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Research Article

# Effect of Palm Olein on Reproductive Parameters in Male Guinea Pig (*Cavia porcellus*)

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## Abstract

Palm olein is a clear, golden, yellow edible oil obtained from crude red palm oil after refining. The aim of this study was to investigate the effects of palm olein on some reproductive parameters in male guinea pigs. 40 adult male guinea pigs weighing 323 ± 84.43g were randomly distributed into 4 groups of 10 animals each. For 75 days, the control group (T0) and the 3 other groups (T1, T2 and T3) were orally given 1ml of distilled water/kg of body weight (b.w), 1, 2 or 3 ml of palm olein/kg of b.w respectively. Results showed that the proportion of males which reacted in the presence of a female was insignificantly ($p > 0.05$) greater in treated animals (100; 100 and 71.43% respectively for T1, T2 and T3) compared to the control (T0) animals (66.67%). Also, the reaction time was not significantly ($p > 0.05$) higher in treated animals compared to T0. The relative weights of genital organs showed no significant difference ($p > 0.05$) among treatments. The caudal epididymal sperm concentration was insignificantly ($p > 0.05$) greater in the control group ($92.18 \pm 78.18 \times 10^6$) compared to the treated groups ($73.50 \pm 39.82 \times 10^6$; $61.26 \pm 51.10 \times 10^6$ and $60.92 \pm 73.84 \times 10^6$ respectively for T1, T2 and T3). Sperm mobility showed no significant increase ($p > 0.05$) in animals orally fed with palm olein (3.94 ± 0.73; 3.64 ± 0.69; 3.50 ± 0.91% respectively for T1, T2 and T3) compared to control group (3.39 ± 0.55%). The percentage of normal spermatozoa was also insignificantly ($p > 0.05$) greater in groups which received palm olein (82.38 ± 5.42; 82.57 ± 6.44 and 78.07 ± 11.65% respectively for T1, T2 and T3) compared to the control group (75.17 ± 6.24%). Total serum cholesterol concentration showed no significant difference ($p > 0.05$) among treatments while an insignificant ($p > 0.05$) increase of testosterone concentration was obtained in animals who ingested palm olein (0.23 ± 0.11; 0.17 ± 0.04 and 0.23 ± 0.10 ng/ml respectively for T1, T2 and T3) compared to the control (0.16 ± 0.07 ng/ml). In sum, palm olein had some positive effects on the studied parameters.

***Keywords:*** *Palm Olein; Caudal Epididymal Sperm; Blood Testosterone; Libido; Guinea Pig*

## Introduction

Vegetable oils are principally used for human consumption but are also used in animal feed, for medicinal purposes, and for certain technical applications. Palm oil has been used in food preparation for over 5000 years. Palm oil obtained from the fruits of the oil palm tree is the most widely produced vegetable oil in the world and its nutritional and health attributes have been well documented [1]. Indeed, palm olein a vegetable oil obtained from the refining of red palm oil [2,3], is composed of saturated fatty acids made of 0.89, 3.51 and 41.54% of myristic, stearic and palmitic acid respectively and unsaturated fatty acids grouped into monounsaturated (42.7 - 43.9%

of oleic acids) and polyunsaturated fatty acids (10.43% of linoleic acids or ω-6 fatty acids) and traces of alpha linolenic acids or ω-3 fatty acids [2,4]). Its high proportion in oleic acids makes it comparable to monounsaturated vegetable oils (olive and sunflower oils) on their effects on cholesterol [5]. In addition, palm olein is rich in natural antioxidants which are tocopherols and tocotrienols [2], which could be good to fight against excess free radicals that might destroy the plasma membrane of sperm cells. Moreover, animals cannot synthesize ω-6 or ω-3 fatty acids de novo because of a lack of the appropriate fatty acid desaturase enzymes [6]. Therefore, the ω -6 PUFA and the ω -3 PUFA need to be provided in the diet as these PUFAs are essential for numerous processes including growth, vision, brain development and reproduction [7]. Like many other vegetable oils, palm olein could be used in animal feed, for medicinal purposes. [8] showed that palm olein did not affect total cholesterol level after 4 weeks in rats. On the other hand, [9] had results showing that dietary palm olein reduced total cholesterol after 12 weeks of treatment in rats. Since, cholesterol plays an important role in sexual steroidogenesis, and looking at the numerous bioactive molecules contained in palm olein, this latter could have positive effects on animal reproduction. Nevertheless, reports on the effects of palm olein on reproductive performances in farm animals to our knowledge are still unknown. Therefore, this study was designed to evaluate the effects of palm olein on the weights of genital organs, sperm characteristics, libido and the concentrations of serum testosterone and total cholesterol.

## Material and Methods

### Study animal, lodging and feeding

Forty (40) adult male guinea pigs averagely weighing 323 ± 84.43g were used in this study. For trial purpose, each animal was identified at the ear and lodged in identical cages of dimensions 1.5m x 0.8m x 0.6m, equipped with feeder and drinker. In each lodge, white saw dust was placed on the floor, which served as litter. Animals were kept in a 12h light-dark cycle and provided *ad libitum* with water and a specific diet.

Animals were orally gavaged with palm olein (Mayor) whose characteristics are the following: energetic value: 90 calories/10g oil; lipids: 16%; cholesterol: 0%; vitamin A: 32.5 µl/g.

### Trial

The animals were distributed into 4 groups of 10 animals each, comparable in body weight. For 75 days, the control group (T0) was orally treated with 1ml distilled water/kg b.w while the groups T1, T2 and T3 orally received respectively 1, 2 or 3 ml of palm olein/kg b.w per day. At the end of the experiment, the animals were slaughtered for data collection.

### Studied parameters and data collection

### Blood cholesterol and testosterone concentration

Twenty four (24) hours after the last gavage, blood was collected by cardiac puncture and introduced in test tubes free from anticoagulant. The serum was collected after 4 hours, centrifuged at 2000 turns per minutes for 15minutes, kept in tubes and preserved at -20°C. Total cholesterol was quantified using the commercial dosage kit from CHRONOLAB. Testosterone was also measured in the serum with AccuDiag™ Testosterone ELISA kit (Diagnostic Automation Inc).

### Sexual desire (libido)

At the 75$^{th}$ day of the essay, each animal was coupled with an adult female. The chronometer was set as soon as the male and female were placed together and stopped when a reaction was observed from the male (pursuit of the female, smell of the ano-genital region of the female). Then the reaction time was recorded. The maximum time of observation of the male in the presence of the female was 5 minutes. Also, the percentage of males which reacted in the presence of the female was calculated.

### Weight of genital organs

The testes, epididymides, vas deferens and accessory glands were collected, cleared from fat and then weighed using an electric scale of 160 ± $10^{-3}$g. Also, the volume of testes was determined by immersion in NaCl solution contained in a graduated cylinder.

### Sperm mobility

After weighing the epididymal tails of each guinea pig, they were dilacerated in a petri dish containing 0.9% NaCl solution at 37°C.

For the gross mobility, 5 ml of NaCl were used; a drop of the solution was placed on a slide, covered with a cover slip and observed under the microscope at 400X. The scoring was done following [10].

For individual mobility, the above preparation was once more diluted in 5 ml of NaCl and the same observation procedure was done. Mobiles and immobile spermatozoa were rapidly counted on at least 200 cells and the percentage of mobile form determined.

### Concentration of spermatozoa

The mother solution was diluted in 10 ml of NaCl, a drop from the prepared sample was placed on the Thoma's cell chamber and spermatozoa contained in were counted. The concentration was calculated according to the following formula:

$C = N \times 4 \times 1000 \times 20/0.1$

Where: N = Number of spermatozoa contained in 4 big squares; 20 = Volume of NaCl; 0.1 = Volume of the chamber in $mm^3$; 1000 = Factor of conversion of $mm^3$ to ml.

### Morphology

To evaluate the morphological abnormalities of spermatozoa, a solution of eosine-nigrosine was used. One drop of semen was mixed with 4 drops of the dye. One minute after, 10 µl of the mixture was spread on the slide prewarmed at 37°C and stored in the oven at 30°C till the time of analysis. Identification of abnormalities was done under a light microscope at 400X. At least 200 cells were counted and the proportion of abnormal cells was expressed into 2 groups: major abnormalities at the level of the head of the spermatozoa and minor abnormalities at level of the middle piece and the tail.

### Statistical analyses

The obtained results were expressed as mean ± standard deviation and in percentages. One way analysis of variance (ANOVA) was used to test the effect of the treatment (palm olein) on the studied characteristics. Duncan's test at 5% was used to separate means when a significant difference existed.

## Results

### Effect of palm olein on blood total cholesterol and testosterone

The concentrations of total cholesterol (Figure 1) decreased in blood of treated animals compared to the control yet no significant difference ($p > 0.05$) was observed among treatments.

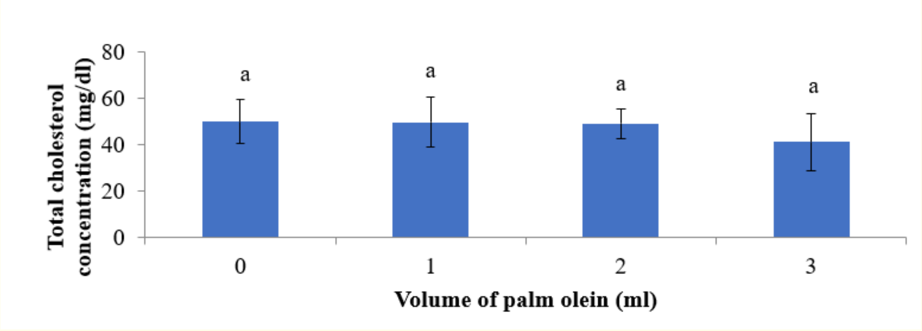


***Figure 1:*** *Variation of the serum total cholesterol according to the dose of palm olein.*
*a: Bars affected with the same letter do not differ significantly (p > 0.05).*

The concentration of serum testosterone (Figure 2) showed no significant difference ($p > 0.05$) among treatments. However, the concentration of this hormone increased in animals receiving 1 and 3 ml palm olein.

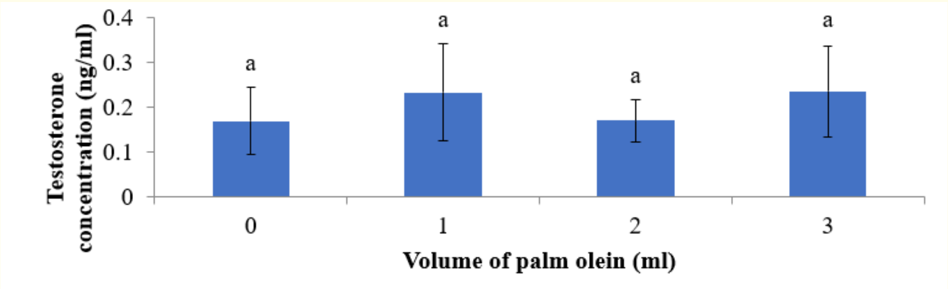


***Figure 2:*** *Variation of the serum testosterone concentration according to the dose of palm olein.*
*a: Bars affected with the same letter do not differ significantly (p > 0.05).*

## Effect of palm olein on the libido

The reaction time (Figure 3) and percentage of male which reacted in the presence of the female (Figure 4) were comparable ($p > 0.05$) among treatments. Nevertheless, animals treated with 1 and 3 ml of palm olein/kg b.w reacted more rapidly in the presence of the female than those receiving 2 ml of palm olein/kg of b.w and distilled water/ kg of b.w (control group). The percentage of males that reacted was greater in treated groups compared to the control group.

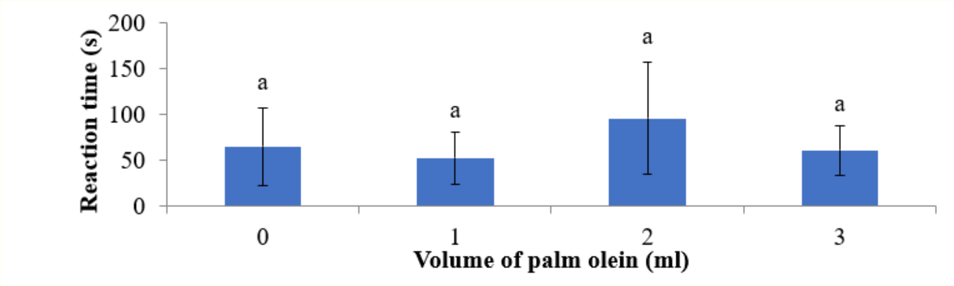


***Figure 3:*** *Variation of the reaction time according to the dose of palm olein.*
*a: Bars affected with the same letter do not differ significantly (p > 0.05).*

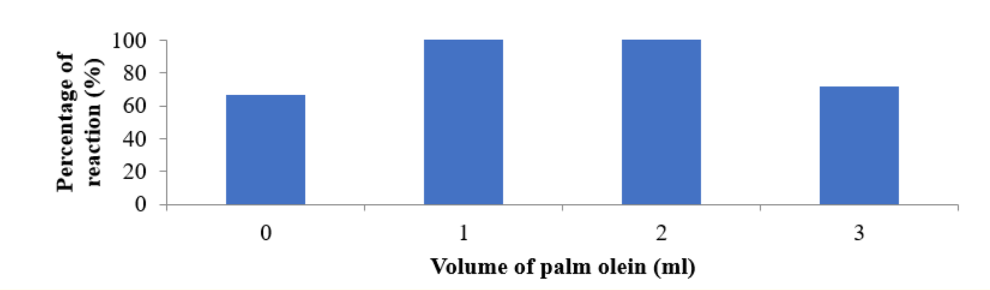


***Figure 4:*** *Variation of the percentage of males reacting in the presence of a female.*

### Effect of palm olein on the weights and volume of genital organs

The relative weights of testes, epididymides, vas deferens and accessory glands and the volume of testes (Table 1) were comparable ($p > 0.05$) among guinea pigs fed with different doses of palm olein. The group treated with 3 ml of palm olein/kg of b.w showed the lowest values for these parameters, whereas the highest values were observed in males that received 2 ml of palm olein/kg of b.w.

| Parameters | Volume of palm olein (ml) | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| | n = 8 | n = 8 | n = 8 | n = 8 |
| **Weight (g/100g bw)** | | | | |
| Testes | 0.34 ± 0.12$^{a}$ | 0.34 ± 0.11$^{a}$ | 0.40 ± 0.12$^{a}$ | 0.30 ± 0.14$^{a}$ |
| Epididymides | 0.07 ± 0.03$^{a}$ | 0.07 ± 0.02$^{a}$ | 0.07 ± 0.02$^{a}$ | 0.06 ± 0.03$^{a}$ |
| Vas deferens | 0.04 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.03 ± 0.01$^{a}$ |
| Accessory glands | 0.26 ± 0.19$^{a}$ | 0.27 ± 0.11$^{a}$ | 0.32 ± 0.18$^{a}$ | 0.21 ± 0.17$^{a}$ |
| Volume (ml) | | | | |
| Testes | 1.77 ± 0.75$^{a}$ | 1.86 ± 0.45$^{a}$ | 2.05 ± 1.01$^{a}$ | 1.30 ± 0.74$^{a}$ |

***Table 1:*** *Effect of the dose of palm olein on the relative weights and volume of genital organs.*
*n: Number of animals; a, b, on the same line, values affected with the same letter do not differ significantly (p > 0.05).*

### Effect of palm olein on caudal epididymal sperm characteristics

The gross as well as the individual mobility of spermatozoa from the caudal epididymis (Table 2) was generally higher in groups that received palm olein compared to the control group. Nonetheless, no significant difference ($p > 0.05$) was observed.

| Sperm characteristics | Volume of palm olein (ml) | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| | n = 8 | n = 8 | n = 8 | n = 8 |
| Gross mobility (%) | 67.80 ± 11.00$^{a}$ | 78.80 ± 14.60$^{a}$ | 72.80 ± 13.80$^{a}$ | 70.00 ± 18.20$^{a}$ |
| Individual mobility (%) | 73.40 ± 10.00$^{a}$ | 81.20 ± 13.60$^{a}$ | 74.20 ± 14.00$^{a}$ | 68.60 ± 14.60$^{a}$ |
| Number/tails of epididymis (x $10^6$) | 92.18 ± 78.18$^{a}$ | 73.50 ± 39.82$^{a}$ | 61.26 ± 51.10$^{a}$ | 60.92 ± 73.84$^{a}$ |
| Number/g of epididymis (x $10^6$) | 278.18 ± 93.96$^{a}$ | 229.26 ± 92.64$^{a}$ | 173.42 ± 101.48$^{a}$ | 188.08 ± 154.54$^{a}$ |
| Morphology | | | | |
| Normal (%) | 75.17 ± 6.24$^{a}$ | 82.38 ± 5.42$^{a}$ | 82.57 ± 6.44$^{a}$ | 78.07 ± 11.65$^{a}$ |
| Major abnormalities (%) | 6.78 ± 3.59$^{ab}$ | 4.65 ± 3.03$^{ab}$ | 3.93 ± 3.63$^{b}$ | 9.29 ± 6.55$^{a}$ |
| Minor abnormalities (%) | 18.06 ± 4.21$^{a}$ | 12.98 ± 3.83$^{a}$ | 13.50 ± 5.79$^{a}$ | 12.57 ± 8,69$^{a}$ |

***Table 2:*** *Variation of sperm characteristics according to the dose of palm olein.*
*n: Number of animals; a, b, on the same line, values affected with the same letter do not differ significantly (p > 0.05).*

The number of spermatozoa per tail and per gram of epididymis generally decreased insignificantly ($p > 0.05$) with the increasing dose of palm olein.

No statistical difference ($p > 0.05$) was observed among groups for the number of spermatozoa morphologically normal and for minor abnormalities, unlike major abnormalities which was significantly high ($p < 0.05$) in guinea pig feeding 3 ml of palm olein and less recurrent in groups that received intermediary dose than the control.

## Discussion

The insignificant increase in the weights of the testes, epididymis, accessory glands and vas deferens noticed in guinea pigs treated with palm olein is in accordance with the findings of [6] in rabbits treated with corn oil. This increase might be due to the rise of testosterone concentration in animals which received palm olein. As a matter of fact, [11] reported in his study a significant increase of the bulbourethral gland mass in *Antechirus stuartii* (a marsupial) when treated with exogenous testosterone. Moreover, low serum testosterone levels have been reported to negatively affect the structure and weight of the testes and epididymis [12]. Hence, the development and secretory function of these reproductive organs are under the influence of androgens, mainly testosterone [13-16]. Apart from testosterone which led to an increase in these organs, another factor could have been the increase in energy contained in palm olein. In general, studies conducted by [17,18] in rams, [19] in bulls reported an increase in testicular and epididymal size resulting from feeding high energy diets. Hence, the fact that treated animals in this study had a little increase in energy due to the ingestion of palm olein seems to equally explain the small increase in the weight of these organs.

In some domestic animals, it has been shown that sperm concentration increases with the ingestion of vegetable oil. It is the case of mice treated with date seed oil [20], rats treated with olive oil [21] and rabbits treated with corn oil [6]. Those findings are controversial to the results of the present study which showed a decrease although not significant in sperm concentration in animals treated with palm olein. In fact, the slight increase in the weight of the testes in this study could have caused an increase in sperm concentration since it is known that the production of spermatozoa is positively correlated with the development of the testes [15,22]. Likewise, according to [16,23], testosterone is required for normal spermatogenesis. Hence, the moderate increase of this hormone concentration could equally have increased sperm count. Therefore, we do not have plausible explanations for these contradictory results.

The increase in sperm mobility in treated animals corroborate with the findings of [20] in mice treated with date seed oil. Increase in sperm mobility could be as a result of an increase in energy level received by treated groups. Indeed, according to a study conducted by [24] in rats, high-energy diets significantly increased sperm mobility.

The lower rate of morphologically abnormal spermatozoa recorded in treated animals as compared to the control group is in agreement with the results of [25] in mice treated with 4ml/kg of pumpkin seed oil. The decline in the abnormal sperm cells could be attributed to the increase of testosterone level. In fact, [26] reported an increase in the number morphologically abnormal spermatozoa due to low level of testosterone. Indeed, according to [23], testosterone is required for the development of normal sperm cells.

Diets rich in saturated fatty acids lead to high levels of serum total cholesterol, whereas diets rich in unsaturated fatty acids tend to be associated with lower levels [6]. Our results support that of [27] in rats fed palm oil and those of [8,28] respectively in rats and mice fed palm olein. In fact, as a matter of fact, palm olein contains almost equal proportions of saturated and unsaturated fatty acids. Hence, the comparability of total cholesterol concentration among treatments in this study might be due to the neutral effect of palm olein on cholesterol level.

The comparable rate of total cholesterol among doses augured the similar rate of testosterone in experimental groups of guinea-pigs. The sexual desire in the different groups expressed as the proportion of males that reacted in the presence of a female and as the reaction time confirmed this comparability. Indeed, according to [29,30] testosterone is responsible for the sexual behaviour. Likewise, this comparability could equally be explained by an increase in energy intake in treated groups. According to [18], testosterone production and libido were negatively affected when rams fed low energy diets. Moreover, [31] equally suggested that energy intake particularly from saturated and monounsaturated fatty acids, elevates testosterone production. Therefore, since palm olein contains a high amount of saturated and monounsaturated fatty acids, it might have led to the slight increase in energy intake which indeed increased testosterone as well as libido in treated animals.

## Conclusion

In conclusion, these results imply the importance of vegetable oil in general and that of palm olein in particular in reproduction though no significant effects on the weight of reproductive organs, sperm characteristics, libido, serum concentrations of testosterone and total cholesterol.

## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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