EFFECTS OF DIETARY ZINC SUPPLEMENTATION ON SPERMATIC CHARACTERISTICS OF RABBIT BREEDERS

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ABSTRACT

In order to study the effects of dietary zinc supplementation on spermatic characteristics, 27 New Zealand White rabbits were used, following a completely randomized design with five treatments (Zn levels). Two treatments had had six replicates, while the rest had five. The rabbits were selected immediately after weaning (4 weeks of age) and used in the experiment that extended till 34 weeks of age. The treatments were respectively, 0, 50, 100, 150 and 200 ppm supplemental zinc in the diet. The animal began receiving ZnO in their diets as soon as they were weaned. First semen collection was performed at 28 wks of age, being collected six times weekly per animal. The volume of each ejaculate was registered and an aliquot was separated for further analyses. Due to a possible error in saline formol concentration, it couldn't be possible to estimate spermatozoa concentration through microscopic counting. The alternative used to bypass this problem was to determine the volume of cell mass (volume of spermatozoa) of every ejaculate. The technique consisted in using Wintrobe tubes (graded tubes with subdivisions of 0.01 ml) to centrifuge semen samples, at 4,000 rpm for 20 minutes, and measure the cellular volume each sample. Mean values of total ejaculate didn't differ ($p > 0.05$) among treatments. Animal fed 50, 100 and 150 ppm ZnO presented higher spermatozoa volume as compared to the other groups.

Key words: semen, spermatozoa, cellular mass volume, concentration, nutrition.

INTRODUCTION

Zinc is a micro-mineral involved in various processes of animal metabolism. Since it was originally demonstrated that zinc is necessary for healthy growth of rats (TOOD *et al*., 1934, cited by UNDERWOOD & SOMERS, 1977), the role of zinc in the animal organism began to gain special attention.

Zinc participates actively in protein synthesis and carbohydrate metabolism. The discovery that the enzyme carbonic anhydrase contains 0.33% of zinc in its molecule (KEILIN & MANN, 1939, cited by UNDERWOOD & SOMERS, 1977) is considered the first acceptable explanation of the mechanism of action of this element. After that, many

other enzymes have been identified as containing zinc: alcohol dehydrogenase, carboxipeptidase and DNA-polymerase, this latest being fundamental in cell division process. This mineral stabilizes the quaternary structure of enzymes; large quantities of zinc were found to provide stability to the structures of RNA, DNA and ribosomes (PRASK & PLOCKE, 1971, quoted by MCDOWEL, 1992).

The high concentration of zinc in organs like prostate, testicles and in the spermatozoa itself (BERTRAND & VLADESCO, 1921, cited by UNDERWOOD & SOMERS, 1988), suggests its importance in reproduction. Zinc requirement for rabbits, indicated in the literature, is 30-60 mg/Kg dry matter, with suggestion of higher levels for breeders (MATEOS & BLAS, 1998). Zinc supplementation enhances spermatogenesis, but the mechanism is not completely known. This mineral is important for spermatogenesis, being directly involved in spermatozoa maturation and preservation of germinative epithelium (UNDERWOOD & SOMERS, 1969). It is also essential to cellular division, synthesis and stability of DNA (DEVENSON, 1993), as well as in cellular differentiation.

Selenium (Se) is part of molecular structure of the enzyme glutathione peroxidase (GTP), which clears hydroperoxides generated during the metabolism of numerous substances, especially in the metabolism of unsaturated fatty acids, converting them into alcohol. HOEKSTRA (1975), cited by PUTMAN & COMBEN (1987) suggested a mechanism linking the antioxidant activity of vitamin E with GTP. According to him, vitamin E molecules attached to cell internal membrane attract polyunsaturated fatty acids and establish with them weak bonds, until these fatty acids are catabolized during normal cellular metabolism. In the meanwhile, GTP removes peroxides formed within the cell. This theory demonstrated that Se and vitamin E play complementary roles in the protection of cellular membranes, avoiding peroxidation of unsaturated fatty acids. He emphasized that, neither of the two elements replaces the other. In the absence of sufficient amounts of Se to form GTP, cells can have excessive concentration of peroxides, which will damages the referred fatty acids even when vitamin E levels are appropriate. On the other hand, reactive oxygen from other sources, present in the cell, can oxidize the polyunsaturated fatty acids if they are not protected by vitamin E.

Reactive oxygen species (ROS) such as the superoxide anion radical ($O_2^{\cdot-}$), H_2O_2 and the hydroxyl radical ($OH^{\cdot}$) are generated as an unavoidable side reaction in aerobic cells that have oxygen as the terminal electron acceptor (FRIDOVICH, 1995). ROS production in semen has been associated with loss of sperm motility, decreased capacity for sperm-oocyte fusion and loss of fertility (GRIVEAU & LE LANNOU, 1997). Nevertheless, researchers suggested an important role of $^{\cdot}O_2$ in sperm hyperactivation and capacitation (O'FLAHERTY *et al.*, 1997). The appropriate balance between superoxide radical generation and superoxide dismutase (SOD) activity is decisive for the functional status of sperm (SIKKA, 2001). The cofactors of this enzyme (SOD) are zinc and copper. According to MAXWELL & STOJANOV (1996), exogenous addition of SOD and catalase enhances the motility, acrosome integrity and fertility of ram spermatozoa. One of the major roles of vitamin C in the metabolism is the recycling (reduction) of vitamin E after its oxidation by free radicals and oxygen. GEVA *et al*. (1996) demonstrated increased *in vitro* fertilization rates following vitamin E and C therapy.

DNA replication is fundamental in the spermatozoa synthesis. Zinc is directly involved in anatomic development and normal function of male reproductive organs; deficiency of zinc in the diet delays testicle development, reduce testosterone production and stops spermatogenesis (SOMERS & ANDERWOOD, 1977). The production of hormones by the pituitary gland is also affected in an animal when the diet is deficient in zinc (HIDIREGLOU & KNIPFEL, 1984; REEVES & ODEEL, 1988).

Studies demonstrated that the use of some natural antioxidants, such as vitamins A and E, can improve semen quality, through protection of spermatozoa membrane (RODE *et al.*, 1995; MALDJIAN *et al.*, 1998). Also, zinc supplementation has been showing its advantages in spermatic production. Ambient temperature is another important factor in the production of spermatozoa. Temperatures around or above 30°C cause drop of quantity and quality of semen (BICUDO & PASCHOAL, 1991; EL MARSAY *et al.*, 1994; FINZI & MACCHIONI, 1994; VIUDES-DE-CASTRO & VICENTE, 1997).

The aim of this experiment was to determine the effects of dietary supplementation of zinc upon total volume and cellular mass volume of rabbit breeder semen, during six weeks, from September till October, with ambient temperature varying between 23 and 28 °C.

MATERIALS AND METHODS

Animals
A total of 27 male rabbits of New Zealand White breed were used. After weaning (at four weeks of age), the rabbits were housed in individual cages in the same room, receiving ration and water *ad libitum*. Semen collection was preformed from 28th week.

Diets
Treatments were made up of basal diets varying in supplemental zinc contents, provided as zinc oxide (ZnO), as described in table 1.

Experimental design
The experimental design was completely randomized, with five treatments, being six replicates of one rabbit in treatments 0Zn and 50ZN, and five replicates in treatments 100Zn, 150Zn and 200ZN.

Semen collection and analysis
Of each animal, six collections of semen were performed, being one per week, using artificial vagina and measuring the volume of each ejaculate with graduate tube (subdivisions of 0.01 ml). Samples of 0.1 ml were taken, fixed in saline formol (1 part of semen in 5 parts of saline formol) and used to determine spermatic concentration.

Table 1. Quantity of ZnO (g/Kg diet) supplied and corresponding estimated zinc supplement per treatment

Treatments	ZnO supplement (g)	Estimated supplemental Zn (ppm)
0	0	0
50	0.064	50
100	0.128	100
150	0.192	150
200	0.256	200

Table 2: Basal diet composition (g/kg)

Ingredients	Quantity
Alfalfa hay	343.08
Wheat bran	250.00
Ground whole corn ears	150.00
Corn (grains)	100.00
Soybean meal	71.72
Soy oil	40.00
Powdered molasses	20.00
Limestone	14.22
Inert material	10.00
Bicalcium phosphate	6.79
Sodium chlorine	5.00
L-lysine	4.87
PREMIX (Zn-free)	3.00
Threonine	1.95
DL-methionine	1.46
Antioxidant (BHT)	0.10

Table 3. Calculated chemical composition of the diet (as fed basis)

Nutrient or energy	Percentage or kcal/kg
Dry matter	88.95 %
Crude protein	16.50 %
Acid detergent fiber	17.02 %
Calcium	1.15%
Phosphorus	0.60%
Lysine	0.80%
Methionine + Cystine	0.60%
Threonine	0.68%
Digestible energy	2,500 kcal/kg

Nevertheless, possible errors in saline formol concentration caused spermatozoa agglutination, which made impossible the evaluation of their concentration in the

samples. In order to bypass this difficulty, the solution adopted was to determine spermatozoa mass of the samples, using spermatocrit, which is an innovating technique for rabbit semen. In the method of Wintrobe or macrohematocrit, blood samples are put into 1 ml tubes with subdivisions of 0.1 ml, centrifuged, and then, the volume of cells in each sample is recorded. In this experiment, semen samples were centrifuged during 20 minutes at 4,000 rpm, in Wintrobe tubes. After this process, the content of each tube presented two layers: the lower one, white colored, indicating the quantity (ml) of cells (spermatozoa), and the upper layer, completely transparent, which contained no considerable amount of cells. The mean value of the six consecutive determinations was calculated for each rabbit, resulting in the measurement for the respective replicate. The treatments were compared for the variables cellular (spermatozoa) mass volume and volume of each ejaculate.

Statistical analyses
Analysis of variance was carried out using SAS (1989), and the treatment means (spermatozoa mass volume and volume of ejaculate) compared by the test of Duncan.

RESULTS AND DISCUSSION

The means of total ejaculate and of cellular mass volume per treatment are shown in Table 4.

Table 4. Volumes (ml) of total ejaculate and of cellular mass according to zinc supplementation levels in diets

Supplemental Zn (ppm)	Mean volume of ejaculate (ml)	Mean volume of cellular mass (ml)
0	0.3273	0.1761[b]
50	0.4527	0.2544[ab]
100	0.4523	0.2533[ab]
150	0.5864	0.3757[a]
200	0.4261	0.2444[b]

[ab] Means with different superscripts in a column are statistically different ($p < 0.05$)

Mean volume of ejaculate ranged from 0.3 to 0.6 ml, and did not increase with zinc supplementation. In contrast, EL-MARSY *et al*. (1994) and MOCÉ *et al*. (2000) registered higher volumes o ejaculates in animals fed supplemental zinc (levels from 35 to 100 ppm) as compared to non-supplemented ones. Also, they observed an increase of spermatozoa quantity (concentration) in the ejaculates of animal fed the supplemental zinc. The present study demonstrated enhancement of cellular mass volume of ejaculates in rabbits supplemented with 50, 100 and 150 ppm zinc, revealing a possible increase of spermatozoa concentration in the respective animals, which is in agreement with the above quoted authors.

Rabbits supplemented with 200 ppm zinc, as well as non-supplemented animals, showed low values of cellular mass volume (Table 4). This suggests that, the positive

effect of zinc supplementation upon semen concentration may be valid until certain limit of concentration of the referred mineral.

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