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Effects of Palm Oil – Containing Diets on Enzyme Activities of Rats

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Abstract: The effect of consumption of palm oil diets on plasma activities of some enzymes used as markers of organ function was investigated in rats. Four-week old male albino rats of the Wistar strain (n = 8 per group) were maintained for 28 days on standard dry rat food (4.7% fat by weight) supplemented (10% and 20% by weight) with red palm oil (RPO), refined palm olein (REFPO) and corn oil (CO). In the study, the effects of the various dietary supplements on plasma activities of lipase (EC 3.1.1.3), alkaline phosphatase (EC. 3.1.3.1), aspartate transaminase (EC 2.6.1.1) and alanine transaminase (EC 2.6.1.2) were compared with those of a control group receiving normal rat mash. The enzyme activities in the plasma of rats fed 10% oil-supplemented diets were comparable to those of the control ($p>0.05$). Plasma enzyme activities indicated dose-effect relationships between the amount of oil in the diet and activities of lipase, alkaline phosphatase (ALP) and alanine transaminase (ALT). The 10% oil-supplemented groups had significantly higher ($P<0.05$) lipase activities (201.60 - 233.80 U/L) than the 20% oil-supplemented dietary groups (147.80 - 165.20 U/L). Alkaline phosphatase levels in test groups (65.70 - 94.46 U/L) were higher than those of the control (63.76 U/L). Increasing the fat contents of the diets induced significant increases ($p<0.05$) in ALP activities. The aspartate transaminase (AST) levels of the control and 10% dietary groups (53.75-68.25 U/L) were higher than those of the 20% oil dietary groups (32.80 - 33.60 U/L). The activities of ALT in the experimental animals decreased with the levels of oil in the diet in a dose - dependent manner. Generally the activities of the transaminases assayed in the 20% oil-fed rats were significantly lower ($p< 0.05$) than those of the control. A determination of ALT/AST ratios indicated values lower than unity in all experimental animals, though the values for 20% oil groups were higher than those of the control and 10% groups. The effects of palm oil containing diets on enzyme profile were comparable to those of corn oil containing diets, as were the effects of RPO when compared with those of REFPO. The results indicated that consumption of palm oil (red or refined) in moderate amounts supports normal enzyme activities.

Key words: Palm oil, enzyme activities, plasma

Introduction

Palm oil, one of the most widely consumed cooking oils in the tropics, is obtained from the mesocarp (pulp) of the fruit of the oil palm *Elaets guineensis*. The oil is consumed fresh (red) or refined at various levels of oxidation. Red palm oil (RPO) is obtained by squeezing the pulp (of palm fruit), which has been boiled and pounded. It contains 50% saturated fatty acids, 40% monounsaturated fatty acids and 10% polyunsaturated fatty acids. Palm oil contains a high proportion of the saturated palmitic acid, but considerable quantities of oleic and linoleic acids, which give it a higher unsaturated content than coconut oil and palm kernel oil (the minor oil obtained from the oil palm) (Gunstone *et al*., 1986). Red palm oil is the richest natural source of β-carotene (500 - 700 mg/L), which is responsible for the characteristic colour of the oil. Most of the β- carotene is destroyed during processing of the oil in palm oil refineries.

Refined palm oil is the light - coloured fraction obtained during the refining, bleaching and deodorization of RPO. This fractionation brings about an enrichment of oleic and linoleic acids in addition to the concomitant reduction in the amount of palmitic acid (King and Sibley, 1984; Gunstone *et al*., 1986; Tan, 1989). Palm oil and its fractions contain less than 1.5% of the hypercholesterolemic lauric and myristic acids. Furthermore, Palm oil and its fractions contain tocopherols. It is especially rich in *Y*- tocotrienol. These substances, which are physiologically active as vitamin E, are useful antioxidants (Sundram and Top, 1994). They delay the time when oxidation in the oil will have proceeded far enough to produce off-flavours and /or odours (Gunstone and Norris, 1983). In addition, they possess free radical scavenger properties, serving to protect biological systems against oxidative and carcinogenic stress (Krinsky, 1994; Manorama *et al*., 1993; Nesaretnam *et al*., 1993; Diplock, 1994).

Several studies have highlighted the effects of dietary palm oils on humans and experimental animals. Little, however, exists in the literature regarding the effects of ingested palm oil on plasma enzymes. A few authors (Ngah *et al*., 1991; Manorama *et al*., 1993; Fernandez and McNamara, 1994) have reported on the effects on

consumption of palm oil diets on some cholesterol and drug metabolizing enzymes. Presently only few feeding studies have examined the relationship between palm oil and the amounts of serum enzymes (such as alkaline phosphatase, alanine and aspartate transaminases) to detect apparent abnormalities in liver (Manorama and Rukmini, 1991;Owu *et al*., 1998). The changes in plasma enzyme activities as a function of the amount of fat ingested, remains unclear due to problems associated with the use of a fixed dose of the oil. Therefore the dose-response relationship has not been established. Thus it was necessary to evaluate the effect of graded levels of palm oil on some enzymes used as markers of organ function.

Materials and Methods

Analysis of the oils used: Red palm oil (RPO) samples were taken from the palm oil mil at the Nigerian Institute for Oil Palm Research (NIFOR), Abak sub station, Akwa Ibom State, Nigeria. Refined palm olein (Turkey Brand) and corn oil (Mazola brand) were purchased from municipal supermarkets in Calabar, Nigeria. The oils were analyzed for their physicochemical variables by the methods of the Association of Official Analytical Chemists (AOAC, 1984). Fatty acids were estimated by gas liquid chromatography (Unicam 4600 gas chromatograph, Cambridge, England) using a polyethylene glycol succinate (PEGS) column on chromosorb W mesh 80-120 with flame ionization detector. Fatty acid composition was expressed as percentages of the total fatty acids (Table 1).

Experimental animals: Albino rats of the Wistar strain bred in the animal house of the College of Medical Sciences, University of Calabar, Nigeria, were used for the study. Male weanling rats aged 28 days were divided into 7 dietary groups (one control and six test groups) of 8 rats each, with body weights evenly distributed across all groups. The control group was placed on commercial rat mash (obtained from Bendel Feed and Flour Mill Limited, Benin City, Nigeria). Animals of the test groups were placed on rat mash supplemented with 10% or 20% (by weight) of RPO, refined palm olein (REFPO) or corn oil (CO). The rats were housed in stainless steel cages in a well -ventilated room at about 27±2^{o}C. Lighting regimen was about 13 hr: 11 hr of light and dark. All the experimental animals had free access to food and water for 4 weeks, after which blood samples were taken for analysis. All animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1985).

Experimental diets: The test diets were prepared (by mixing each of the oils with commercial rat mash) to contain 10% or 20% oil. The 10% oil diet was prepared by adding 5.6g oil to 94g rat mash, while 16.1g oil was added to 84g rat mash to produce the 20% oil diet. The commercial rat mash is scientifically formulated to be rich in all essential nutrients, vitamins and micro elements. It contained 16.3% protein, 4.7% fat, 7.0% ash, 7.9% fibre and 64.1% available carbohydrate.

Collection of blood samples and measurement of plasma enzyme activities: The animals were rats were anaesthetized with chloroform. While under chloroform anaesthesia, the animals were exsanguinated by cardiac puncture through the abdominal aorta of the heart. Sterile syringes and needles were used to collect whole blood into labeled tubes containing 2 drops of heparin as anticoagulant. The contents of each tube were shaken to mix and thereafter centrifuged at 1500 x g for 15 minutes to separate the plasma which was used for analysis of lipase, alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT).

Lipase activities were measured using a commercially available kit (Sigma Diagnostics, St. Louis, MO) based on the method of Tietz and Fiereck (1966). Alkaline phosphatase was determined using a commercially available kit based on the recommendations of DGKC (1972). Aspartate transaminase and ALT activities were determined using enzyme kits (Randox laboratories Ltd., Crumlin, North Ireland, U.K.) based on the method of Reitman and Frankel (1957). Enzyme activity was expressed in International Units per litre (U/L). The results were compared with those obtained using an oil with known hematological effects (corn oil).

Statistical analysis: Results are expressed as the means ± SE. The significance of differences between mean values was determined by Student's t-test and one-way analysis of variance (ANOVA).When ANOVA indicated significant differences, group means were compared by Student's t-tests. Differences were considered significant at $p<0.05$.

Results

The physicochemical properties of the oils used are shown in Table 1.

Table 2 shows the food intake, body weight and body weight gains (BWG) of the rats on the various experimental diets. The daily food consumption for the control rats was 11.8g/rat. Food consumption of rats fed 10% oil-containing diets was comparable to those of the control. The groups on 10% and 20% RPO supplemented diets had consumption of 11.90 and 9.81g/rat/day respectively, while the corresponding values for 10% REFPO and 20% REFPO groups were 12.30 and 9.41g/rat/day. The 10% CO and 20% CO group had food consumption (11.92 and 9.35g respectively), which were comparable to these of 10%

Table 1: Physicochemical Variables and Fatty Acid Composition of the Oils Used*

Physicochemical Variables	Red Palm oil	Refined Palm Olein	Corn Oil
Relative Density	0.92	0.91	0.93
Iodine Value	55.83	61.54	112.27
Saponification Value	199.27	193.20	188.53
Free Fatty Acid	3.50	0.92	0.51
Fatty Acid Composition g/100g fatty acids			
Lauric 12:0	0.1	0.1	-
Myristic 14:0	1.1	1.0	0.1
Palmitic 16:0	44.0	42.1	11.5
Stearic 18:0	4.6	4.1	2.2
Oleic 18:1	39.8	41.5	31.1
Linoleic 18:2	9.8	10.4	52.6
Linolenic 18:3	0.2	0.3	1.1
Arachidic 20:0	0.3	0.3	0.4
Saturated Fatty acids	50.1	47.6	14.2
Unsaturated fatty acids	49.8	52.2	84.8

Average of duplicate determinations

and 20% RPO fed groups. More of a 10% fat diet (23.2 energy %) was consumed compared to a 20% fat diet (40.0 energy %). The average BWG of control rats was 59.94g. The values of BWG (g) were 61.83 and 27.43 for 10% RPO and 20% RPO groups respectively. The 10% and 20% REFPO groups had BWG (g) of 68.08 and 24.85 respectively, while the CO groups had corresponding values of 61.98 and 29.95 respectively. The BWG for 20% oil-fed rats were significantly lower ($p < 0.05$) than those of other dietary groups, but BWG of 10% oil groups were not significantly higher ($p > 0.05$) than those of control. For each level of dietary fat, the weights of corresponding groups of rats were not significantly different ($p > 0.05$).

The effects of palm oil supplementation on the activity of plasma enzymes are illustrated in Table 3. Lipase activities ranged between 147.80 and 233.80 U/L. The control rats had significantly higher lipase activities (201.60 U/L) than those of 20% oil-fed rats ($p < 0.05$). Animals fed 20% oil diets had lower lipase levels (147.80 - 165.20 U/L) than those fed 10% oil diets (201.60 - 233.80 U/L). Lipase levels in rats fed REFPO diets at 10-20% supplementation were not significantly different from corresponding levels in RPO-fed rats or CO-fed rats ($p > 0.05$). The ALP activities of all test groups were higher than those of the control 63.76U/L. Increasing the percentage of oil in the diet produced dose-related alterations in ALP values. The plasma AST activity was 53.8 U/L in the control group. The value was lower than that of the 10% oil-supplemented group (61.14-67.00 U/L), but higher than that of the 20% (31.00-33.60 U/L) oil-supplemented groups. At each test dietary level, the AST activities of animals fed RPO were comparable to these fed REFPO or CO. The groups fed 20% oil-containing diets had significantly lower AST activities ($p < 0.05$) than other experimental groups. Alanine transaminase (ALT) values (in U/L) varied from 12.80 (20% CO) to 19.60 (control). Animals fed 10% oil diets had higher ALT activities (17.29 - 19.25 U/L) than those fed 20% oil diets (12.80-14.40 U/L). Increasing the level of oil in the diets led to dose-dependent decreases in plasma ALT activities of the experimental the reductions in ALT activities brought about by inclusion of oil at 20% levels reached statistical significance ($p < 0.05$).

The ALT/AST ratios of the experimental animals were lower than 1.00. The ratios for animals fed on 20% oil-supplemented diets (0.43) were higher than those of the 10% oil - treated or control groups (0.31-0.37). At each level of dietary fat, similar ratios were observed between animals fed on corn oil and palm oil diets (red or refined). The differences between ALT/AST ratios of 20% oil-fed group and those of other experimental groups did not reach statistical significance ($p > 0.05$).

Discussion

The changes in lipase activities showed inverse dose-effect relationships with the groups fed 20% oil-supplemented diets having lower values than other dietary groups. The enzyme activity changes between one test dietary level and the other were significant ($p < 0.05$). A low level of lipase activity is indicative of heightened activities in the gastrointestinal tract resulting from sustained high influx of triacylglycerols. The normal range of lipase activities is 18 to 280 U/L (Baron, 1973). Since the levels of lipase were in the normal range, it is likely that no treatment-related damage was caused to the pancreas. The differences between the alkaline phosphatase (ALP) activities of all test animals (except 10% RPO and 10% REFPO) and those of the control were significant ($p < 0.05$). ALP activity is affected by diet. Induction of ALP appears to be lipid-dependent. Ingestion of fat leads to an increase in ALP synthesis by rat intestinal mucosa (Glickman *et al.*, 1970; Izui, 1971; Kramer and Hoffman, 1997). Plasma ALP is a sensitive detector for early intrahepatic and extrahepatic bile obstruction, the presence of infiltrative diseases of the liver (Owu *et al.*, 1998) and all bone diseases associated with osteoblastic activities (e.g. osteomalacia and rickets among others). However the ALP levels observed in these studies were within the normal physiological range of 20 to 90 IU/L (Harper, 1975; Kaneko *et al.*, 1997). Thus it is likely that the levels of fat used in the study did not adversely interfere with the calcification and other metabolic activities mediated by ALP.

The differences observed between the aspartate transaminase (AST) activities in rats fed 10% oil-enriched diets and the control was not significant ($p > 0.05$). From the results, refining of palm oil did not seem to have significant effects ($p > 0.05$) on the levels of AST, through animals fed REFPO diets had lower activities than those fed RPO diets. One of the causes for variation

Table 2: Nutritional Performance of Rats Fed the Various Experimental Diets*

Parameter	Experimental Group						
	Control	10% RPO	20% RPO	10% REFPO	20% REFPO	10% CO	20% CO
Average food intake g/rat/day n = 8	11.87	11.90	9.81	12.30	9.41	11.92	9.35
Average daily energy intake (kcal/rat) n=8	43.20	46.71	44.16	50.15	42.41	48.53	42.13
Average protein intake (g/rat/day) n=8	1.94	1.83	1.35	1.89	1.29	1.83	1.28
Initial body wt (g)	55.39±3.22	58.45±3.77	61.94±2.68	56.55±4.83	61.03±4.51	56.46±4.51	88.20±4.25
Final Body wt(g)	115.33±4.88^{a}	120.28±5.79^{a}	92.69±4.90^{b}	124.63±5.54^{a}	87.34±5.27^{b}	118.44±5.40^{a}	58.25±4.82^{b}
Body wt gain (g)	59.94±2.39^{a}	61.83±4.09^{a}	30.75±3.38^{b}	68.08±3.37^{a}	26.31±1.43^{b}	61.98±2.59^{a}	29.95±1.77^{b}
Feed efficiency ratio	0.18±0.01^{a}	0.19±0.01^{a}	0.11±0.01^{b}	0.20±0.01^{a}	0.10±0.01^{b}	0.19±0.01^{a}	0.12±0.01^{b}

*Values are means ± S E (n=8). Values in a horizontal now not sharing a common superscript are significantly different (p<0.05).
Legend: RPO = red palm oil. REFPO = refined palm olein. CO = corn oil

Table 3: Enzyme Activities of Rats fed Palm Oil and Corn Oil –Containing Diets*

Parameter U/L	Experimental Group						
	Control	10% RPO	20% RPO	10% REFPO	20% REFPO	10% CO	20% CO
Lipase	201.60±0.00^{a}	201.60±11.59^{a}	147.80±5.69^{b}	233.80±5.77^{a}	165.20±6.96^{b}	205.80±2.32^{a}	166.88 ± 9.13^{b}
ALP	63.76±2.07^{a}	77.35±5.52^{a}	88.30±5.17^{b}	65.70±0.86^{a}	94.46±6.71^{bc}	77.20±1.15^{bd}	79.70±2.72^{b}
AST	54.80±5.27^{a}	68.25±5.55^{a}	33.60±2.35^{b}	61.14±7.91^{a}	32.80±2.53^{b}	67.00±7.12^{a}	31.00±3.03^{b}
ALT	19.60±1.47^{a}	17.29±1.17^{ab}	14.40±0.98^{b}	18.71±2.05^{a}	13.60±0.75^{b}	19.25±2.51^{a}	12.80±0.20^{b}
ALT/AST	0.37± 0.04	0.31±0.04	0.43±0.02	0.30±0.06	0.43±0.05	0.32±0.06	0.42±0.05

*Values are means ± SE (n = 8). Values in same row not sharing a common superscript are significantly different (p< 0.05).
Legend: ALT = Alanine Transaminase, AST = Aspartate Transaminase, ALP = Alkaline phosphatase.
RPO = Red Palm Oil, REFPO = Refined Palm Olein, CO = Corn Oil.

in transaminase activities is the dietary intake of pyridoxine (vitamin B_6), which is an essential cofactor for transamination reactions (apart from its functions in amino acid decarboxylations, transulfuration and transfer of amino acids into cells among others) (Murray *et al*., 2000). Decreased transaminase activities follow a decreased pyridoxine intake. Thus the low levels of AST in rats fed 20% oil-containing diets could be attributed to dietary factors, especially when it is observed that the 20% groups consumed lower amounts of protein than the 10% groups, which had a higher dietary consumption than the control rats.

The activities of ALT in plasma of rats fed 20% test diets were significantly different from the control ($p < 0.05$) in spite of the type of dietary fat. This could be attributed to reduced food consumption by these groups of rats.

Although variations occur in plasma levels of both AST and ALT in conditions that affect liver integrity, ALT is the more liver-specific enzyme. It is mainly present in liver with only small amounts in other organs. Liver is by far the richest source of ALT. The transaminases are of value as indices of possible liver damage, in detecting the presence of toxicity to the liver or alterations in membrane architecture of the cells of the liver. More important than the absolute ALT and AST values is the ALT/AST ratio. Stroev and Makarova (1984) reported that a high ALT/AST ratio indicates pathology involving the liver. ALT/AST values greater than 1.00 indicate alterations involving the liver cells (Tietz, 1982). The results indicated no statistically significant difference ($p > 0.05$) between ratios in control rats and those fed 10% or 20% oil-treated diets and could therefore be considered to be of no toxicological significance.

From the foregoing, there were inverse does-effect relationships between the level of oil supplementation (in animal diets) and the activities of lipase, and ALT. The ALT/AST ratios did not indicate possible adverse pathological effects involving the livers of the test rats. It does appear that moderate consumption of palm oil supports normal enzyme activities.

References

AOAC, 1984. Official methods of analysis. Association of Official Analytical Chemists. 14th edn. Washington DC. Method No. 28.014.

Baron, D.N., 1973. A Short Textbook of Chemical Pathology, English Language Book Society, London, p. 219.

DGKC, 1972. Recommendations of the Deutsche Gesellschaft fur Klinische Chemie (German Society for Clinical Chemistry). Optimized standard colorimetric method for the determination of alkaline phosphatase. J. Clin Chem. Clin. Biochem., 10: 182.

Diplock, A.T., 1994. Antioxidants and disease prevention. Mol. Aspect Med., 15: 293-376.

Fernandez, M.L. and D.J. McNamara, 1994. Dietary fat saturation and chain length modulate guinea pig hepatic cholesterol metabolism. J. Nutr., 124: 331-339.

Glickman, R.S., D.M. Alpers, G.H. Drammey and K.J. Isselbacher, 1970. Increased lymph alkaline phosphatase after fat feeding: effect of medium chain triglycerides and inhibition of protein synthesis. Biochem. Biophys. Clin. Chem., 12: 871.

Gunstone, F.D. and F.A. Norris, 1983. Lipids in Foods: Chemistry, Biochemistry and Technology, Oxford: Pergamon Press, pp: 40, 141,144-146.

Gunstone, F.D., J.I. Harwood and F.B. Padley, 1986. The Lipid Handbook. Chapman and Hall, London, pp: 76-78.

Harper, H.A., 1975. Vitamins. In: Review of Physiological Chemistry, Lange Medical Publications, California, pp.88-125.

Izui, K., 1971. Lipid requirement for induction of alkaline phosphatase, one of the periplasmic enzymes in E. coli. Biochem. Biophys. Res. Comm., 45: 1506.

Kaneko, J.J., J.W. Harvey and M.L. Bruss, 1997. Clinical Biochemistry of Domestic Animals. 5th edn., New York, Academic Press, pp: 895-899.

King, B. and I. Sibley, 1984. Authenticity of edible vegetable oils and fats. Part II : Palm oil and palm oil fractions. Surrey, U.K, Leatherhead Food Research Association (LFRA research report no. 642).

Kramer, J.W. and W.E. Hoffman, 1997. Clinical Enzymology. In: "Clinical Biochemistry of Domestic Animals" (Kaneko, J.J., Harvey, J. W. and Bruss, M.L.,eds.),5th edn., New York, Academic Press, p: 315.

Krinsky, N.I., 1994. Mechanism of action of biological antioxidants. Proc. Soc. Exp. Biol. Med., 200: 248-254.

Manorama, R. and C. Rukmini, 1991. Nutritional evaluation of crude palm oil in rats. Am. J. Clin. Nutr., 53: 1031 S -1033 S.

Manorama, R., N. Chinnasamy and C. Rukmini, 1993. Effect of red palm oil on some hepatic drug-metabolizing enzymes in rats. Food Chem. Toxicol., 31: 583-599.

Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, 2000. Harper's Biochemistry. 25th ed., McGraw Hill (Health Professional Division), New York, pp: 633-634.

Nesaretnam, K., T.P. Devasagayam, B.B. Singh and Y. Basiron, 1993. The influence of palm oil or its tocotrienol - rich fraction on the lipid peroxidation potential of rat liver mitochondria and microsomes. Biochem. Mol. Biol, Int., 30: 159-167.

Ngah, W.Z.W., Z. Jarien, M.M. San, A. Marzuki, G.M. Top, N.A. Shaman and K.A. Kadir, 1991. Effect of tocotrienols on hepatocarcinogenesis induced by 2-acetylaminofluorene in rats. Am. J. Clin. Nutr., 53: 1076 S-1081 S.

NRC, 1985. National Research Council. Guide for the care and use of laboratory animals. Publication no 85-23 (rev). National Institutes of Health, Bethesda, MD.

Owu, D.U., E.E. Osim and P.E. Ebong, 1998. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. Acta Tropica, 65: 65-73.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28: 56-60.

Stroev, E.A. and V.G. Makarova, 1984. Laboratory Manual in Biochemistry. Mir Publishers, Moscow, pp: 162-164.

Sundram, K. and A.G.M. Top, 1994. Vitamin E from palm oil. Its extraction and nutritional properties. Palmas, 15: 77- 82.

Tan, B.K., 1989. Palm carotenoids, tocopherols and tocotrienols. J. Am. Oil Chem. Soc., 66: 770-776.

Tietz, N.W., 1982. Fundamentals of Clinical Chemistry, W.B. Saunders Company, Philadelphia, pp: 562-698.

Tietz, N.W. and E.A. Fiereck, 1966. Specific method for serum lipase determination. Clin. Chim. Acta., 13: 351-358.