SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2025v46n1p199

Does supplementation with white copaiba essential oil improve seminal quality in rabbits?

A suplementação com óleo essencial de copaíba branca melhora a qualidade seminal de coelhos?

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Highlights

White copaiba essential oil administered orally decreases sperm vigor in rabbits.
Copaiba essential oil administered orally decreases sperm concentration in rabbits.
White copaiba essential oil increases major sperm defects in rabbits.

Abstract

In this study, we aimed to evaluate the effects of oral supplementation with antioxidant β-caryophyllene-rich white copaiba essential oil (EO) on seminal quality in rabbits. Eight adult rabbits were randomly assigned to two treatments (T), and the ejaculate was used as an experimental unit: T1 (control, *n* = 20), no supplementation with white copaiba EO; and T2 (*n* = 20), supplementation with white copaiba EO. White copaiba EO was diluted in fractionated coconut oil at a concentration of 1%, and oral supplementation (0.002 mL of pure essential oil/day) took place for 95 days. Five seminal samples were collected from each sire. Seminal physical and morphological parameters, functional and physical integrity of the plasma membrane, acrosomal membrane integrity, mitochondrial activity, and sperm chromatin integrity were assessed. The normality of the data was checked using the Shapiro-Wilk test, and the normally distributed data were analyzed using the analysis of variance (ANOVA) test; the Mann-Whitney test was used for analysis of non-normally distributed variables. For all analyses, a *p*-value < 0.05 was considered statistically significant. Statistically significant differences were observed for sperm vigor (T1, 4 ± 1.5; T2, 23 ± 1.7), sperm concentration (T1, 272.5×10^6 ± 76.1×10^6; T2, 168.2×10^6 ±

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Received: Sept. 10, 2024 Approved: Dec. 17, 2024

85.5×10^6 sperms/mL), and percentage of major defects (T1, 6.1 ± 3.1%; T2, 12.7 ± 8.5%). No differences were found regarding sperm motility (T1, 85 ± 5%; T2, 80 ± 15%), percentage of minor defects (T1, 55.8 ± 22.8%; T2, 63.3 ± 11.4%), and percentage of total defects (T1, 62 ± 21.7%; T2, 76.1 ± 10.4%). No differences were observed for structural (T1, 88.7 ± 6.5%; T2, 82.1 ± 14.8%) and functional integrity of the plasma membrane (T1, 32.8 ± 29.4%; T2, 47.3 ± 25.7%), acrosomal membrane integrity (T1, 84.7 ± 5%; T2, 86.4 ± 4.1%), mitochondrial activity (T1, 97.5 ± 6.5%; T2, 94.8 ± 4.7%), and chromatin integrity (T1, 97.8 ± 2.6%; T2, 98.4 ± 3.4%). Overall, oral supplementation with white copaiba EO at a concentration of 1% did not improve seminal physical and functional parameters in rabbits, with a negative impact on important parameters, such as sperm vigor, sperm concentration, and major sperm defects.
Key words: Acrosomal integrity. Mitochondrial activity. Sperm motility.

Resumo

O objetivo com esse estudo foi avaliar o efeito da suplementação oral com óleo essencial de copaíba branca, rico em antioxidante β-cariofileno, sobre a qualidade seminal de coelhos. Utilizou-se oito coelhos adultos distribuídos aleatoriamente em dois tratamentos (T); o ejaculado foi considerado como unidade experimental, sendo: T1 (controle, *n*=20): sem suplementação com o óleo essencial (OE) de copaíba branca e T2 (*n*=20): com suplementação de OE de copaíba branca. O OE de copaíba branca foi diluído em óleo fracionado de coco, na concentração de 1% (0,002 mL de óleo essencial puro/dia) e a suplementação oral ocorreu por 95 dias. Foram realizadas cinco coletas seminais em cada reprodutor, totalizando 20 ejaculados por tratamento. Foram avaliados os parâmetros físicos e morfológicos seminais, a integridade funcional e física da membrana plasmática, integridade da membrana acrossomal, atividade mitocondrial e integridade da cromatina espermática. A normalidade dos dados foi verificada pelo teste de Shapiro-Wilk, aqueles com distribuição normal foram submetidos à ANOVA e para as variáveis que não apresentaram distribuição normal, utilizou-se o teste Mann-Whitney, com 5% de significância para todas as avaliações. Houve diferença para o vigor espermático (T1, 4,0±1,5 x T2, 3,0±1,7); para a concentração espermática (T1, 272,5x10^6±76,1x10^6 x T2, 168,2x10^6±85,5x10^6 espermatozoides/mL); e para a porcentagem de defeitos maiores (T1, 6,1±3,1% x T2, 12,7±8,5%). Não foram encontradas diferenças para motilidade espermática (T1, 85,0±5,0% x T2, 80,0±15,0%); defeitos menores (T1, 55,8±22,8% x T2, 63,3±11,4%); defeitos totais (T1, 62,0±21,7% x T2, 76,1±10,4%). Não foram observadas diferenças para integridade estrutural (T1, 88,7±6,5% e T2, 82,1±14,8%) e funcional (T1, 32,8±29,4% e T2, 47,3±25,7%) da membrana plasmática, acrossoma íntegro (T1, 84,7±5,0% e T2, 86,4±4,1%), atividade mitocondrial completa (T1, 97,5±6,5% e T2, 94,8±4,7%) e cromatina íntegra (T1, 97,8±2,6% e T2, 98,4±3,4%). A suplementação oral com óleo essencial de copaíba branca na concentração de 1% (0,002 mL de óleo essencial puro/dia) não melhorou parâmetros físicos e funcionais seminais de coelhos, com impacto negativo em parâmetros importantes, como vigor espermático, concentração espermática e defeitos maiores. Desta forma, a suplementação diária de coelhos machos com OE de copaíba branca, via oral, no volume supracitado, não se mostrou eficaz.
Palavras-chave: Atividade mitocondrial. Integridade acrossomal. Motilidade espermática.

Introduction

The essential oil (EO) of white copaiba (*Copaífera officinalis*), extracted from the trunk of the copaiba tree, is used for several pharmacological and non-pharmacological purposes (Veiga & Pinto, 2002). Aromatic and phytotherapeutic plant extracts and EOs have been shown to exhibit an antioxidant potential (Dicastillo et al., 2017). Sesquiterpene and diterpene acids constitute >50% of white copaiba oil (Veiga et al., 2005), with β-caryophyllene as the most important constituent. This composition confers the antioxidant property of the oil as it contains precursors of vitamin E. These antioxidant characteristics have been shown to eliminate reactive oxygen species (ROS), with a positive effect on spermatogenesis (Tonini, 2016).

Additionally, these compounds were shown to be capable of protecting biological systems, especially lipid membranes, from oxidative stress-induced damage (Cozzi et al., 1997); moreover, they could reduce sperm susceptibility to lipid peroxidation, preserving the polyunsaturated fatty acids and thereby improving seminal quality and fertility (Tonini, 2016). Furthermore, Bezerra et al. (2016) showed that copaiba oil could optimize the seminal volume and thus improve the reproductive rate in male animals.

The effects of these natural antioxidant compounds have been previously evaluated, aiming to replacing the synthetic products, which might pose some negative effects to the body (Miranda et al., 2021). In addition, these natural compounds provide the advantage of a higher availability, lower toxicity, and strong activity, compared to the synthetic products (Tonini, 2016).

Therefore, in this study, we aimed to evaluate the effects of oral supplementation with the antioxidant β-caryophyllene-rich white copaiba EO on the seminal quality in rabbits.

Material and Methods

The study was carried out at the Federal University of Reconcavo da Bahia (UFRB), in the municipality of Cruz das Almas/Bahia/Brazil, at latitude 12°39'54.23" S and longitude 39°4'41.988" W in autumn. The project was approved by the Ethics Committee on the Use of Animals (CEUA) of UFRB (protocol no. 23007.00000315/2022-93).

Eight adult rabbits with an average body weight of 2.79 ± 0.09 kg and an average body condition score (BCS) of 2.46 ± 0.19 (scale from 1-5) (Burkholder, 2000) were used. The animals were kept in an intensive breeding system and housed in individual galvanized wire cages measuring 80×60×45 cm, with galvanized steel feeders and ceramic drinkers. Each rabbit was fed 100 g of commercial feed per day (Table 1), according to Couto (2002). Roughage (*Boehmeria nivea*) and water were provided *ad libitum.*

Table 1
Nutritional composition of the commercial feed used during the experimental period

Warranty levels	
Moisture (maximum)	130 g/kg (13%)
Crude protein (minimum)	120 g/kg (12%)
Ether extract (minimum)	20 g/kg (2%)
Crude fiber (maximum)	150 g/kg (15%)
Acid detergent fiber – ADF (maximum)	120 g/kg (12%)
Mineral matter (maximum)	120 g/kg (12%)
Calcium (maximum)	20 g/kg (2%)
Calcium (minimum)	12 g/kg (1,2%)
Phosphorus (minimum)	5000 mg/kg (0,5%)
Vitamin A (minimum)	1600 UI/kg
Vitamin D3 (minimum)	200 UI/kg
Vitamin E (minimum)	2.4 UI/kg
Vitamin K3 (minimum)	0.1 mg/kg
Vitamin B2 (minimum)	0.58 mg/kg
Vitamin B12 (minimum)	1.0 mcg/kg
Calcium pantothenate (minimum)	1.86 mg/kg
Niacin (minimum)	2.99 mg/kg
Iodine (minimum)	0.06 mg/kg
Manganese (minimum)	6.5 mg/kg
Copper (minimum)	0.75 mg/kg
Zinc (minimum)	4.89 mg/kg
Selenium (mínimo)	0.04 mg/kg

A completely randomized design (CRD) was used, with the ejaculate as an experimental unit. The animals were randomly divided into two treatments (T): T1 (n = 20) without supplementation with white copaiba EO and T2 (n = 20) with white copaiba EO supplementation. White copaiba EO was orally administered daily, whereas water was administered at the same time to the rabbits in the control group. The solution was administered for 58 days before seminal collection, as well as throughout the collection period, with a total of 95 days of supplementation.

White copaiba EO (Therra by Laszlo, Brazil) (Figure 1) was used at a concentration of 1% after dilution in fractionated coconut oil (DoTerra, USA), and 0.2 mL of the solution was daily administered to each rabbit. Therefore, the volume of white copaiba EO in the solution was 0.002 mL, and that of the major compound (β-caryophyllene) was 0.000776 mL.

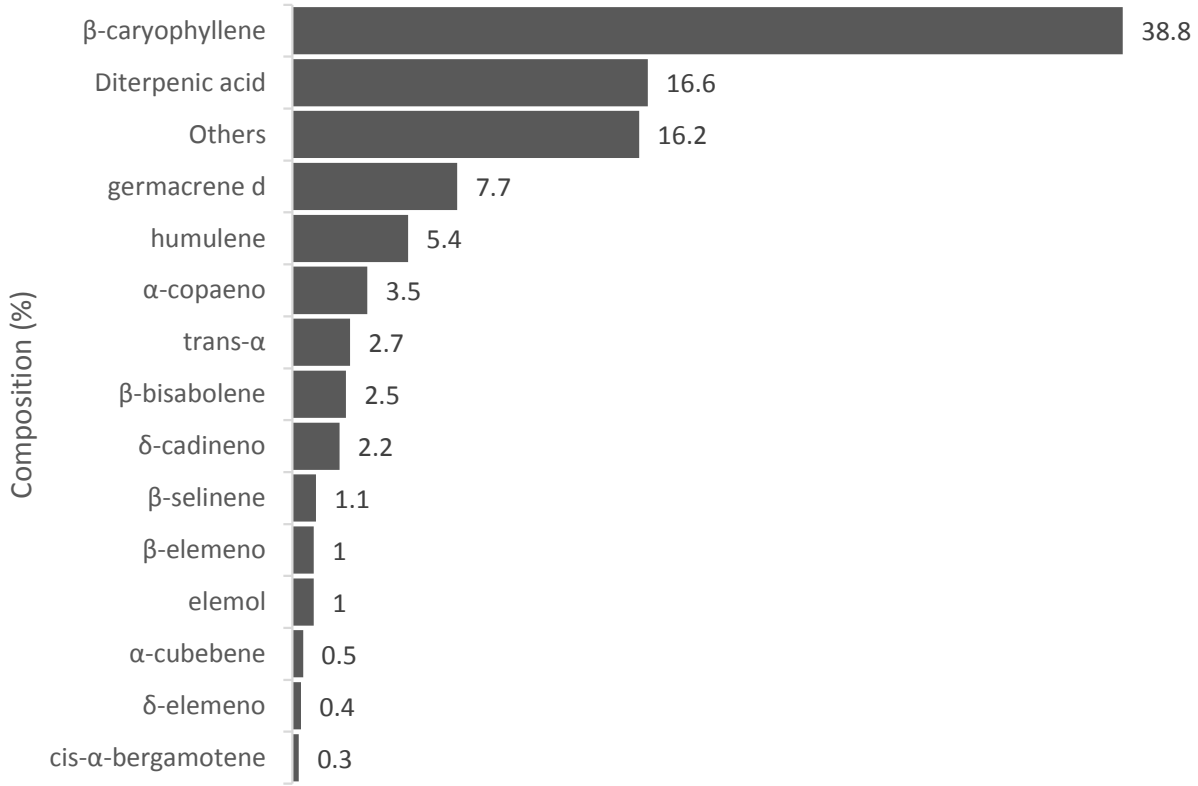

Figure 1. Chemical composition of the essential oil of white copaiba (*Copaifera officinalis*) used in this study, obtained by means of high-resolution gas chromatography.

Semen collection was performed twice weekly using an artificial vagina and a mannequin as a female. Seminal physical (progressive sperm motility, sperm vigor, and sperm concentration) and morphological aspects (minor defects, major defects, and total defects) were evaluated; in addition, complementary tests to assess the functional and physical integrity of the plasma membrane, integrity of the acrosomal membrane, mitochondrial activity, and sperm chromatin integrity were performed.

Sperm concentration was measured using a Neubauer chamber, with a dilution of 1:50. Sperm morphology was assessed using a wet slide under a phase-contrast microscope (Olympus, Japan) at a magnification of 1,000× under immersion.

The functional integrity of the plasma membrane was assessed using the hypo-osmotic swelling test (HOST), as described by Jeyendran (1984) and Kumi-Diaka (1993). Semen (10 µL) was added to 1 mL of a fructose-based hypo-osmotic solution (100 mOsmol.kg^{-1}) and incubated in a water bath at 37 °C for 30 min. Subsequently, slides were prepared for evaluation under the phase-contrast microscope (Olympus, Tokyo, Japan) at 400× magnification. A total of 200 cells were counted and classified as reactive and non-reactive spermatozoa, and the percentages of both were calculated.

The structural integrity of the plasma membrane was assessed as described by Björndahl et al. (2003) after seminal staining with eosin-nigrosin dye. Equal volumes of the semen and dye were mixed, and one drop of the solution was used to prepare the seminal smear. The smear was then air-dried and evaluated directly using a light microscope (Olympus, Japan) at 1000× magnification under oil immersion, where 200 sperm cells were counted. Pink- or red-stained sperm was classified as dead, whereas unstained (white) sperm was classified as viable.

To assess the integrity of the acrosomal membrane, 100 mL of a saturated aqueous Congo red solution and 100 mL of a 0.5% aqueous gentian violet solution were prepared. A thin smear slide with fresh semen was prepared and air-dried. The slide was immersed in the Congo red solution for one minute, gently washed with running water and air-dried, followed by immersion in the gentian violet solution for 30 s, washing and drying again. The assessment was performed using light microscopy (Olympus, Japan) at 1000× magnification under immersion, where 200 cells were counted and classified as 1) intact acrosome; 2) irregular acrosome; 3) partial detachment of the acrosome; or 4) total detachment of the acrosome, as described by Cerovsky (1976).

Sperm mitochondrial activity was assessed according to the method described by Hrudka (1987). Semen (20 µL) was added to 20 µL of 3,3'-diaminobenzidine (DAB) in 1 mg/mL phosphate-buffered saline (PBS) and incubated at 37 °C for 60 minutes in the dark. After incubation, the smears were prepared and fixed for 10 minutes in 10% formaldehyde in the dark, followed by washing the slides with distilled water and air drying. The samples were then evaluated using phase-contrast microscopy (Olympus, Japan) at 1000× magnification under immersion. Two hundred sperms were counted and classified according to the deposition of the dye in the intermediate part as class I (the intermediate part fully stained), II (≥ 50% of the intermediate part stained), III (≤ 50% of the intermediate part stained), or IV (the intermediate part not stained).

To assess chromatin integrity, seminal smears were prepared and fixed in Carnoy's solution for 1 minute, followed by fixation in 70% ethanol for 3 minutes and hydrolysis of the smears in hydrochloric acid for 15 min. These smears were then washed with distilled water and dried at room temperature. After drying, a drop of 0.025% toluidine blue in McIlvaine buffer, pH 4 was placed between the slide and coverslip. A total of 500 cells were counted and evaluated using phase contrast microscopy (Olympus, Japan) at 1000× magnification under immersion, and classified as intact chromatin (light blue staining of the head region) or fragmented chromatin (dark blue or violet staining of the head region) (Beletti & Mello, 2004).

The normality of the data was checked using the Shapiro-Wilk test, and the normally distributed data were analyzed using the analysis of variance (ANOVA) test, whereas the Mann-Whitney test was used for the analysis of non-normally distributed variables. For all analyses, a p-value < 0.05 was considered statistically significant.

Results and Discussion

Oral supplementation of rabbits with white copaiba EO did not significantly affect the progressive sperm motility or percentage of minor defects and total defects ($p < 0.05$); however, significant differences were observed with regard to the sperm vigor, sperm concentration, and percentage of major defects ($p < 0.05$) (Table 2).

The progressive sperm motility was 85 ± 5% and 80 ± 15% in the white copaiba EO-supplemented and control rabbits, respectively ($p = 0.093$). According to Alvariño (1993), raw rabbit semen is considered to be of good quality when the progressive sperm motility is equal to or greater than 60%.

In a study carried out by Tonini (2016) to evaluate the effects of addition to the diet on the seminal characteristics in roosters, copaiba oil-resin had no significant effects on the progressive sperm motility. Similarly, Bezerra et al. (2016) did not observe any differences in the progressive sperm motility upon addition of copaiba oil (*Copaifera* sp.) to the diet of semi-heavy breeding roosters. However, the simultaneous use of antioxidant protein compounds and micronutrients that directly impact the health and metabolism of male rabbits is expected to improve sperm motility.

Table 2
Seminal physical parameters and sperm morphology of rabbits supplemented with white copaiba essential oil orally

Parameters	Without copaiba oil (n=20)	With copaiba oil (n=20)	*P* Value
Motility (%)[1]	85,0±5,0	80,0±15,0	0,093
Sperm vigor (0-5)[1]*	4,0±1,50[a]	3,00±1,7[b]	0,046
Concentration (sptz/mL)[2]**	272,5x10^6±76,1x10^{6a}	168,2x10^6±85,5x10^{6b}	0,015
Minor defects (%)[2]	55,8±22,8	63,3±11,4	0,395
Major defects (%)[2]**	6,1±3,1[a]	12,7±8,5[b]	0,045
Total defects (%)[2]	62,0±21,7	76,1±10,4	0,099

[1]Nonparametric data refer to median ± interquartile range. *There was a difference between treatments by the Mann-Whitney test, 5% significance. [2]Data refer to mean ± standard deviation. **There was a difference between treatments by the Dunnett test, 5% significance.

In addition, rabbits that received 1% white copaiba EO showed lower sperm vigor than the controls (4 ± 1.5 and 3 ± 1.7, respectively; $p = 0.046$; Table 2). However, the sperm vigor values in both groups remained within the standard that is considered ideal for rabbits (3 on a scale of 0-5), according to Alvariño (1993). In contrast to our findings,

Bezerra et al. (2016) did not observe any differences in roosters supplemented with copaiba oil-including diet at different concentrations (0.10, 0.15, 0.2, 0.25, 0.30, 0.35, and 0.40%); however, it is noteworthy that the white copaiba EO concentration was lower than that used in our study (1%).

The sperm concentration was lower in the rabbits supplemented with white copaiba EO ($168.2\times10^6 \pm 85.5\times10^6$ sptz/mL) than that in the control rabbits ($272.5\times10^6 \pm 76.1\times10^6$ sptz/mL; $p = 0.015$; Table 2). It has been previously shown that the rabbit sperm concentration varies between 150×10^6 and 900×10^6 sptz/mL, with an ideal value $\geq250\times10^6$ sptz/mL (Alvariño, 1993). Similarly, Bezerra et al. (2016) showed that the inclusion of copaiba oil (at concentrations ranging from 0.1% to 0.4%) in the diets resulted in a gradual reduction in the sperm concentration in semi-heavy breeding roosters.

However, these findings are not in line with the results of Tonini (2016) showing insignificant differences in the sperm concentration upon addition of copaiba oil-resin (25 mg/day) to the diet of breeding roosters.

The major active compound in white copaiba EO, β-caryophyllene exhibits antioxidant effects and is therefore expected to increase cell division and meiotic efficiency, resulting in a beneficial effect on spermatogenesis and sperm concentration; however, an opposite effect was observed. This finding might be attributed to the concentration of EO used in our study, which might have a pro-oxidative effect. The capacity of the antioxidants to have anti- and/or pro-oxidant activity depending on the concentration was previously described by Prado (2012).

The percentages of the minor and total defects were not significantly different between the copaiba EO and control groups ($p = 0.395$ and 0.099, respectively; Table 2). However, the percentage of total sperm defects in both groups (76.1 ± 10.4% and 62 ± 21.7% in the copaiba EO-supplemented and control rabbits, respectively) exceeded 25%, which is the accepted value in rabbits (Sinkovics et al., 1983). A higher percentage of major sperm defects was observed in the rabbits supplemented with white copaiba EO (12.77 ± 8.59%) than in the control rabbits (6.16 ± 3.1%; $p = 0.045$; Table 2). However, in both groups the percentage of major defects remained below 15%, which is considered the upper normal value in rabbits (Sinkovics et al., 1983). Rabbits with ejaculates that exceed these limits of sperm defects should be re-evaluated as several factors can interfere with spermatogenesis, leading to temporary or permanent abnormal sperm production. In rabbits, heat stress can result in a high percentage of sperm abnormalities, mainly tail alterations, owing to the sensitivity of sperms to high environmental temperatures (Finzi et al., 1995).

The physical integrity of the sperm plasma membrane was not significantly different between the two groups (82.1 ± 14.8 vs. 88.7 ± 6.5% in the copaiba EO and control groups, respectively; $p = 0.88$; Table 3). Ptaszynska & Molina (2007) showed that rabbits exhibited an average of 84% of viable sperms in the fresh semen. Thus, the values obtained in the present study are within the expected range.

Table 3
Structural and functional integrity of the plasma membrane and sperm chromatin of rabbits supplemented with white copaiba essential oil orally

Parameters (%)	Without copaiba oil (n=20)	With copaiba oil (n=20)	*P* Value
Structural integrity of plasma membrane *			
Alive	88.7±6.5	82.1±14.8	0.880
Dead	11.2±6.7	17.8±14.8	0.880
Functional integrity of plasma membrane (HOST)*			
Reactives	32.8±29.4	47.3±25.7	0.126
Non-Reactive	67.1±29.4	52.6±25.7	0.126
Chromatin integrity **			
Intact	97.8±2.6	98.4±3.4	0.408
Fragmentada	2.2±2.6	1.6±3.4	0.408

*Data refer to mean ± standard deviation. There was no difference by Analysis of Variance, 5% significance. **Non-parametric data refer to median ± interquartile range. There was no difference between treatments by Mann-Whitney test, 5% significance. HOST *(Hypo-osmotic swelling test)* = hypo-osmotic swelling test.

Ginger supplementation (200 mg/kg of diet) resulted in an increase in the percentage of viable sperms in breeding rabbits (El-Ratel et al., 2021). In addition, they showed that the structural integrity of the plasma membrane was significantly different in the treatment group (80.3 ± 1.065%), compared to that in the control.

In contrast to El-Ratel et al. (2021), there was no significant difference in the functional integrity of the sperm cell membrane between the two groups in the present study (Table 3). HOST analysis showed that the average percentage of reactive cells was 40.11 ± 27.57%. Therefore, <50% of the sperms in both groups had an intact plasma membrane.

Hosny et al. (2020) also studied the functional integrity of the sperm plasma membrane upon supplementing rabbits with organic selenium (OSe; 0.3 mg/kg of dry matter) for 12 consecutive weeks. The functional integrity of the sperm plasma membrane improved (79.16%) in the OSe-treated rabbits, which may be related to the antioxidant activity of OSe, protecting the sperm plasma membrane against the negative effects of free radicals and lipid peroxidation.

Regarding the rabbit sperm chromatin compaction, there was no differences between the two groups (Table 3). The rabbits receiving the EO showed 98.4 ± 3.4% sperms with intact chromatin and 1.6 ± 3.4% sperms with fragmented chromatin, showing that supplementation with white copaiba EO did not affect the rabbit sperm DNA. According to Evenson (2016), evaluation of sperm chromatin compaction is essential since DNA damage is detrimental to the pregnancy rate and embryonic development.

Chromatin is essential for the morphological structure of the sperm head. Furthermore, chromatin condensation protects DNA against mutagens, free radicals, and nucleases. Thus, changes in sperm chromatin compaction may lead to fertility problems in males (Kanayama & Beletti, 2011). Gally (2023) observed no differences in the percentage of intact and fragmented chromatin between the ginger EO-supplemented (0.5%) and control rabbits, whereas the percentage of intact chromatin was slightly higher in the rabbits receiving ginger EO (95.3 ± 3.66%) than that in the control rabbits (95.26 ± 3.1%), which might be attributed to the antioxidant potential of ginger oil.

The acrosomal integrity of sperms was not different between the two groups, with an average of 85.6 ± 4.57% sperms exhibiting an intact acrosomal membrane (class I; Table 4). According to Ptaszynska & Molina (2007), fresh rabbit semen normally have an average of 88% sperms with normal acrosomes. In addition, the percentage of classes representing damaged acrosomes showed no significant differences between the two groups.

Table 3
Acrosomal integrity and sperm mitochondrial activity of rabbits supplemented orally with white copaiba essential oil

Parameters (%)	Without copaiba oil (n=20)	With copaiba oil (n=20)	*P* Value
Acrosomal integrity*			
Class I	84.7±5.0	86.4±4.1	0.294
Class II	7.8±2.7	6.2±2.0	0.061
Class III	3.3±1.3	3.2±0.9	0.751
Class IV	3.9±1.5	4.0±1.8	0.883
Mitochondrial activity**			
Class I	97.5±6.5	94.8±4.7	0.899
Class II	1.5±4.5	2.0±3.2	0.911
Class III	1.0±1.0	1.0±1.3	0.626
Class IV	0.0±0.0	0.0±0.2	0.827

*Parametric data refer to mean ± standard deviation. Class I = Intact acrosome; Class II = Irregular acrosome; Class III = Partial detachment of the acrosome; Class IV = Total detachment of the acrosome. There was no difference between treatments by ANOVA at 5% significance. **Nonparametric data refer to median ± interquartile range. There was no difference between treatments by Mann-Whitney test, 5% significance. Class I = Complete mitochondrial activity; Class II = Average mitochondrial activity; Class III = Low mitochondrial activity; Class IV = Absence of mitochondrial activity.

Anciuti et al. (2022) showed that the topical use of copaiba oil at any concentration in rats negatively interfered with the acrosomal integrity of the sperm cells. At concentrations of 0.01% and 0.1%, the acrosomal integrity was 40.8 ± 6% and 50.4 ± 7%, respectively. The authors attributed this finding to the chemical composition of copaiba oil with approximately 72% hydrocarbons and 28% carboxylic acids (50% of each type of terpenes), which might result in acrosomal quality reduction since these acids could affect the lipid composition of the acrosomal membrane and increase the membrane fluidity.

In contrast, Hosny et al. (2020) showed a higher percentage of sperms with intact acrosomes (96.86%) upon supplementing the diet of rabbits with OSe (0.3 mg/kg of dry matter). In general, supplementation with OSe significantly improved the semen quality and reproductive performance of rabbits.

El-Ratel et al. (2021) evaluated the effects of natural antioxidants on the semen characteristics in rabbits, showing that supplementation with ginger (200 mg/kg of diet) increased the percentage of sperms with intact acrosomes (85.5 ± 1.013%; $p < 0.05$), compared to that in the control group. Addition of ginger to the diet reduced oxidative stress and improved the quantitative and qualitative characteristics of semen, suggesting that ginger supplementation might be an option to increase the reproductive efficiency of breeding rabbits.

Regarding the sperm mitochondrial activity, there was no differences between the groups for all the classes evaluated (Table 4). Both groups showed high levels of sperms with complete mitochondrial activity (class I; 94.85 ± 4.75% and 97.5 ± 6.5%, in the copaiba EO and control groups, respectively). In addition, the percentages of sperms with class II, III, and IV mitochondrial activity were also comparable between the groups.

Gally (2023) demonstrated that ginger EO (0.5%) did not alter the sperm mitochondrial activity in rabbits. Rabbits supplemented with ginger EO and control rabbits exhibited 91 ± 7.25 and 89.5 ± 11 sperms with complete mitochondrial activity (class I), respectively. According to Angrimani et al. (2015), ginger EO exerts antioxidant action and have the potential to protect the mitochondria from oxidative damage. Therefore, evaluation of the mitochondrial activity is important since the mitochondria are responsible for the production of cellular energy to maintain sperm motility and vigor, as well as other various cellular processes affecting the sperm quality and fertility.

Conclusion

Oral supplementation with white copaiba EO at a concentration of 1% (0.002 mL of pure essential oil/day) did not improve the physical and functional characteristics of seminal fluid in rabbits with a negative impact on important parameters, such as sperm vigor, sperm concentration, and major defects, although all sperm parameters were within the normal limits. Therefore, daily oral supplementation of male rabbits with white copaiba EO at the given concentration is not effective in improving the seminal quality.

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