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EFFECT OF DIETARY ZINC AND VITAMIN SUPPLEMENTATION ON SEMEN CHARACTERISTICS OF HIGH GROWTH RATE MALES DURING SUMMER SEASON

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ABSTRACT

Thirty adult males selected by high growth rate were used to study the effect of dietary zinc and vitamin supplementation in some semen traits during summer season. Starting from a comercial diet with 11.1 MJ ED/kg DM, 179 g CP/kg DM and 197 g ADF/kg DM (diet C), two supplemented diets were formulated by adding: 100 mg of retinyl acetate/kg and 100 mg of α-tocopheryl acetate/kg (diet AE), or 245 mg of $ZnSO_4$ (100 mg Zn)/kg (diet Z). The sperm parameters were subject to seasonal fluctuations due to changes in the environmental temperature, affecting negatively the total sperm production and in the incidence of some sperm abnormalities of semen collected two months after. Males fed with Z diet had slightly lower food intake than rabbits fed with C and AE diets. Males given a diet supplemented with 100 mg Zn/kg showed a significantly higher total sperm production (+30.2 · 10^6 spermatozoa per ejaculate) than those given a commercial diet or a diet supplemented with vitamins A and E. Dietary treatment did not affect the semen quality parameters evaluated. Commercial diets seem to provide enough amounts of vitamin A and E for an adequate semen production even under heat stress conditions, but an increase of dietary Zn could slightly reduce the depression of semen production usually observed in autumn.

INTRODUCTION

As it has been stated in a previous work (LAVARA *et al.*, 2000), one of the main problems for the use of the semen of rabbit males selected by high growth is the relatively low sperm count of this animals (200-600·10^6), especially under high ambient temperature (150-200·10^6). When animals are subject to high ambient temperature (30ºC) the semen amount and its quality decrease (BICUDO and PASCHOAL, 1991; EL-MASRY *et al.*, 1994); these could be due to changes in the physiological balance (hormonal pattern, mineral imbalance,...) and to the lower food intake of males. The duration of spermatogenesis in rabbit is about 42 days so, the high temperatures of August usually affect semen production of males in October.

Only a few studies have been realized to increase the amount and quality of sperm produced by males under high environment heat (EL-MARSY *et al.*, 1994) by means of the addition to the diet of vitamins and zinc. As it is well known in other species, the use of some natural antioxidants (vitamins A and E) could improve the quality of semen, mainly due to their role in the protection of the sperm membrane integrity, in view of the high susceptibility to peroxidation of their unsaturated fatty acids (RODE *et al.,* 1995; MALDJIAN *et al*., 1998). On the other hand, zinc directly affects the male and female reproductive organs through the pituitary-gonadal axis. In males, zinc controls the release of pituitary gonadotropins, androgenes and testosterone (HIDIROGLOU and KNIPFEL, 1984; REEVES and ÓDEEL, 1988) and its deficiency stops the development of testes and causes atrophy of the seminiferous epithelium.

The primary objective of the present experiment was to determine the effect of vitamins A and E or zinc dietary supplementation in the production and quality of semen of males selected by high growth rate, during summer and under not controlled environmental conditions.

MATERIAL AND METHODS

Animals

Thirty adult males (about 1.5 years old) from line R were used in this experiment carried out during 20 weeks (from August to December). Line R was selected on the basis of growth rate from weaning to slaughter (28-63 days of age, ESTANY *et al.*, 1992). Males were assigned randomly to diet groups and were housed in individual cages with light alternating on a cycle of 16 light hours and 8 dark hours. Animals were kept in traditional building under not controlled environmental conditions. Maximum environmental temperatures were registered throughout the experiment (varying from 14 to 32ºC).

Diets

Three diets were formulated with different vitamin and Zn contents. Usually, rabbit males used for artificial insemination are fed with reproductive doe diets. So, starting from a typical comercial pelleted diet (Table 1) with 11.1 MJ ED/kg DM, 179 g CP/kg DM and 197 g ADF/kg DM (diet C), two supplemented diets were formulated by adding: 100 mg of retinyl acetate/kg and 100 mg of α-tocopheryl acetate/kg (diet AE), or 245 mg of $ZnSO_4$ (100 mg Zn)/kg (diet Z). They were offered *ad libitum* during the experimental period.

Table 1. Ingredients of basal diet (g/kg).

	Control diet
Lucerne hay	200
Barley straw	130
Barley grain	330
Soya meal (44%)	160
Coarse wheat bran	135
Animal fat	10
Methionine	1
Calcium hydrogen phosphate	24
Sodium chloride	7.2
Robenidine	0.8
Vitamin/mineral mixture[1]	2

[1] Contains (g kg^{-1}): thiamin, 0.25; riboflavin, 1.5; calcium pantothenate, 5; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2; cholecalciferol, 0.1; α-tocopherol, 15; phytylmenaquinone, 0.5; cyanocobalamin 0.006; choline chloride, 100; $MgSO_4 \cdot H_2O$, 7.5; ZnO, 30; $FeSO_4 \cdot 7H_2O$, 20; $CuSO_4 \cdot 5H_2O$, 3; KI, 0.5; $CoCl_2 \cdot 6H_2O$, 0.2; Na_2SeO_3, 0.03.

Semen collection and evaluation

From October to December, data from 10 weeks of sexual activity were recorded. Two ejaculates per male were collected each week using an artificial vagina. The following measurements were taken from semen:

a) Volume of semen was measured in a graduated conical tube.
b) Concentration of sperm per milliliter was calculated with a Thoma – Zeiss counting cell chamber.
c) Acrosomal integrity, abnormal sperm and protoplasmatic droplets: spermatozoa were fixed with glutaraldehyde 2% in Dulbecco's phosphate buffered saline (Pursel and Johnson, 1974) and the proportion of sperm with normal intact acrosome, abnormal sperm morphologically and protoplasmatic droplets were estimated using interference contrast optics at a magnification of x750.

Statistical analysis

A chi-squared with Yate´s correction was used to analyse the collection rate (number of eyaculates/number of collections –eyaculates, eyaculates with urine, urine) between diets. Food intake of males and semen parameters were analysed by variance analysis, using a mixed procedure (PROC MIXED) of SAS (STATISTICAL ANALYSIS SYSTEM INSTITUTE, 1996) and according to a repeated measures design that take into account the variation between animals and covariation within them. The model included as fixed effects the diet (3 levels) and the week (10 levels), using the present temperature and the temperature of two months previous to the collection as covariate. Covariance structures of mixed procedure were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by LITTELL *et al.* (1998).

RESULTS AND DISCUSSION

The results in Table 1 indicate that sperm parameters are subject to seasonal fluctuations because of changes in the environmental temperature (Figure 1). Usually, heat stress reduces all parameters of economic importance in rabbit production due to the changes in the food intake and in the physiological balance of the animals. The high ambient temperature affected negatively the total sperm production and in the incidence of some sperm abnormalities of semen collected two months after. In fact, the mean total sperm production of males ($140 \cdot 10^6$) was clearly lower than those values obtained by other authors (VIUDES-DE CASTRO and VICENTE, 1997) for line R males ($200\text{-}600 \cdot 10^6$). Similar results were obtained by other authors in rabbits (FINZI *et al.*, 1994) and in other species (SARLOS and MOLNÁR, 1995), that observed a decrease of the volume and concentration 2 months after the hot season.

On the other hand, males fed with Z diet had slightly lower food intake than rabbits fed with C and AE diets (Figure 1). These results are unclear and no trials have been conducted to quantify the possible zinc toxicity in rabbits, but rats and weaning pigs fed with diets containing more than 1000 mg Zn/kg induced no discernible effects, and only higher Zn levels depressed growth and appetite. HATCH *et al.* (1987) also showed a significant decrease of feed intake and growth of lambs fed with a diet supplemented with 47 mg of Zn /kg. They proposed that high levels of dietary zinc might have aggravated a deficiency of another mineral, resulting in a reduction in performance.

Table 1. Simple correlation matrix between the different variables analysed.

	AI	PD	SA	DFI	Tmax	Tmax2
TSP	0.346***	-0.316***	-0.264***	-0.104	-0.149*	-0.227***
AI		-0.004	-0.453***	-0.093	-0.077	0.004
PD			0.141*	-0.082	0.218**	0.353***
SA				0.113	-0.018	-0.074
DFI					-0.368***	-0.110
Tmax						0.860***

TSP: total sperm production; AI: acrosoma integrity; PD: protoplasmatic drop; SA: sperm abnormalities; DFI: daily food intake; Tmax: T^a^ maximum; Tmax2: T^a^ maximum 2 months before.
* P<0.05; ** P<0.01; *** P<0.001.

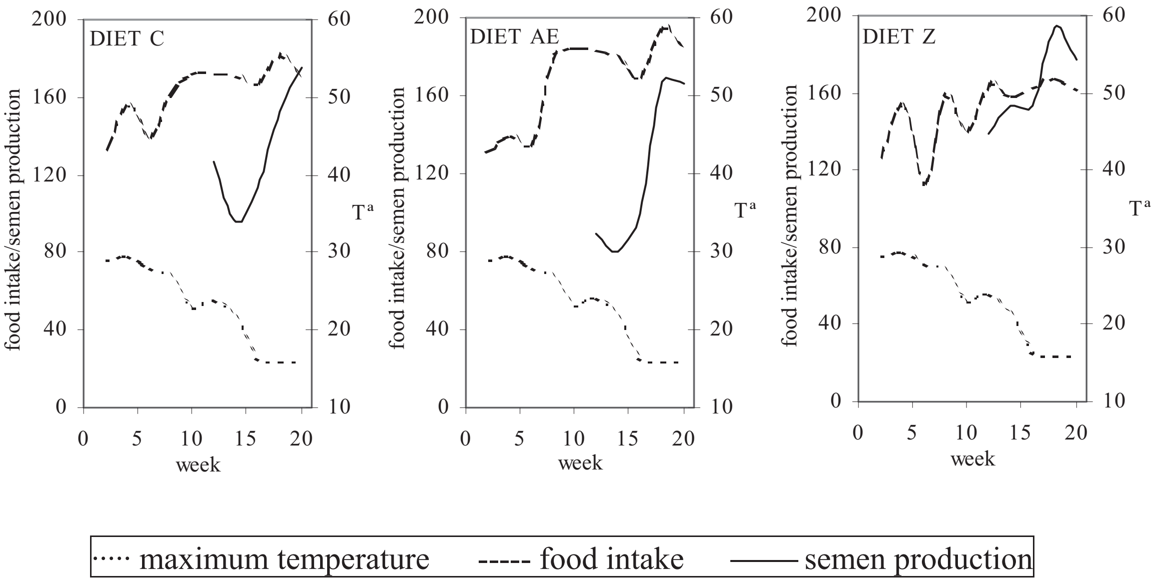

Figure 1. Evolution of the maximum temperature (Tª), and the food intake (g/day) and the semen production (10^6 spermatozoa) of rabbit males given the experimental diets (C, AE and Z) throughout the experimental period.

With respect to the effect of dietary supplementation with vitamins or Zn in the amount and concentration of semen of males during summer, under not controlled environmental conditions, the results of Table 3 indicate that males given a diet supplemented with 100 mg Zn/kg showed a significantly higher total sperm production ($+30.2 \cdot 10^6$ spermatozoa per ejaculate) than those given a commercial diet or a diet supplemented with vitamins A and E. Dietary treatment did not affect the semen quality parameters evaluated. Regarding to the vitamins effect, we are partially agree with those obtained by other authors (EL-MASRY *et al.,* 1994; CASTELLINI *et al.,* 1999) who did not find any significant effect of vitamin E addition on semen volume, sperm concentration and semen quality parameters.

Commercial diets seem to provide enough amounts of vitamin A and E for an adequate semen production even under heat stress conditions, but an increase of dietary Zn could slightly reduce the semen production decrease usually observed in autumn. EL-MASRY *et al.* (1994) also found a clearly improvement of total semen production of males under high environmental conditions (133 to 344 $\cdot 10^6$ spermatozoa for a control and a Zn supplemented diet, respectively) when 35 mg of Zn was added to a control diet that presented a relative low vitamin-mineral premix percentage (0.04%).

Table 3. Effect of dietary vitamin AE and Zn supplementation on some qualitative semen parameters.

	Diets			SE	Significance
	C	AE	Z		
Total sperm production (10^6)	132.8[a]	120.1[a]	163.0[b]	10.07	***
Acrosoma integrity (%)	87.02	85.11	85.24	1.660	NS
Sperm abnormalities (%)	12.78	10.71	11.58	1.443	NS
Protoplasmatic drop (%)	16.35	18.62	19.21	1.119	NS

SE: standard error.
NS, not significant; *** $P<0.001$.
[a,b] Means within a row with different letters are significantly different at $P<0.05$.

However, when the maximum ambient temperature fell below 22ºC the total sperm production obtained 2 months after was similar for the different diets evaluated (Figure 2). These results could indicate that the dietary Zn supplementation could reduce the total sperm production depression of rabbit males after a heat stress season, but it does not seem to improve the semen production under normal environmental conditions.

Figure 2. Effect of maximum temperature two moths before (Tªmax2) on the increase of the sperm production (10^6 spermatozoa) obtained with the AE and Z diets respect to values obtained for Control diet.

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