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THE EFFECT OF LONG TERM CONSUMPTION OF THERMOXIDISED PALM OIL DIET ON SOME REPRODUCTIVE PARAMETERS IN MALE WISTAR RATS

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ABSTRACT

The effects of long term ingestion of thermoxidised palm oil diet on some reproductive parameters in male wistar rats were studied. Most palm oil is consumed in the thermoxidised form which has been associated with toxicity and impairment of function of several body tissues. Male wistar rats weighing 80 – 125 g and aged 19-23 weeks were divided into a control group and a thermoxidised palm oil diet-fed group of five rats each. Thermoxidised palm oil diet was prepared by mixing thermoxidised palm oil (TPO) and animal feeds in a ratio 15g:85g for TPO and animal feeds respectively. Control group was fed on normal rat feeds while the other group was fed on TPO diet. Animals were fed daily for 13 weeks after which they were euthanized, epididymal semen and blood samples collected for determination of various reproductive parameters. The results showed significant decreases in sperm count ($p<0.001$), serum testosterone ($p<0.001$) and luteinizing hormone ($p<0.001$) in the TPO diet-fed rats compared with the control group. There were no significant differences in the seminal pH, sperm motility and viability, serum level of follicle stimulating hormone and morphological defects in the TPO diet-fed group compared with control rats. We conclude that long term consumption of TPO diet impairs reproductive function in male wistar rats.

KEYWORDS: Thermoxidised palm oil, Seminal fluid parameters, Testosterone, FSH, LH.

INTRODUCTION

Palm oil is an edible vegetable oil extracted from the mesocarp of ripe palm fruits primarily of the *Elaeisis guineensis*, the African variety and to a small extent the America and Manipa

species. In its raw form, palm oil is red in color and contains 50% saturated, 10% polysaturated and 40% unsaturated fatty acids.[1] Palm oil is the most widely consumed vegetable oil in most parts of Africa especially Nigeria[2,3] because of its low cost and high oxidative stability.[4] Triglycerides and small amounts of di- and mono- glycerides form the major components of the oil while the minor components include free fatty acids, phytonutrients like carotenoids, tocotrienols, tocopherols, co-enzyme Qio, flavonoids, squalenes, phenolic acid, chlorophyll, phospholipids and[5,6] Palm oil is one of the few highly saturated vegetable fats and is semi-solid at room temperature. Domestically, raw or crude palm oil is used for cooking and shortening. Industrially, it is used for the manufacture of soaps chocolate, cookies dietary, creamers, margarine, ice cream as well as for the manufacture of methyl esters and hydrodeoxygenated biodiesels.

Crude palm oil has several health benefits. The carotenoids, vitamin C and tocotrienols present in palm oil are members of a biological antioxidants network which converts highly reactive radicals to less active species and so help to protect tissues against oxidative damage.[7,8] Phospholipids, the main building blocks in all living cells have synergistic antioxidant effects with other antioxidants.[9] Crude palm oil improves memory, nutrient absorption, digestion and lipid transportation.[10] Tocopherols especially the gamma and delta isomers have a cholesterol synthesis lowering effect.[11] Crude palm oil has been used to treat vitamin A deficiency[12] and to attenuate the oxidative stress-induced sperm damage.[13] Palm oil improves immunity and is said to have antitumorogenic[14,15] and antiatherogenic effects.[11] Unfortunately, much of the oil consumed is not in the raw or crude form but in the thermoxidised form in which many of the beneficial components have been destroyed and toxic substances produced.[16,17]

Thermoxidation is the oxidation of oils or lipids following application of heat. This is usually done by subjecting the oil to repeated cycles of heating at high temperatures, allowing the oil to cool in between heatings.[18] Thermal oxidation of oil causes several changes in the physicochemical properties of the oil. These changes include reduction in iodine value, increase in carbonyl value, increase in peroxide and acid values[16], increase in free fatty acid content, density and moisture and saponification values as well as linoleic acid content. Thermoxidation destroys B-caroteines and several other phytonutrients and antioxidants in the oil, making it susceptible to peroxidation.[17] Peroxidation of the oil results in the formation of toxic substances including hydroperoxides, malondialdehyde, reactive aldehydes

and free radicals that have damaging effects on cells.[19,20,21] Lipid peroxidation of testicular tissue is known to result in testicular injury with resultant testicular dysfunction.[22]

Long term consumption of TPO has been associated with growth retardation, fatty liver, thrombosis[2], hemotoxicity[23], peptic ulceration[24], hepatotoxicity[25], discoloration of uteri, ovaries and testes[26] and reduced glomerular filtration rate.[27] Chronic ingestion of TPO diet has also been linked with distortion of villi morphology, malabsorption of fluid and glucose[28], increase in serum sodium, chloride, bicarbonate and creatinine concentrations. An oil which is known to have these varying toxic effects on body tissues, may also have some effect on this vital system, the male reproductive system which plays a critical role in fertility and continuity of species as well as economic and social consideration. Unfortunately, TPO is the major form in which palm oil is consumed, finding its use in the food frying industry and domestic consumers.

Infertility is a global problem and concern not only because of the need for continuity of species but also due to its economic, social and psychological implications.[29,30] In slightly less than half of these cases, it is attributed to the male factor. The main clinical manifestation of infertility include reduced sperm count (oligozoospermia), reduced sperm motility (asthenozoospermia) and sperm morphological abnormality (teratozoospermia).[31] Despite known effects of the consumption of this form of palm oil on several body functions, there is paucity of information on its effects on male reproductive function. It therefore became necessary to evaluate the possible effects of consumption of this oil on some male reproductive parameters which include (serum pH, sperm count, motility, morphology, serum testosterone, luteinizing hormone and follicle stimulating hormone).

MATERIALS AND METHODS

Experimental animals

Ten male wistar rats weighting 80-125g and aged 19-23 weeks were acclimatized in the Animal house of the Department of Physiology, University of Calabar, Calabar for two weeks. They were housed in cages at room temperature and a 12 hour day/night cycle.

Experimental design

The ten male rats were randomly divided into two groups – a control group and a TPO diet-fed group with each group made up of five rats. The control group was given normal rat chow while the TPO-fed group received TPO diet daily. Both groups had free access to tap water

and their respective diets. The duration of feeding was 13 weeks, at which time the rats were nineteen to twenty three weeks old. At the end of the feeding period, the animals were euthanized and blood samples were collected through cardiac puncture for assay of necessary serum hormones while the gonads were harvested and used for epididymal seminal analysis. Approval for the use of the animals was obtained from the College Ethical Committee of the Faculty of Basic Medical Sciences, Abia State University Uturu, in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Preparation of thermoxidised palm oil diet

Thermoxidised palm oil was prepared as described by Isong *et al.*[32] and used by Ani *et al.*[33] In summary, the red palm oil was heated in a stainless steel pot over a heating mantle at about $150^{O}C$ for about 20-25 minutes. Heating was done for five times and oil allowed to cool in between heating sessions. The TPO diet was prepared by mixing 15g of the TPO with 85g of rat feed as used by Obembe *et al*.[24]

Determination of epididymal seminal parameters

Seminal fluid parameters were evaluated according to World Health Organization (WHO) standard.[34] A summary is given below.

pH

Semen was aspirated from the epididymis with a sterile syringe and its pH measured using a hand-held pH meter.

Sperm motility

One drop of well mixed semen was placed on glass slide and covered with a cover slip. Using x10 and x40 objective lenses, motility of the sperm cells was observed and expressed in percentage.

Viability

One drop of semen was mixed with a drop of 0.5% Eosin solution on a slide. The preparation was left for 2minutes. The semen sample was counted under the microscope (x40) and the percentage of viable (unstained) and non-viable (stained) spermatozoa was determined.

Sperm count

The semen was diluted in 1 in 20ml sodium bicarbonate-formalin diluting fluid and mixed. An improved Neubauer-ruled chamber was used for the counting with x10 objective with condenser. Spermatozoa in an area of 2 square mm were counted and number of spermatozoa in 1ml of semen calculated by multiplying the number counted by 100,000.

Morphology

A thin smear of well mixed semen was made on a slide and while still wet fixed with 95% v/v ethanol for 10 minutes and allowed to air-dry. The smear was then covered with carbol Fuchsin (1 in 20) and allowed to stain for 3minutes. The stain was washed with water. The smear was counter stained with diluted Loeffler methylene blue for 2 minutes. Using x40 objective lens, morphology of sperm cells for abnormalities was assessed.

Determination of serum hormones concentration

FSH

Serum follicle stimulating hormone (FSH) level was determined in triplicates samples by radioimmunoassay (RIA) technique using rats FSH kits obtained from Biocode Company Belgium according to the protocol provided with the kit.[35]

Serum luteinizing hormone (LH) concentration was assessed by enzyme-immunoassay (EIA) technique using rat LH kit from Immunometrics, London, UK. Optical density was determined using Jenway 6300 Spectrophotometer at a wave length between 492-550nm.[36]

Testosterone

Serum testosterone levels were measured by enzyme-immunoassay (EIA) using rat testosterone kit from Immunometrics, London, UK. Using a Jenway 6300 Spectrophotometer, the optical density was determined at a wave length of 492-550nm.[36]

Statistical analysis

Data obtained were expressed as mean $\pm$ standard error of mean (SEM) and analyzed using the Student's t-test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Sperm count

The results demonstrated a significant decrease ($p<0.001$) in sperm count (x1 million\ml) in the TPO diet-fed rats (3.18±0.24) compared with control group (5.78±0.24) as in figure1.

Serum testosterone concentration

Serum testosterone level (ng\ml) was significantly lower ($p<0.001$) in the TPO-fed group (3.76±0.09) compared with control rats (5.04±0.11) as shown in figure 2.

Serum luteinizing hormone (LH) concentration

Serum LH level (iu\ml) was significantly reduced ($p<0.001$) in TPO-fed rats (3.32±0.06) compared with the control group (4.42±00) as in figure 3.

Epididymal pH, sperm motility and viability, morphology and serum FSH concentration

There was however no significant difference in the pH of epididymal seminal fluid of TPO-fed (6.74±0.24) and control (6.62±0.4) groups as in table 1. Comparison of sperm motility (%) in TPO diet-fed rats (49.00±10.17) and control group (59.00±7.65) did not show any significant difference between the two as in table 1. The percentages of non-viable sperms in the TPO-fed rats (51.00±10.17) and control group (41.00±7.65) were not significantly different as in table 1. There was no significant difference in the number of sperms with total morphological defects (%) in the TPO diet-fed (11.75±1.18) and the control rats (14.00±1.87) as in table 2. Serum FSH levels (ng/ml) were not significantly different in both TPO diet-fed (12.20±0.11) and control (12.28±0.15) groups as in fig 4.

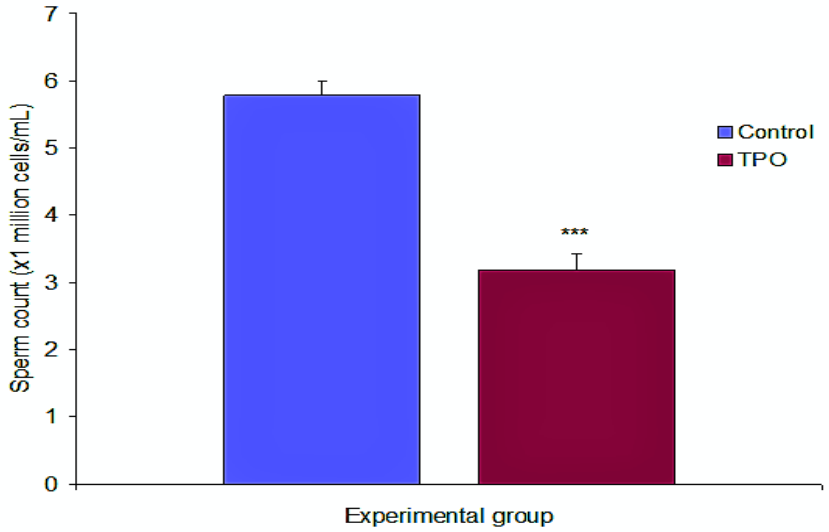

Fig. 1: Sperm count in control and thermoxidized palm oil fed male rats. Values are expressed as mean ±SEM, n = 5.

*** = significantly different from control at p<0.001

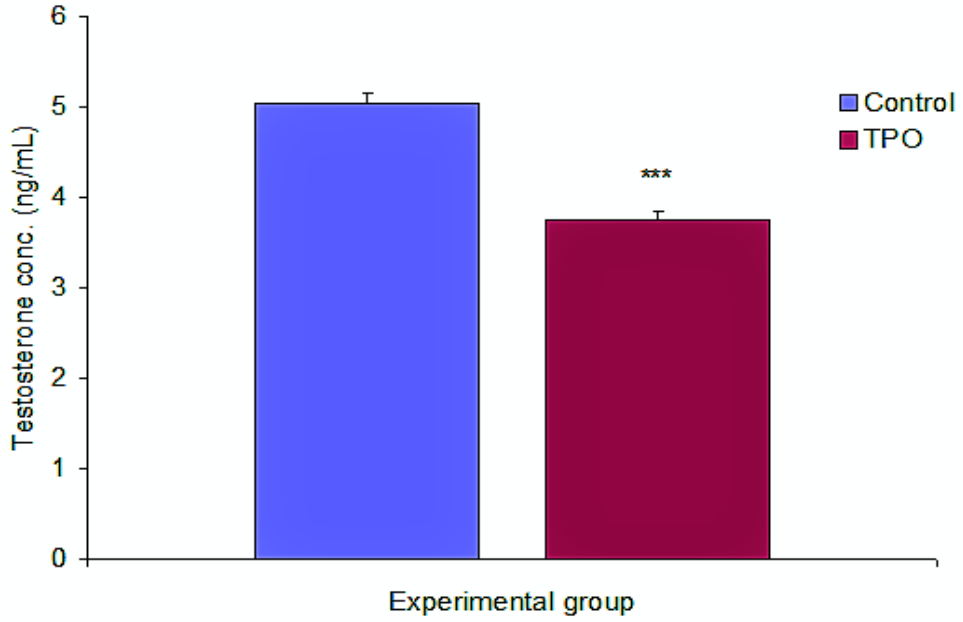

Fig. 2: Testosterone levels in control and thermoxidized palm oil fed male rats. Values are expressed as mean ±SEM, n = 5.

*** = significantly different from control at p<0.001

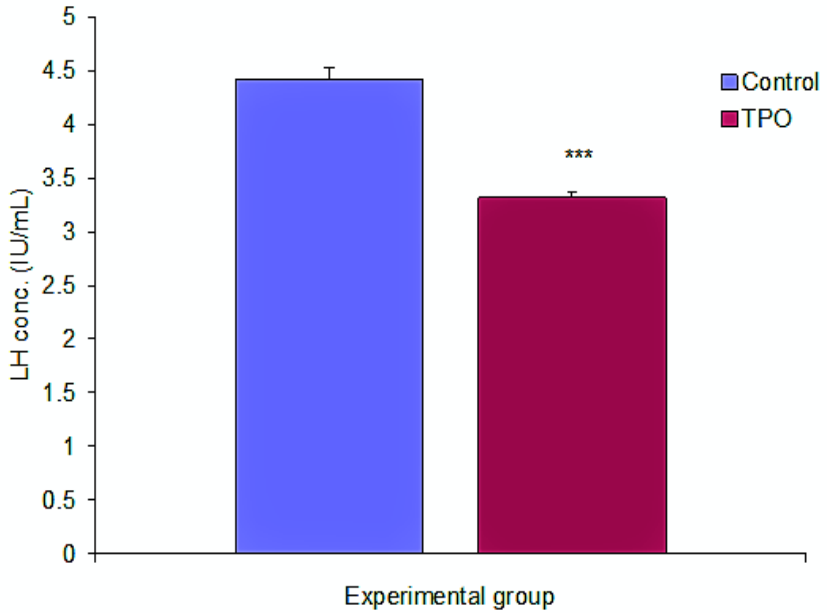

Fig. 3: Luteinizing hormone levels in control and thermoxidized palm oil fed male rats. Values are expressed as mean ±SEM, n = 5.

*** = significantly different from control at p<0.001

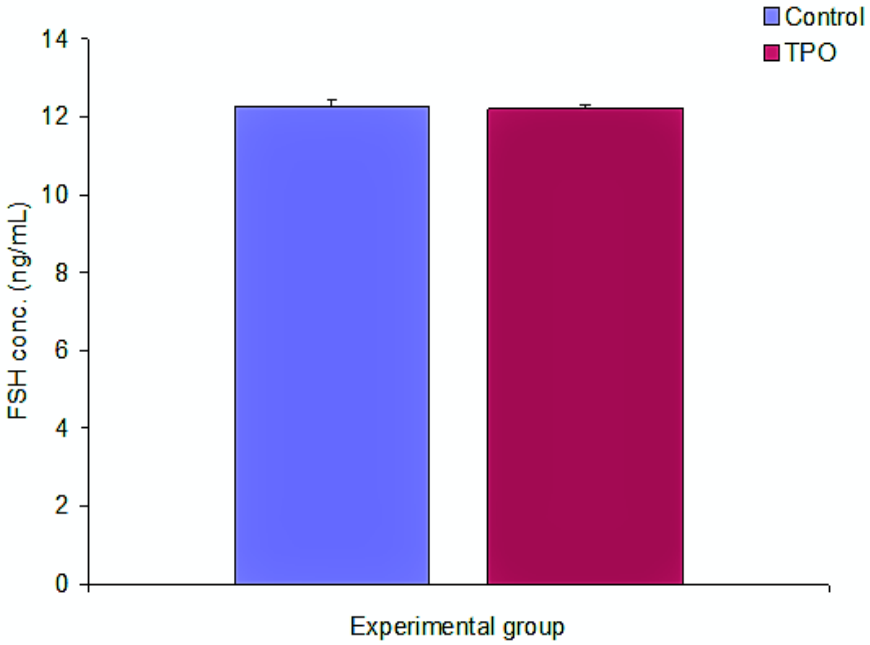

Fig. 4: Follicle stimulating hormone levels in control and thermoxidized palm oil fed male rats. Values are expressed as mean ±SEM, n = 5.

No significant difference between the two experimental groups

Table 1: Sperm pH, motility and viability in control and thermoxidized palm oil-fed rats.

	pH	Motility	RPFM	SPFM	RSM	NMS
Control	6.62	59.00	7.00	50.00	5.75	41.00
	±0.14	±7.65	±1.22	±6.12	±1.34	±7.65
TPO	6.74	49.00	10.00	34.00	9.25	51.00
	±0.24	±10.17	±1.58	±7.81	±1.34	±10.17

Values are expressed as mean ±SEM, n = 5.

No significant differences among groups

RPFM = Rapid progressive forward movement

SPFM = Slow progressive forward movement

RSM = Residual sperm movement

NMS = Non-motile sperm

Table 2: Sperm morphology in control and thermoxidized palm oil fed rats.

	Total defect	Head defect	Middle piece defect	Tail defect
Control	14.00	4.40	2.50	7.60
	±1.87	±0.40	±0.26	±1.21
TPO	11.75	5.50	2.00	4.75
	±1.18	±0.65	±0.58	±1.18

Values are expressed as mean ±SEM, n = 5.

a. No significant differences among groups

DISCUSSION

Palm oil is widely consumed in Nigeria and many African countries. Most of the palm oil is rather consumed in the thermoxidised form for economic reasons and since this is said to improve the oil's palatability. Unfortunately consumption of this form of palm has been associated with cytotoxicity in several body tissues. Information on its effects on reproductive function is quite scanty. It therefore became necessary to evaluate its effects on function of this all-important system – the reproductive system. The results show that long term consumption of thermoxidised palm oil diet causes significant impairment of reproductive function in male wistar rats.

Sperm count in TPO diet-fed male rats was significantly lower compared with the control group. Causes of low sperm count include varicoceles, orchitis[37], antisperm antibodies, irradiation and chemotherapy. Other causes are tubular defects with defective sperm transport, chromosomal abnormalities[38] as well as testicular toxicity, pituitary abnormality

and DNA methylation. The observed decrease in sperm count in the TPO-fed group could in part be due to the reduction in serum level of luteinizing hormone noticed in the TPO-fed rats. Luteinizing hormone which is secreted by the pituitary gland following stimulation by hypothalamic gonadotropin-releasing hormone stimulates Leydig cells in testes to secrete testosterone and together with follicle stimulating hormone modulates Sertoli cells function. Testosterone is essential for spermatogenesis.[39] Primary testicular toxicity by the TPO diet with resultant failure in spermatogenesis could also have contributed to the low sperm count in the group compared to control. Long term consumption of TPO diet has been associated with impairment of several body functions.[25,27] The testes might not have been an exception to the generalized cytotoxicity reported of TPO consumption.

Serum testosterone level in the TPO-fed group was significantly lower than in the control group. This observed decrease could have been due to testicular failure following toxicity from products of lipid peroxidation Lipid peroxidation is known to cause testicular injury with resultant testicular failure.[22] Most of the testosterone is synthesized in the testes from cholesterol by Leydig cells while a little quality comes from acetate.[40] Very little of it comes from adrenal glands. The failure of synthesis of this hormone therefore may point to loss or deficiency of Leydig cell function.[41] Krivenkova and Treschuk,[42] had suggested the possible testicular toxicity induced by inclusion of thermally oxidized fats in the diet of rats. Testosterone secretion by Leydig cells in testes is stimulated by luteinizing hormone. The observed reduced level of testosterone in the TPO-fed rats might also have been due to the low luteinizing hormone in this group which suggests a possible anterior pituitary abnormality.[43]

The significant decrease in this anterior pituitary glycoprotein, LH concentration observed in our study strongly suggests a primary pituitary toxicity from the TPO. The low LH could not have been due to negative feedback effect by testosterone since the level of testosterone is also low.[44] Serum concentration of LH is regulated by gonadotropin-releasing hormone, pituitary sensitivity as well as negative feedback effect from high serum testosterone.

Epididymal seminal fluid pH is weakly acidic.[45] It is not just a vehicle to transport sperms but also shields the sperm from hostile environment, provides nourishment aside helping to keep the sperms immobile.[46] There was however no significant change in the pH of seminal fluid in the two groups of rats.

There was no significant change in the percentage of motile sperms in the two groups. Sperm motility is reduced by structural abnormality of sperms, pH of semen[46], as well as semen temperature above body temperature.[47] The insignificant differences in morphological defects and seminal pH might have contributed to the non-significant change in sperm motility in both groups.

The results did not show any significant change in sperm viability in both control and TPO diet-fed groups suggesting that TPO might not have had significant effect on sperm viability. In conclusion, this study has shown that long term ingestion of TPO diet has deleterious effect on reproductive function (sperm count, serum testosterone concentration and serum luteinizing hormone level) in male rats.

Conflict of Interest and Source of Funding

The authors have no conflict of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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