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Normal Diet and Linoleic-Enriched Sunflower Oil Improved Sperm Variables but Not Reproductive Hormones in Male Wistar Rats

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ABSTRACT: Male reproductive processes are impacted by edible fats. The present work studied the effect of the consumption of a sunflower-enriched diet on sperm variables and reproductive hormones in male rats. A total of twenty male Wistar rats were used in this study. The Control group received the regular rat chow while the Treated group received the regular rat chow supplemented with 25% linoleic sunflower oil. After 28 days sperm variables, and reproductive hormones were assessed. There was no significant difference in the serum testosterone and prolactin levels in the group fed sunflower-enriched diet compared to the control group. The serum LDH and the seminal vesicle fructose levels were significantly increased in the animals fed sunflower enriched diet compared to the control animals. The sperm count and sperm motility levels were significantly higher in the rats fed sunflower enriched diet compared to the control rats. The current findings imply that while 25% linoleic sunflower oil might increase sperm characteristics, it might not have the same effect on testosterone levels.

Keywords: Testosterone, Sperm count, Edible fats, Sunflower oil, Fructose

Introduction

Most of the energy in a diet comes from carbohydrates. Yet, as components of the cellular membranes, lipids offer energy and are crucial for biological processes. Linoleic acid and other omega-6 (n-6) polyunsaturated fatty acids (PUFA) lower blood pressure, plasma cholesterol, and to some extent triglycerides (Djuricic and Calder, 2021). Omega-6 fatty acids can be found in foods including rapeseed, soybean, and sunflower oil (Alagawany *et al.,* 2019). There are three different kinds of sunflower oil: high oleic, mid oleic, and linoleic. Sunflower oil is one of the oils used in the preparation of the majority of popular fried meals. Typical cooking oil with high quantities of polyunsaturated fat is linoleic sunflower oil. It is also renowned for its lack of trans fat and clean flavor (Mishra and Manchanda, 2012).

The male reproductive tract, and hence fertility, are regulated by dietary fatty acids and oils (Domínguez-Vías *et al.,* 2017). It is recognized that fatty acids and oils might contribute to metabolic problems (Sokoła-Wysoczańska *et al.,* 2018). Understanding how fatty acids and oils in typical diets affect male reproductive systems has recently attracted attention. Recently, we investigated how male reproductive systems were impacted by oleic acid and olive oil (Aisoni *et al.,* 2022). This study looked at how male rats' sperm and hormone levels changed after eating standard food enriched with sunflower oil.

Materials and methods

Experimental animals: At the College of Medicine, University of Lagos, male Wistar rats weighing between 100 and 120g were kept in the Animal Facility. Two groups of ten (10) rats each were created. The Control group received the regular rat chow while the Treated group received the regular rat chow supplemented with 25% linoleic sunflower oil. Studies have reported that administering less than 30% edible oils to male Wistar rats has reproductive beneficial effects on metabolic abnormalities (Carvajal-Zarrabal *et al.,* 2014; Del Toro-Equihua *et al.,* 2016).

The experiment lasted for 28 days. The animals were housed in plastic cages at a constant temperature of 27 to 30°C with a photoperiod of 12 hours of light and 12 hours of darkness. The National Academy of Sciences Handbook for the Care and Use of Laboratory Animals was followed during the experimental process (National Academy of Sciences, 2011). After the expiration of the 28 days, the animals were fasted overnight, and the next morning the animals were sacrificed by cervical dislocation.

Biochemical Analyses: Using an enzyme-linked immunoassay (ELISA) and in accordance with the manufacturer's instructions, the levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and prolactin in the serum were all determined. ELISA kits (batch code EIA-6K2A2) were purchased from Monobind Inc (California, USA).

Fructose in the seminal vesicle and serum lactate dehydrogenase (LDH) levels were tested using enzyme colorimetric techniques. The reagents used for the assay were purchased from Randox Laboratories Ltd. (Antrim, UK). LDH activity was assayed using the catalytic feature of LDH that causes reversible oxidation of L-lactate to pyruvate, which is mediated by the hydrogen acceptor, NAD+. Spectrophotometric monitoring can be used to track the conversion of pyruvate to lactate or the reverse reaction of oxidizing L-lactate to pyruvate. Following is an analysis of fructose levels. After thoroughly combining 20 µl of seminal plasma was mixed thoroughly with 220 µl distilled water, the mixture was deproteinized using 50 µl of ZnSO4 and 50 µl of NaOH.

Sperm analysis: The analysis of sperm function was conducted according to the method of Adekunbi *et al.,* 2016.

Statistical analysis: The data were shown as means ± SEM. When a pair-wise comparison was made between the groups, Analysis of Variance (ANOVA) was used, backed by the Newman-Keuls test (GraphPad Software, San Diego California, USA). The p-value used to determine the statistical significance was 0.05.

Results

There was no significant difference in the serum testosterone levels in the group fed a sunflower-enriched diet compared to the control group. However, the serum estradiol level was significantly reduced in the animals fed sunflower enriched diet compared to the control animals (Fig 1).

There was no significant difference in the serum prolactin level in the rats fed a sunflower-enriched diet compared to the control rats (Fig 2). The serum LDH and the seminal vesicle fructose levels were significantly increased in the animals fed a sunflower-enriched diet compared to the control animals (Fig 3). The sperm count and sperm motility levels were significantly higher in the rats fed a sunflower-enriched diet compared to the control rats (Fig 4).

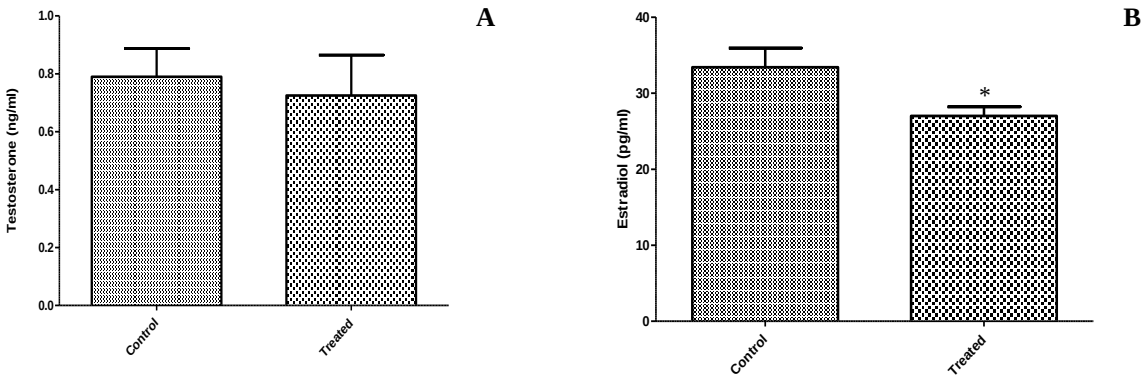

Fig. 1: **(A)** Testosterone and **(B)** Estradiol levels of rats fed sunflower-enriched diet for 28 days. Values are expressed as mean ± SEM, n=10 per group. *p< 0.05 was significant when compared with control.

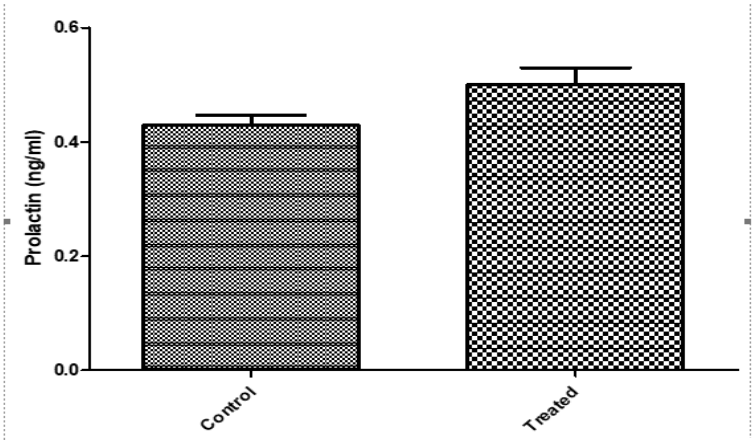

Fig. 2: Prolactin level of rats fed sunflower enriched diet for 28 days. Values are expressed as mean ± SEM, n=10 per group.

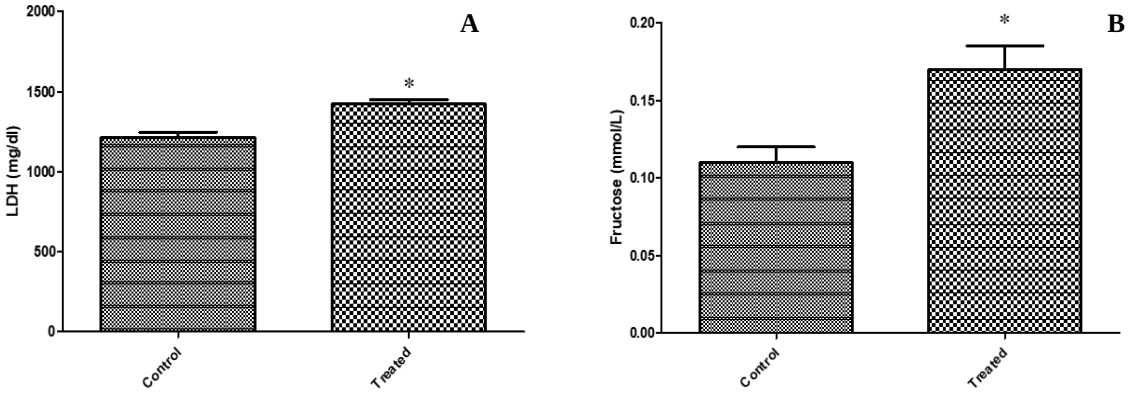

Fig. 3: **(A)** LDH and **(B)** Fructose levels of rats fed sunflower-enriched diet for 28 days. Values are expressed as mean ± SEM, n=10 per group. *p< 0.05 was significant when compared with control.

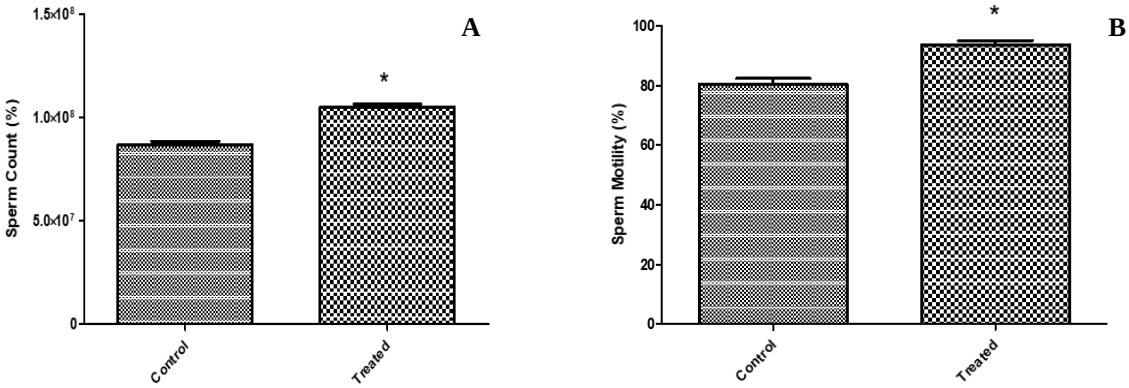

Fig. 4: **(A)** Sperm count and **(B)** Sperm motility of rats fed sunflower-enriched diet for 28 days. Values are expressed as mean ± SEM, n=10 per group. *p< 0.05 was significant when compared with the control.

Discussion

Sperm count and motility significantly increased in this study. This reveals that there are some dietary lipids that are crucial for preserving and enhancing the quality of semen in both humans and animals (Esmaeli *et al.*, 2015, Safarinejad, 2011), there are however other lipids that affect the male reproductive axis negatively (Rato *et al.*, 2014).

Polyunsaturated fatty acids (PUFAs) are necessary for the reproductive system and some n-6 fatty acids cannot be synthesized by humans or animals due to a lack of suitable fatty acid desaturase enzymes, linoleic acid, for example, must be given in the diet (Kochhar, 2002). High levels of n-6 PUFA have been found in testicular cells and spermatozoa, and omega-6 is concentrated more in the epididymis, where sperm are stored than in the testis (Saether *et al.,* 2007). Thus, as the spermatozoa are transferred to the epididymis, the testis is continuously drained of PUFA acid (Esmaeli *et al.,* 2015). In this study, normal rat chow was supplemented with sunflower oil and this enhanced the epididymis increasing sperm count and motility significantly.

Rats given a regular diet enhanced with 25% sunflower oil had testosterone levels that were similar to those of rats given a normal diet without sunflower oil (Control). This is similar to another study by Segarra *et al.,* (2008). In that study, the animals' diets were supplemented with 10% of the oils used. In comparison to other oils, such as sunflower, the testosterone levels in that study were reported to be 2-4 times higher compared to animals fed a supplemented diet of Iberian pig fat. Iberian pig lard has a higher concentration of monounsaturated fatty acids (MUFA- 62%) than PUFA (9.5%), with oleic acid making up most of the fatty acid. In a different study, oleic acid was again given to animals who were fed a normal diet, and there was no discernible difference in the testosterone levels of the group fed oleic acid-enriched feed compared to the control group (Aisoni *et al.,* 2022). The results of this study and other studies indicate that before any significant improvement can be seen in testosterone levels, there must be a synergy between MUFA and PUFA supplementations in a normal diet.

In the present study, the animals given a sunflower-enriched diet had considerably lower estradiol levels than the control animals. Serum oestradiol levels can hamper testosterone production thus in the presence of high aromatase enzyme activity, there could be reduced testosterone production (Aguirre et al., 2015). This was however not the case in this study.

When compared to the control group, the prolactin levels in the animals fed the sunflower-enriched diet were not significantly different. When prolactin levels in the serum are normal, it has acceptable effects on the male reproductive system (Shibli-Rahhal and Schlechte, 2009).

The metabolic mechanisms that give energy for spermatozoa's survival, motility, capacitation, and fertility depend on lactate dehydrogenase (LDH) (Sirat *et al.,* 1996). LDH has been suggested as a reliable biomarker of sperm viability (Stamatiads *et al.,* 1984). Therefore, the increase in sperm variables noted in this study may be related to the elevated LDH levels in rats fed a diet enriched with sunflower seeds as opposed to control rats.

The main source of energy for sperm is fructose, which is exclusively generated in seminal vesicles. The seminal vesicle's fructose concentration can be used to deduce how well it performs its secretory function, which is regulated by testosterone (Veena and Preeti, 2017). It has been proposed that seminal fructose develops from glucose through the glycogen phosphohexose route when phosphohexoisomerase and alkaline phosphatase are present. Testicular androgens initiate and regulate the fructose production process in seminal vesicles (Veena and Preeti, 2017). The increase in sperm count and motility seen in this study was caused by a higher fructose concentration in animals fed a diet supplemented with sunflower seeds as opposed to the control animals. This is because fructose provides the sperm with energy. The increase in fructose level is an indication of its support either in vas deferens or the epididymis (Gonzales and Villena, 1997; Gonzales, 2001).

The present results suggest that while linoleic sunflower oil may be beneficial in terms of sperm variables improvement this may not be the same for the testosterone level.

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