). Similar effects were observed when cool drinking water was given to rabbits under heat- stressed conditions (MARAI *et al.*, 1999). In another experimental trial where salt was added to

drinking water (2000-5000 mg/kg), the total protein plasma level decreased in young rabbits reared under high environmental temperature (HABEEB *et al.*, 1997).

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

	dEB1	dEB2	Error variance (17 d. f.)
AST (U/l)	18.10	20.59	49.5362
ALT (U/l)	30.05	23.42	98.5623
$\gamma$ -GT (U/l)	8.17	6.99	2.0203
Calcium (mmol/l)	3.71	3.65	0.0210
Phosphorus (mmol/l)	1.47	1.39	0.0201
Sodium (mmol/l)	138	138	7.3542
Potassium (mmol/l)	5.34	5.23	3.9878
Chloride (mmol/l)	102	101	2.3645

Plasma enzyme and mineral profile of the bucks' figures in Table 4. The dEB2 rabbits presented level of alanine and aspartate aminotransferase similar to those of dEB1 group. Also the level of  $\gamma$ -GT did not differ between the groups. Plasma minerals studied were similar between the groups. HABEEB *et al.* (1997) observed that drinking tap water supplemented with high levels of NaCl (2000-5000 mg/kg) induced an increase of some blood electrolytes such as Na, K and on Ca and P in growing rabbits, particularly under hot summer conditions.

**Table 5. Plasma levels of cortisol and thyroid hormones.**

	dEB1	dEB2	Error Variance (17 d. f.)
Cortisol ( $\mu$ g/dl)	0.58	0.44	0.0410
T3 (ng/dl)	183	167	775
T4 ( $\mu$ g/dl)	3.78	3.60	0.1476
FT3 (pg/ml)	7.11	6.37	0.9490
FT4 (ng/dl)	1.97	2.76	0.9177

Table 5 shows the plasma levels of some hormones. The dEB2 rabbits presented tendentially lower cortisol, but the difference was not significant. MARAI *et al.* (1999) observed in rabbits under high temperature and a fed diet containing 5% natural clay, that plasma cortisol decreased.

The dietary electrolyte balance did not substantially modify the thyroid hormone profile of the bucks. Plasma values of T3 and T4 was not significantly different between the dEB1 and dEB2 rabbits as well as the level of FT3. The biologically active fraction of T4 (FT4) presented a slight increase in dEB2 rabbits.

Literature reports higher T3 and T4 plasma levels in the cold winter in comparison to the hot summer (SHEATA *et al.*, 1998); under hot conditions the drinking water containing high levels of salt (2000-5000 mg/kg) induced a reduction of T3 (HABEEB *et al.*, 1997).

In reproductive rabbits, thyroid hormones are particularly important, given the relationships between hormonal levels and reproductive performance. FOOTE (2002)

demonstrated that in males with hyperthyroidism, induced by supraphysiologic doses of triiodothyronine, the spermatogenesis was depressed. Anyway, the effect of induced hyperthyroidism on sperm production was transient.

## CONCLUSIONS

This study adds other indications on the effects of the dietary electrolyte balance in the productive and physiological response of rabbits.

The treatment performed on three reproductive cycles and based on two dEB levels ranging from 27 to 35 meq/100 g demonstrated that at the third cycle the animals of the two groups presented similar body conditions and reproductive performance.

The metabolic profile, together with the enzyme and mineral plasma content did not change with the higher dEB level. The lack of the effect on reproductive performance is in agreement with the results obtained also on the endocrine status of the animals.

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