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Effect of Daily Food Supplementation with Essential Fatty Acids on Canine Semen Quality

AA da Rocha[1], ICN da Cunha[1], BB Ederli[2], AP Albernaz[2] and CR Quirino[1]

[1]*Laboratório de Reprodução e Melhoramento Genético Animal;* [2]*Laboratorio de Sanidade Animal, Universidade Estadual do Norte Fluminense (UENF), Campos dos Goytacazes, Rio de Janeiro, Brazil*

Contents

Polyunsaturated fatty acids are important membrane components that influence membrane integrity and fluidity. In the present study, the effect of oral supplementation for 60 days with essential fatty acids (omega 3, 6 and 9) and vitamin E on canine semen quality was evaluated. Sixteen dogs were selected for the experiment; eight were used as the control group and eight received the fatty acid supplemented diet for 60 days. Semen samples were taken every 15 days during the entire experimental period and were analyzed for volume (ml), motility (%), vigour (0–5), concentration ($\times 10^6$/ml), morphology of spermatozoa (%), plasma membrane integrity (%; using the hyposmotic swelling test) and thermoresistance (motility and vigour after 4 h at 38°C). We concluded that, daily supplementation with omega 3, omega 6 and omega 9 fatty acids, together with vitamin E, for a period of 60 days, significantly increased the semen volume of the treated group after 15 days of supplementation; the vigour and concentration of spermatozoa were superior after the first month of supplementation, while the percentage of morphologically abnormal spermatozoa decreased and the cells were protected against thermal stress.

Introduction

Fatty acids are fundamental units in most lipids, and the majority of them are synthesized by mammals from fats consumed in their diet. However, some unsaturated fatty acids are not synthesized by the organism, and must be obtained from the diet (Carr 2000). Omega factors are fatty acids and nowadays are considered as fundamental units in the protection of numerous vital functions in organisms (Abdalla 1993). The metabolism of fatty acids generates arachidonic acid whose degradation activates inflammatory mediators, which are responsible for lesions on cell membranes. Omega 3 supplementation induces a decrease in the enzymatic action of arachidonic acid, possibly because of their metabolic competition for the same enzymes.

Vitamin E, known as tocopherol, is an essential nutrient, whose main function is to inhibit the action of free radicals. For this reason, it is usually associated with omega fatty acids in dietary supplements.

It is known that, spermatogenesis is directly related to the adequate function of Leydig cells, and that the function of these cells is regulated through synthesis and release of LH by cyclic AMP (cAMP) metabolic pathways. It has been confirmed that the pathways of some messengers involved in cAMP formation are related to arachidonic acid and its metabolites (Marinero et al. 1996). Some studies have demonstrated that, LH induces the rapid release of arachidonic acid from Leydig cells, and that the metabolites of this acid are directly related to the induction of spermatogenesis and also to the production of testosterone, induced by elevation of plasma concentrations of GnRH (Dix et al. 1984). These results indicate that the supplementation of omega fatty acids could interfere with the production of hormones and consequently in the production of sperm cells by testicular tissue.

Concerning the maintenance of sperm quality during spermatogenesis, epididymal storage and ejaculation, some studies indicate that there is a relationship between the action of vitamin E and a reduction in cell damage. The formation of free radicals after lipid peroxidation of sperm cell membranes causes serious injuries, which decrease the quality and viability of spermatozoa. The oxidative activity of these metabolites interferes with membrane fluidity and cell locomotion and thus fertilizing abilities. Vitamin E has been used as the main antioxidant for these purposes because of its ability to pass through sperm membranes and to interrupt chain reactions that lead to free radical formation (Suleiman et al. 1996).

There is significant interest in dietary supplements aimed at improving seminal quality, and the aim of this study was to evaluate the effect of dietary supplements of omega 3, omega 6 and omega 9 fatty acids, with the addition of vitamin E, on the semen quality of dogs, during and after a period of 60 days of supplementation.

Materials and Methods

We used 16 clinically healthy male dogs, aged between 1 and 7 years old and of different breeds: Belgium Shepherd (1), Bull Terrier (2), Dachshound (1), French Bulldog (1), Labrador retriever (5), mongrels (2), Pitbull (1), Yorkshire Terrier (1) and White Shepherd (2). All animals were housed by their owners and were given water *ad libitum* and commercial dog food. There was no change in their initial conventional diets during the experiment. Before the beginning of treatment, all dogs had several semen collections and evaluations with 2–3 days between collections.

The animals were randomly divided into two groups: the control group (eight dogs) received no supplement; the treated group (eight dogs) received daily supplementation of linoleic acid (omega 3) – 7.2 mg per body weight, linolenic acid (omega 6) – 25 mg/kg, oleic acid (omega 9) – 10.1 mg/kg and vitamin E – 1 UI/kg.

Semen collection and evaluation of all dogs were performed by the same people and were undertaken at the beginning of treatment (D0) and after 15 (D15), 30

(D30), 45 (D45) and 60 (D60) days. Supplementation in the treated group began on day 0 and ended on day 60. The second fraction of semen was collected after manual stimulation and the samples were taken within 30 min to the Laboratory of Animal Reproduction and Genetic Improvement of North Fluminense State University (UENF) for analysis. Each semen sample was collected and evaluated for volume (ml), subjective motility (%), subjective vigour (score from 0 to 5, meaning the linearity and quality of spermatic movement), concentration (spermatozoa/ml), spermatozoa morphology (%), integrity of membranes (by hyposmotic swelling test) as well as motility and vigour after 4 h of incubation at 38°C (thermoresistance test).

After descriptive analysis, the data were submitted to ANOVA in order to determine whether there were differences in the semen characteristics because of supplementation with fatty acids. The average scores were compared using the SNK test with a p-value of 0.05 indicating statistical significance (SAS 1999).

Results

Besides the parameters evaluated in this study, and within the time period of 60 days, we found that there was no statistical difference between treated and control groups ($p > 0.05$) in terms of the rate of motility of spermatozoa and membrane integrity.

The volume (ml) of the second fraction of the ejaculate of the treated group was higher after 15 days of dietary supplementation. The vigour and spermatozoa concentration rates were greater ($p < 0.05$) after the first month of supplementation, while abnormal sperm significantly decreased ($p < 0.05$; Table 1). With regards to the thermoresistance test and cell vigour, we found values were better in samples collected after 15 days of supplementation. Furthermore, we observed a progressive improvement of longevity during thermoresistance test within the supplemented group with a significant increase in motility after 30 days of intake and in vigour after 15 days ($p < 0.05$; Table 1).

Discussion

Supplementation with omega fatty acids and vitamin E for at least 60 consecutive days induced an improvement in the semen quality of tested dogs. Spermatogenesis is mediated by hormones, mainly influenced by GnRH and LH, the latter inducing testosterone synthesis by stimulation of the Leydig cells. Arachidonic acid is involved in the synthesis of cAMP messengers, which are directly related to the proper function of Leydig cells.

Other studies have documented that, after oral supplementation of omega 3 fatty acids, inflammatory mediators become less harmful, as the metabolism of omega 3 and omega 6 competes for the same enzymatic systems needed for the production of inflammatory mediators, which thus become less active (Dix et al. 1984). This hypothesis may be supported by the fact that, non-steroidal anti-inflammatory drugs block the formation of inflammatory mediators such as prostaglandins, through the inhibition of lipoxygenases. This was confirmed by investigation of the effect of non-steroidal anti-inflammatory drugs on mouse testicular cells, which were found to influence spermatogenesis. The production of testosterone through the induction of LH was reduced in Leydig cells of animals treated with anti-inflammatory drugs (Dix et al. 1984). The supplementation of dogs' food with fatty acids may stimulate the action of lipoxygenases, which do not use the pathway of the arachidonic acid metabolism. These enzymes do not contribute to the production of inflammatory mediators, which decreases cell injury. Supplementation still increases the production of hormones that support the function of the testicular cells and that would account for the higher semen quality during the use of supplements containing omega fatty acids. Because the supplementation increases the capacity for semen production by testicular cells, there is also an increase in spermatozoa concentration and a reduction in the rate of morphological abnormalities, as indicated during the present study.

As for the improvement of vigour and motility after supplementation of fatty acids and vitamin E, as observed during the thermoresistance test, we suggest that docosahexaenoic acid (DHA) – the active form of omega 3 – is involved in the regulation of free fatty acids used by the sperm cell. The biophysical properties of DHA contribute to sperm membrane fluidity and to the flexibility of the spermatozoan tail (Conquera et al. 2000). Therefore, daily supplementation

Table 1. Mean values of semen volume (vol.), spermatozoa motility (mot.), vigour (vig.), concentration (cone.), pathology (pathol.), membrane integrity (integ.) and thermoresistance of samples from dogs treated or not treated with fatty acids for 60 days

	Day	Vol (ml)	Mot (%)	Vig (0–5)	Cone ($\times 10^6$/ml)	Pathol (%)	Integ (%)	Thermoresistance longevity	
								Mot (%)	Vig (0–5)
Control	0	1.92	77.5	3.5	208.5	11.0	77.0	15.0	0.75
	15	1.96	76.8	3.25	207.4	10.9	75.0	13.1	0.62
	30	1.96	75.0	3.0	207.0	10.9	74.9	11.9	0.37
	45	1.96	74.0	3.0	206.5	10.6	74.6	11.9	0.37
	60	2.0	74.0	3.0	205.6	10.2	74.4	11.2	0.37
Supplemented	0	1.89^{a}	87.0	3.37^{a}	205.9^{a}	12.9^{a}	79.2	14.4^{a}	0.75^{a}
	15	1.97^{ab}	86.0	3.37^{a}	207.0^{a}	12.1^{a}	79.7	17.5^{a}	1.37^{b}
	30	2.07^{bc}	83.0	3.87^{ab}	218.0^{ab}	7.5^{b}	84.2	33.1^{b}	2.0^{c}
	45	2.11^{c}	81.0	4.25^{b}	229.0^{b}	7.1^{b}	88.5	46.2^{c}	2.2^{cd}
	60	2.15^{c}	81.0	4.37^{b}	234.0^{b}	7.0^{b}	89.1	52.5^{c}	2.5^{d}

Within a column, values with different letters between days in the same group are different using the Duncan test with a 5% significance level.

with omega 3, which is converted to DHA may contribute to higher spermatozoa vigour. Similar results were observed in dogs in which supplementation with vitamin E also raised the rates of vigour and motility, as shown by the thermoresistance test (Leite Neto et al. 2007).

Studies with humans showed significant improvements in semen quality after the use of vitamin E as an anti-oxidant (Suleiman et al. 1996), indicating that an increase in lipid peroxidation affects motility. Thus, with the reduction in cell injury caused by the peroxidative processes, there may be a reduction in the percentage of abnormal spermatozoa, as demonstrated in this study. Furthermore, sperm motility and vigour depend directly on mitochondrial integrity, in which the major components are the phospholipids. As fatty acids from conventional animal food induce the oxidation of phospholipids, there would be an increase in the rates of free radical formation, and a subsequent increase in abnormal cells and a reduction in motility.

Based on the results obtained in this study, it is possible to conclude that supplementation with omega 3, omega 6 and omega 9 fatty acids, together with vitamin E, for a period of 60 days, significantly increases the quality of canine ejaculates as it increases semen volume, vigour and concentration, and decreases the proportion of abnormal sperm.

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Author contributions

AAdR - 1) substantial contributions to conception, design, acquisition of data, analysis and interpretation of data; 2) drafting the article and revising it critically for important intellectual content; 3) final approval of the version to be published; 4) technical and logistic support; ICNdC -1) substantial contributions to conception, design, analysis and interpretation of data; 2) drafting the article and revising it critically for important intellectual content; 3) final approval of the version to be published; 4) administrative, technical and logistic support; 5) obtaining funding; 6) guarantor of the study; BBE -1) substantial contributions to conception and acquisition of data; 2) final approval of the version to be published; 3) technical and logistic support; APA -1) substantial contributions to conception; 2) final approval of the version to be published; 3) administrative, technical and logistic support; 4) obtaining funding; CRQ -1) substantial contributions to design, analysis and interpretation of data; 2) drafting the article and revising it critically for important intellectual content; 3) final approval of the version to be published.

Conflicts of interest

The authors have declared no conflicts of interest.

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Author's address (for correspondence): Isabel Candia Nunes da Cunha, Laboratório de Reprodução e Melhoramento Genético Animal, Universidade Estadual do Norte Fluminense (UENF), Av. Alberto Lamego 2000, CEP 28013-602 Campos dos Goytacazes, Rio de Janeiro, Brazil. E-mail: cunhaicn@uenf.br