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Effects of crude palm oil consumption on sperm parameters in growing rats

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Abstract

Palm oil is by far the most widely used vegetable oil in the world. Despite its controversial health effects, few studies have examined its impact on reproductive function. This study aims to evaluate the effects of crude palm oil consumption on sperm parameters in growing rats. Twelve male *Rattus norvegicus* rats, aged 21 days and weighing between 47 and 51 g, were divided into two groups of six subjects: a control group fed a diet containing sunflower oil and an experimental group receiving a diet based on crude palm oil. The diets, based on the AIN-93G model, were administered *ad libitum* for 60 days. The animals' body weight was monitored weekly, and at the end of the experiment, the reproductive organs were harvested to determine their relative weight. Sperm parameters were also assessed using epididymal suspension. The results show that starting on the 14th day of the experiment, rats in the experimental group exhibited a significant increase ($p<0.05$) in body weight compared to control rats. In addition, a significant decrease in the weight of the testes ($p<0.001$) and epididymis ($p<0.01$) was observed in animals fed the crude palm oil diet. Analysis of sperm parameters also revealed a significant decrease ($p<0.001$) in sperm concentration and motility in the experimental group compared to the control group. Thus, prolonged consumption of crude palm oil may have a negative effect on certain parameters of male reproductive function in growing rats.

Keywords: Crude Palm Oil; Sperm motility; Sperm concentration; 2.4.4. Sperm morphology; Growing Rats

1. Introduction

Palm oil is by far the most widely used vegetable oil in the world [1]. It is found in about half of all everyday food products, ranging from snacks to cosmetics [2]. It is produced from the fleshy mesocarp of the fruit of the oil palm (*Elaeis guineensis*) [3]. This plant is native to the Gulf of Guinea and is widely cultivated in tropical regions, particularly in Asia, Latin America, and Africa [4]. Palm oil is prized for its high availability, high oil yield per hectare, as well as its versatility and relatively low production costs [5]. For the 2024/2025 crop year, the Foreign Agricultural Service of the U.S. Department of Agriculture estimates global palm oil production at approximately 80 million tons [6]. Indonesia and Malaysia are the leading producers, followed by Thailand and Colombia. In Africa, Nigeria and Côte d'Ivoire are the largest producers of palm oil [6]. Although demand for palm oil has accelerated with the emergence of new markets in the biofuel sector [7], nearly 80% of its use remains concentrated in the food industry [8]. Commonly used in its crude form in Côte d'Ivoire (commonly known as red oil), crude palm oil is characterized by its particularly high content of carotenoids and tocotrienols [9]. These compounds play an essential role in preventing vitamin A deficiency in at-risk populations [10]. They also contribute to the protection of brain tissue, the maintenance of cognitive functions, and may reduce the risk of cognitive decline or brain damage [11]. Furthermore, they help reduce oxidative stress, the risk of atherosclerosis, and hypertension [12]. Finally, they strengthen the immune system and provide cellular protection [13]. Furthermore, studies on the nutritional value of this oil have focused on its fatty acid composition. Crude palm oil

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contains 44% palmitic acid and 4.5% stearic acid among its saturated fatty acids. It also contains unsaturated fatty acids such as oleic acid (39%) and linoleic acid (10%) [14,15,16]. However, despite the growing number of scientific publications recommending the consumption of palm oil due to its nutritional, antioxidant, and therapeutic benefits [17,18], other studies disagree and link its high saturated fat content to adverse health effects [19,20].

Thus, despite the abundance of available data on palm oil consumption and its health effects, few studies have specifically examined its impact on reproductive function, particularly in growing organisms. However, puberty and adolescence are critical periods during which spermatogenesis is particularly sensitive to dietary and environmental factors.

In this context, it appears essential to examine the influence of crude palm oil consumption on sperm parameters. This study is conducted with this objective in mind, aiming to evaluate the effects of crude palm oil consumption on sperm parameters in growing male rats.

2. Materials and Methods

2.1. Biological Materials

The species *Rattus norvegicus* (Muridae) was used in this study. The animals were obtained from the vivarium of the École Normale Supérieure (ENS) in Abidjan (Côte d'Ivoire). A total of 12 growing male rats aged 21 days, weighing between 47 g and 51 g, were selected. They were divided into two groups of six rats, comprising an experimental group and a control group. They were caged and housed in a room at the aforementioned institution with a temperature ranging between 27 and 29 °C, relative humidity of 65%, and a photoperiod of 12 hours of natural light and 12 hours of darkness.

To formulate the diets, the following ingredients were required: dried fish (herring), corn, crude palm oil, sunflower oil, rice husks, and Vitaflash, which were purchased at the local market and pharmacy.

2.2. Diet preparation

Growing rats, just weaned (21 days old), were fed *ad libitum* a formulation based on the AIN-93G diet (American Institute of Nutrition, 1993) [21], the composition of which was adapted by replacing the traditional sources with: dried fish meal (herring) for protein, corn meal as the primary source of carbohydrates, crude red palm oil (experimental diet) or sunflower oil (control diet) as the lipid source, Vitaflash as the vitamin complex, and ground rice hulls as the fiber source.

2.2.1. Diet of the experimental group

The diet of the experimental group, or diet containing crude palm oil (DCpo), was prepared using corn meal, dried fish meal, crude palm oil, ground rice hulls, and Vitaflash.

The formulation for 1 kg of feed consisted of 620 g of corn meal, 250 g of fish meal, 70 g of crude palm oil, 30 g of ground rice husks, and 30 g of Vitaflash. The dry ingredients were first homogenized using a spatula for 5 minutes, then the crude palm oil, previously liquefied in a water bath at less than 40 °C, was gradually incorporated. The well-homogenized mixture was heated over low heat for 10 minutes. The final mixture was formed into small balls, dried at less than 40 °C, cooled, and then stored in opaque, airtight bags, protected from light and moisture.

2.2.2. Control group diet

The control group diet, or diet containing sunflower oil (Dso), was prepared using corn meal, dried fish meal, crude palm oil, ground rice hulls, and Vitaflash.

The formulation for 1 kg of feed consisted of 620 g of corn meal, 250 g of fish meal, 70 g of sunflower oil, 30 g of ground rice hulls, and 30 g of Vitaflash. The dry ingredients were first homogenized using a spatula for 5 minutes, then the sunflower oil was gradually incorporated. The well-homogenized mixture was heated over low heat for 10 minutes. The final mixture was formed into small balls, dried at less than 40°C, cooled, and then stored in opaque, airtight bags, protected from light and moisture.

2.3. Experimental design

The rats, divided into two groups of six subjects, one experimental group and one control group, were fed their respective diets every morning around 8:00 a.m. for 60 days. Water was provided *ad libitum* and changed daily. The animals were weighed at the start of the experiment and then once a week. At the end of the experiment, the animals were weighed one last time, then anesthetized for sperm collection, and subsequently euthanized for the collection, weighing, and fixation in 10% formalin of certain organs of the reproductive system: testes, right epididymis, seminal vesicle, and prostate.

2.4. Measurement of sperm parameters

2.4.1. Sample preparation

To prepare the epididymal suspension containing sperm, the animals were euthanized in accordance with ethical guidelines for the protection of animals used for scientific purposes [22]. Immediately after sacrifice and dissection, the tail of the left epididymis was removed by opening the scrotum and placed in 5 mL of saline solution (0.9% NaCl) that had been pre-warmed in a water bath at 36 °C. The spermatozoa were then allowed to diffuse in the medium [23,24].

2.4.2. Sperm motility

Sperm motility was assessed immediately after sacrifice. A small drop of the crude epididymal suspension was placed and lightly spread on a slide that had been pre-incubated at 36 °C. The slide was placed on an optical microscope (Olympus CX31RBSF, Philippines) at a magnification of ×100 [25]. The spermatozoa were filmed and recorded using an AmScope camera (London, United Kingdom). Motile and immotile spermatozoa were counted in five randomly selected fields of view, each comprising ten spermatozoa. The motility percentage was then calculated based on the number of motile spermatozoa relative to the total observed [26].

2.4.3. Sperm concentration

For sperm counting, 50 µL of the crude epididymal suspension was transferred to a tube containing 950 µL of 0.9% NaCl, resulting in a 1:20 dilution [27]. The mixture was gently homogenized to achieve a uniform distribution of sperm. Then, 10 µL of the diluted suspension was placed on a Malassez slide and covered with a coverslip [28]. Counting was performed using a photonic microscope (Olympus CX31RBSF, Philippine) (×400) across five grid squares of the Malassez slide [23,29]. The concentration was calculated using the formula:

$$Concentration\ (sperm/mL) = \frac{N}{5} \times 20 \times 10^4$$

N represents the total number of sperm counted in five fields, 20 is the dilution factor, and 10^4 is the conversion factor based on the volume of the field.

2.4.4. Sperm morphology

The morphological abnormalities considered included fusion, head separation, and deformities of the head and/or flagellum. A total of 200 spermatozoa were examined in liquid medium across three random microscopic fields, and the percentage of normal forms was determined [26,30].

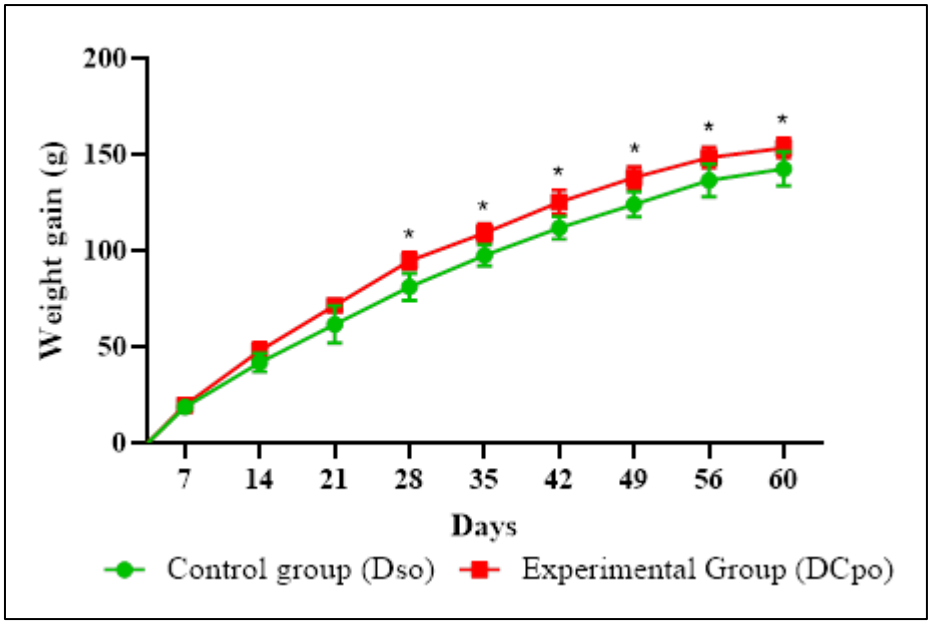
2.5. Statistical analyses

The various values obtained were expressed as the mean followed by the standard error of the mean (Mean ± SEM). The significance of the differences observed between the animal groups was assessed using the Student's t-test, with the GraphPad Prism 8.0.1 software (California, USA).

3. Results

3.1. Body weight of rats

At the start of the experiment, the average body weights of the rats in the control and experimental groups were comparable. This lack of difference persisted until the seventh day. Starting on the 14th day, a significant increase in body weight was observed in the rats of the experimental group compared to the control group ($p<0.05$). This trend persisted until the 60th day (Figure 1).



Dso: diet containing sunflower oil; DCpo: diet containing crude palm oil.; Values are M ± SEM (n = 6). (*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

Figure 1 Changes in body weight in rats over the 60-day experimental period

3.2. Relative weight of the reproductive organs

After 60 days of experimentation, the rats in the control group had a relative testicular weight of 0.95 ± 0.006, while those on the DCpo diet (0.88 ± 0.004) had a significantly lower value ($p < 0.05$). Similarly, the relative weight of the epididymis was significantly reduced ($p<0.01$) in animals in the experimental group (0.20 ± 0.004) compared to the control group (0.23 ± 0.005). In contrast, no significant difference was observed in the relative weights of the seminal vesicles and prostates between the two groups (Table 1).

Table 1 Relative weights of the reproductive organs at the end of the 60-day experiment

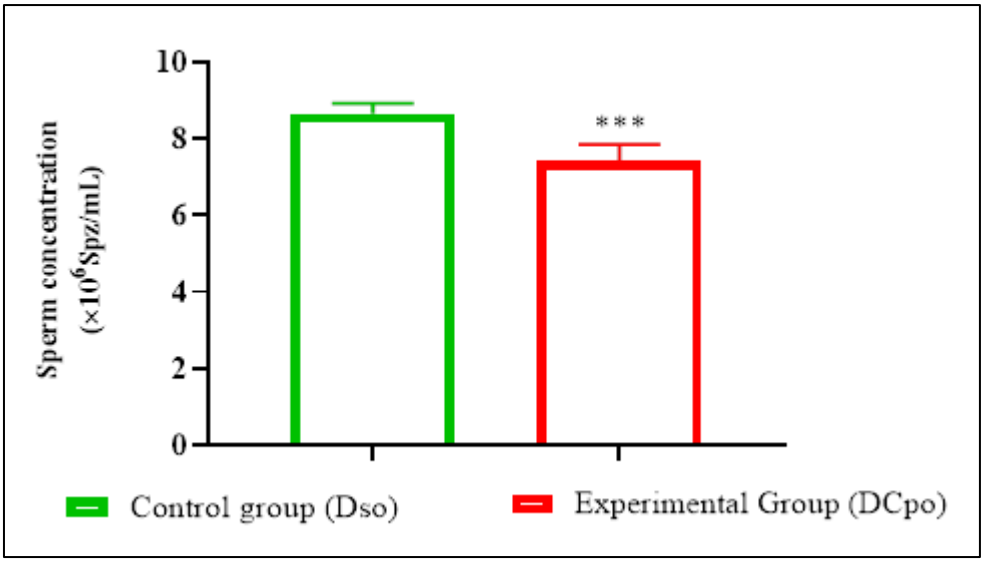
Groups of rats	**Relative weight (g/100g)**			
	Testes	Right epididymis	Seminal vesicle	Prostate
Control group (Dso)	0,95 ± 0,006	0,23 ± 0,005	0,35 ± 0,003	0,16 ± 0,006
Experimental group (DCpo)	0,88 ± 0,010 ***	0,20 ± 0,004 **	0,34 ± 0,005	0,15 ± 0,004

Dso: diet containing sunflower oil; DCpo: diet containing crude palm oil.; Values are M ± SEM (n = 6). (*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

3.3. Semen parameters

3.3.1. Sperm concentration

Analysis of sperm concentration at the end of the 60-day experimental period revealed a significant decrease ($p < 0.001$) in animals fed the DCpo diet compared to the control group fed the Dso diet. Indeed, the mean sperm concentration expressed in spermatozoa/mL was $8.65 \times 10^6 \pm 0.27$ in the control group, compared to $7.43 \times 10^6 \pm 0.42$ in the experimental group, corresponding to a relative reduction of 14.10% (Figure 2).

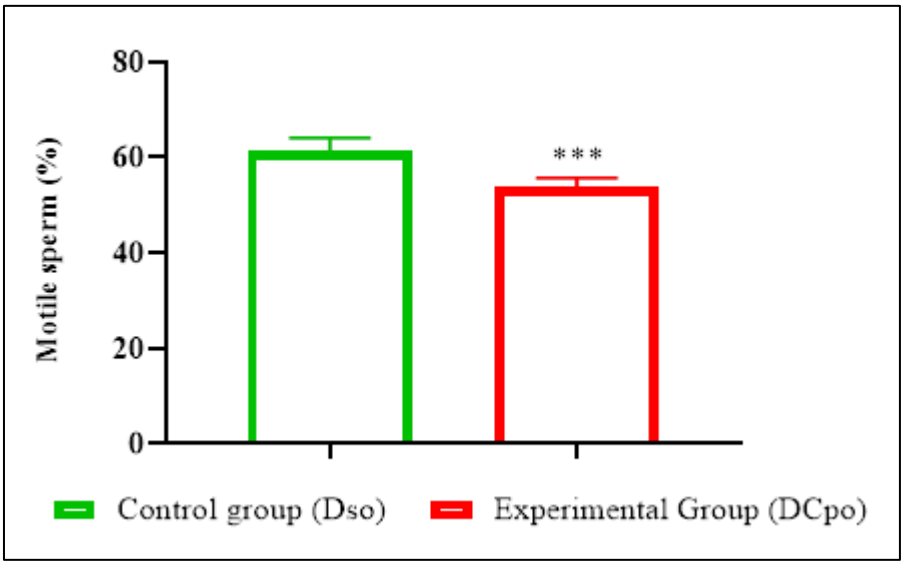


Dso: diet containing sunflower oil; DCpo: diet containing crude palm oil.; Values are M ± SEM (n = 6). (*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

Figure 2 Sperm concentration at the end of the 60-day experiment

3.4. Sperm motility

At the end of the 60-day treatment period, analysis of sperm motility revealed a relative decrease of 12.49% in the animals of the experimental group, compared to the rats in the control group fed sunflower oil. The average percentage of motile sperm was 61.33 ± 0.03% in the control group, whereas it was only 53.67 ± 0.02% in the experimental group (Figure 3).

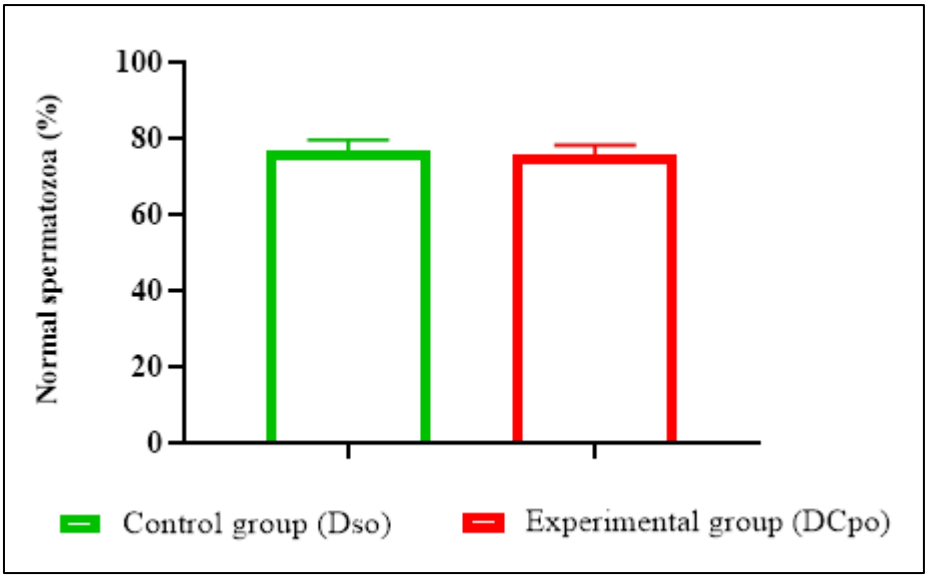


Dso: diet containing sunflower oil; DCpo: diet containing crude palm oil.; Values are M ± SEM (n = 6). (*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

Figure 3 Sperm Motility at the end of the 60-day experiment

3.5. Sperm morphology

The evaluation of sperm morphology after 60 days of the experiment revealed no significant differences ($p > 0.05$) between the animals on the two diets. Nevertheless, a slight relative decrease of 1.63% in the percentage of normal spermatozoa was observed in the experimental group (75.56 ± 0.02%), compared to the control group (76.81 ± 0.03%) (Figure 4).



Dso: diet containing sunflower oil; DCpo: diet containing crude palm oil.; Values are M ± SEM (n = 6). (*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

Figure 4 Sperm Morphology at the end of the 60-day experiment

4. Discussion

The results show that prolonged consumption of crude palm oil by growing male rats leads to changes in body weight as well as certain sperm parameters.

With regard to body weight, rats fed a diet containing crude palm oil showed a significant increase in weight starting on the 14th day compared to the control rats. This weight gain could be linked to the high energy density and high saturated fat content of palm oil. Indeed, according to the work of Sundram et al. [31]and Hariri and Thibault [32], palm oil is a significant source of lipids that can increase body weight when consumed regularly. Similarly, Fattore and Fanelli [33] report that diets high in saturated fatty acids can promote an increase in body mass and metabolic changes in laboratory animals.

With regard to the reproductive organs, a significant decrease in the relative weight of the testes and epididymis was observed in rats fed a diet containing crude palm oil. This decrease may reflect an impairment in spermatogenic activity or testicular endocrine functions. Similar results were reported by Aitken and Roman [34], who indicate that certain dietary changes can affect the structure and function of testicular tissues. Furthermore, Rato et al. [35] emphasize that a high-fat diet can disrupt testicular metabolism and influence sperm production.

Analysis of sperm concentration also reveals a significant decrease in rats fed a diet containing crude palm oil. This reduction could be linked to a disruption of the spermatogenesis process or an alteration of the epididymal environment. According to Sharpe [36] and Gabrielsen and Tanrikut [37], spermatogenesis is particularly sensitive to environmental and nutritional factors. Furthermore, Funes et al. [38] indicate that oxidative stress induced by certain high-fat diets can lead to a decrease in sperm production.

Sperm motility was also affected in our study, with a notable decrease in rats from the experimental group. Sperm motility is a key indicator of male fertility and depends heavily on the integrity of cell membranes and oxidative balance [39]. According to Agarwal et al. [40], increased oxidative stress can alter the structure of sperm membranes and reduce sperm motility.

In contrast, analysis of sperm morphology did not reveal any significant differences between the two groups, although a slight decrease in the percentage of normal sperm was observed in rats in the experimental group. This relative morphological stability could be explained by the fact that certain alterations in reproductive function first appear in sperm concentration and motility before affecting the morphological structure of spermatozoa. Similar results were reported by Cooper et al. [41], who indicate that morphological changes may occur at more advanced stages of impaired spermatogenesis.

5. Conclusion

Rats fed the diet containing crude palm oil showed a significant increase in body weight from the 14th day of the experiment through the 60th day. In addition, a significant decrease in the relative weight of the testes and epididymis was observed in the rats of the experimental group. Analysis of sperm parameters also revealed a significant decrease in sperm concentration and motility in animals exposed to the crude palm oil diet. In contrast, no significant changes in sperm morphology were observed between the groups, although a slight reduction in the percentage of normal sperm was noted in the experimental group.

Thus, prolonged consumption of crude palm oil may have adverse effects on certain parameters of male reproductive function in growing rats, particularly by reducing sperm production and motility.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The animals used in this study were treated in accordance with the ethical principles of the Canadian Council on Animal Care regarding the use of animals in science.

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