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Influence Of Thermoxidized And Fresh Palm Oil Diets On Some Mineral Contents Of The Femur Bone Of Growing Wistar Rats.

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ABSTRACT

The effect of thermoxidized and fresh palm oil diets on some mineral contents of the femur bone of growing Wistar rats was studied. Twenty-four rats were divided into three groups (A, B and C) of eight rats each, and fed on thermoxidized palm oil, fresh palm oil and normal (control) rats diets respectively for fourteen weeks. Each of the palm oil diets contained 15 % (w/w) thermoxidized or fresh palm oil. The calcium concentration of the femur of the thermoxidized palm oil group (85.50 0.82 ppm) was significantly ($P < 0.01$) lower than that of the control (103. 67 0.12 ppm) and that of the fresh palm oil group (100.56 0.2ppm). The magnesium concentration of the femur of the thermoxidized palm oil group (15.81 1.11 ppm) was significantly ($P < 0.01$) lower than that of the control (18.73 0.8 ppm) and that of the fresh palm oil group (18.00 1.21 ppm). The copper concentration of the femur of the thermoxidized palm oil group (8.85 0.8 ppm) was significantly ($P < 0.01$) lower than that of the control group (12.5 0.5 ppm) and that of the fresh palm oil group (11.6 0.65 ppm). Our results suggest that chronic consumption of thermoxidized palm oil diets may interfere with the normal mineralization process in bones of growing rats.

Keywords: Fresh palm oil; Thermoxidized palm oil; Magnesium; Calcium;Copper; Bone.

The tocopherols and tocotrienols in palm oil act as antioxidants making the oil relatively stable to oxidation (Gapor *et al*, 1989). Fresh palm oil has low oxidation values (Rossel, 1983), but on heating, the values changes. Generally people heat oil to render it more palatable, but heating causes thermoxidation. Literature abounds with reports that thermoxidation has a deteriorative effect on oils (Perkin and vanAkkerren, 1965; Peers and Swoboda, 1982; Isong, 1988). Thermal oxidation of oils results in the formation of reactive, cytotoxic and destructive products in tissues (Plea, 1975; Frankel 1980; Ziombski, 1982). And the oxygen derived free radicals and dihydroxy esters in thermally oxidized oil causes injury to cells, tissues and organs (O'Sara *et al*, 1969; Frankel, 1980; Meredith, 1984)

Literature is replete with evidence that chronic consumption of thermally oxidized palm oil diets usually result in a concomitant toxic effect in various tissues. Chronic consumption of thermally oxidized palm oil diet has been demonstrated to cause derangement of several tissues notably the lungs, liver and kidney (Osim *et al.*, 1994); damage to intestinal mucosa (Igiri *et al.*,1994); inducement of reproductive toxicities(Isong. *et.al.*, 1997): anaemia, leucocytosis, thrombocytopaenia and bleeding tendencies (Mesembe,2002): and damage to testes (Mesembe *et al* 2004) in rats.

There is therefore, a possibility that the chronic consumption of thermoxidized and fresh palm oil diets will adversely affect the concentration of mineral content of the femur bone of growing Wistar rats. This study examines this possibility.

MATERIAL AND METHODS

Palm oil diet

Palm oil was purchased from Akim market in Calabar, Nigeria. The palm oil was divided into two equal portions. One part was thermoxidized (Isong, 1988; Osim *et al.*, 1994) whereas the other one was used in its fresh form since these are the two common forms of palm oil used for cooking. The thermoxidized palm oil was obtained as follows.

Fresh palm oil was heated at 150°C in a stainless steel pot intermittently for five times with each lasting twenty minutes. At the end of each heating session, the oil was allowed to cool for five hours. Since the level of palm oil in most West African dishes is about 15% (Umoh, 1972), fifteen grams of the cooled thermoxidized oil was mixed with eighty five grams of rat feed and rats were fed on it as the first test diet. Another fifteen grams of the fresh palm oil was mixed with eighty-five grams of rat feed and given as the second test diet. The diets were stored in black containers at 4°C to prevent further oxidation of the oil component. The control group was placed on rat feed.

Rats

Albino rats of the Wistar strain were bred in the animal house of the Department of Anatomy, University of Calabar, Nigeria. Twenty-four rats were divided into three groups,(A, B, C) of eight rats each. The rats were about the same age (55 60 days old) and each weighed between 50 55g at the start of the feeding experiment.

The rats in the first group were fed on thermoxidized palm oil diet. The second group were fed on fresh palm oil diet while the third group served as the control group. The rat chow was obtained from Livestock Feeds Nig. Ltd. Lagos, Nigeria. Rats in all the three groups were fed *ad libitum* for fourteen weeks .At the end of the experimental feeding period, the animals were sacrificed using chloroform. With the aid of dissecting forceps, dissecting needle and surgical blade, the femur was removed and the muscles, fats and ligaments were removed from the femur. The bones were dried in an Astell Hearson hot air dryer oven at 60^0 C for 24 hours. They were later ground with the laboratory mortar and the powdery form was digested in 5mls concentrated nitric acid and 1ml perchloric acid. The solution was then analyzed for calcium, magnesium and copper by the use of HACH DR 3000 Spectrophotometer.

Statistical analysis

Data are expressed as the mean standard error of mean (SEM). One way analysis of variance (ANOVA) was applied for data analysis. This was followed by a post-hoc student t-test for F values that were significant. Values of p less than 0.01 and 0.05 were considered significant.

RESULTS

The results obtained are shown in table 1. The calcium concentration of the femur of the thermoxidized palm oil group (85.50 0.82 ppm) was significantly ($P<0.01$) lower than that of the control (103.67 0.12ppm;); and significantly ($p<0.01$) lower than that of the fresh palm oil group (100.56 0.2ppm). On the other hand, the calcium concentration of the femur of fresh palm oil group was not significantly different from that of the control.

The magnesium concentration of the femur of thermoxidized palm oil group (15.81 1.11 ppm) was significantly ($P<0.01$) lower than that of the control (18.73 0.8ppm); and significantly ($p<0.01$) lower than that of the fresh palm oil group (18.00 1.21 ppm). However, the magnesium concentration of the femur of the fresh palm oil group was not significantly different from that of the control.

The copper concentration of the femur of the thermoxidized group (8.85 0.8 ppm) was significantly ($p<0.01$) lower than that of the control (12..5 0.5 ppm); and significantly ($p<0.01$) lower than that of the fresh palm oil group (11.6 0.65 ppm). Whereas , the copper concentration of the femur of the fresh palm oil group was significantly ($p<0.05$) lower than that of the control group

DISCUSSION

Mineralization, a vital process in the development of bones involves the impregnation of minerals in the bone matrix (Singh, 2002). The bone matrix is made up of collagen fibres which are embedded in the gelatinous ground substance. The matrix is strengthen by salt present in it. These salts include hydroxyapatites which contain calcium, and phosphate, sodium potassium, magnesium and carbonate. (Sembulingam and Sembulingam, 2002). Any impairment in the process of mineralization would reflect in the concentration of the minerals in the bone. Consumption of thermally oxidized palm oil diet resulted in a significantly lower concentration of calcium, magnesium and copper in the femur of growing rats. This may be as a consequence of malabsorption, due to intestinal mucosal damage (Igiri *et al.*, 1994). Which led to poor absorption of nutrients, thereby resulting in the lower concentration of the minerals.

It is possible that the free radicals and hazardous substance that are produced in oxidized oil may have inhibitory effect on the mineralization process in the bones as shown in our results. This is in agreement with previous studies, which showed that thermoxidized palm oil diet caused degeneration of several tissues (Osim *et al.*, 1994; Igiri *et al.*,1994; Isong *et al.*, 1999 ;Mesembe,2002; Mesembe *et al* .,2004).Furthermore, experiments of (O'Sara *et al.*, 1969; Tappel 1973; Gabriel *et al.*, 1979; Frankel, 1980; and Meredith, 1984) demonstrated that the constituents of oxidized oil caused injury tocells, tissue

Table 1: INFLUENCE OF THERMOXIDIZED AND FRESH PALM OIL DIETS ON SOME MINERAL (CA,MG,CU) CONCENTRATION OF THE FEMUR BONE OF GROWING WISTAR RATS.

Group (N=8)	Diet type	Calcium conc. (ppm)	Magnesium conc. (ppm)	Copper conc. (ppm)
A	Thermoxidized palm oil	85.50 ± 0.82 ppm...	15.81 ± 1.11 ppm...	8.85 ± 0.8 ppm...
B	Fresh palm oil	100.56 ± 0.2 ppm	18.00 ± 1.21 ppm	11.6 ± 0.65 ppm..
C	Control	103.67 ± 0.12 ppm	18.73 ± 0.8 ppm	12.5 ± 0.5 ppm

Values presented as mean SEM
...$p < 0.01$ compared to the control and fresh palmoil group
.. $p < 0.05$ compared to the control

and organs.
The various hazardous constituents may have impaired and suppressed the normal functioning of osteoblast and chondroblast. These cells play a major role in calcification (Bernard and Pearse, 1969; Anderson, 1969). The resultant compromised functioning of osteoblasts may cause a reduced matrix deposition and concomitant reduction in the deposition of calcium salts (Natelson and Natelson, 1975). This is significant because any substance that increases matrix production increases mineralization (Baron 1973)
Chronic consumption of thermoxidized palmoil diet has been reported to induce physiological stress response as evidenced by an increased white blood cell count (Mesembe, 2002), probably as a consequence of tissue damage (Osim *et al.*, 1994). The physiological stress may have caused the decrease in magnesium concentration (Gunther *et al.*,1981). The reduced magnesium concentration may also be a consequence of malabsorption (Nestle, 1985). Magnesium is required for the synthesis of protein, nucleic acid, nucleotide, lipids and carbohydrates (Martin,1985). The concentration of magnesium is highly Correlated with bone resorption and formation process (Alfrey and Miller, 1973) This probably also contributed to a concomitant reduction in calcium concentration (Gunther *et al.*,1981).

The decreased concentration of copper may be a consequence of malabsorption (Nestle, 1985). The deficiency of copper leads to abnormal bone formation (Martin, 1985); because, copper deficiency affects cross- linking of bone collagen (Rucket *et al* 1975).
The decreased concentration of these minerals in the thermoxidized palm oil group indicates a possible interference with the normal osteoblastic and chondroblastic activities and mineralization process in bones of growing rats.

REFERENCES

Alfrey, A. C; and Miller, N. L; (1973) Bone magnesium pools in uremia. Journal of Clinical Investigation. 52: 3019 - 3029.

Anderson , H. C., (1969). Vesicle associated with calcification in the matrix of epiphyseal cartilage. Journal of Cell Biology 41: 59 - 72.

Baron, D. N., (1973). Chemical Pathology English Universities Press. 3rd ed. Pp.95 - 129.

Bernard, G. W., and Pearse, D. C., (1969). An electron microscopic study of initial intramembranous osteogenesis. American Journal of Anatomy 125 : 281 -290.

Frankel, E. N. (1980) Lipid oxidation. Prog. Lipid Res. 19: 1-22.

Gapor, A. B., Ong, A.S. H., Kato, A., Watanabe, I. H., kawada, T., (1989) Antioxidant activities of palm vitamin E with special references to tocotrienols. The int J Oil Palm Res Dev 1: 63 - 67.

Gunther, T., Ising, H., Mohn Nawroth, F., Chahound, I., Meker, H. J., (1981) Embryotoxic effects of magnesium deficiency and stress on rats and mice. Teratology 24 (24): 225 - 233.

Hassan, A. H. (1988) Palm Oil and Health. The planter. Pp 505 519.

Igiri, A. O.; Ibegbu, A. O. and Osim E. E. (1994) The Morphological and histological changes in the small intestine induced by chronic consumption of palm oil diets in rats. Tropical Journal for Applied Sciences. 3 (4) : 144 - 153.

Isong, E. U. (1988) Biochemical and nutritional studies on rats fed thermally oxidized palm oil. Ph.D Thesis. University of Calabar, Nigeria.

Isong, E. U., Ebong, P. E., Ifon E. T., Umoh, I. B., Eka, O. U., (1997) Thermoxidized palm oil induced reproductive toxicity in healthy and malnourished rats. Plant foods Hum Nutr 51: 159 - 166.

Martin, D. W., (1985) water and minerals. In : Harper's Review of Biochemistry 20th ed. ,
Eds Martin DW, Mayes PA, Rodwell VW, Granner D K. Lange. California pp 649 - 660.

Mesembe, O. E., (2002). The effects of fresh and thermoxidized palm oil diets on some haematological indices in the rat. MSc thesis, University of Calabar, Nigeria.

Mesembe, O. E., Igiri, A. O., Akpa, AO, Fischer, V. A., Ekong, M.. B.,Anibeze C. I. P ,(2004) Histological changes and demonstration of glycogen distribution in the gonads of male wistar rats following long term ingestion of mixed palm oil diets. J. Exp and Clin Anat. In Press.

Meredith, S., (1984) Free radicals . Friend or foe ? Medicine Digest 10 (4): 23 - 25.

Natelson. S., and Natelson, E. A., (1975) Maintenance of fluid and electrolyte balance. In: Principle of applied Clinical Chemistry. Chemical Background Medical Applications. Vol 1. Plenum Press. London. New York Pp 144 - 184.

Nestle, M., (1985). Nutrition. In: Harpers' review of Biochemistry. 20th ed. Eds Martin DW, Mayes P A, Rodwell VW, Granner DK, Lange. California Pp678 .

O'sara; R. W., Stewart, L., Brown, J., Harph, W. C., (1969) carcinogenicileg of heated fats and fat fractions. J. Natl. Cancer. Inst. 42 : 275 - 278.

Osim, E. E.; Owu, D. W, Isong, E. U. and Umoh, I. B. (1994) Influence of chronic, consumption of thermoxidized and fresh palm oil diets on basal metabolic rate, body weight and morphology of tissues in rats. Discov. Innov. 6: 389 - 396.

Peers K E and Swoboda PAT (1982): Deterioration of sunflower seed oil under stimulated frying conditions and during small scale frying of potato chips. J. Sci. food Agric 33 (4): 389-395.

Perkins EG and Van Akkeren IA (1965): Heated Fats, IV: Chemical changes in fats subjected to deep fat frying processes. J.AM Oil Chem. Soc. 42: 161-166.

Plea RM (1975): Protein synthesis and absorption in rats fed on oxidized oil. Practitioner 224: 306-421.

Rosell JB (1983): Measurement of rancidity. In: rancidity in foods. Eds. Allen JC, Hamilton RJ. Applied Sci Pub Ltd. England. Pp 21 46.

Rucket, R.. B, Parker, H. E, Rogler. J. C., (1969). Effect of copper deficiency on chick bone collagen and selected bone enzymes. Journal of Nutrition 98: 57 - 63.

Sembulingam, K., Sembulingam, P., (2002) Essentials of Medical physiology 2nd ed. Jaypee Brothers. New Delhi pp 304 - 305.

Singh, I., (2002) Textbook of Human Histology. 4th ed. Jaypee New Delhi. Pp 94 - 166.

Tappel. A. L., (1973) Lipid peroxidation damage to cell components. Fed. Proc. 32: 1870-1874.

Umoh, I. B., (1972) Changes in the nutritive values of some Nigerian diets after cooking by certain South Eastern traditional methods. PhD. Thesis. University of Ibadan, Nigeria.

Ziomski, H., (1982). Chemical and nutritional changes in heated fats III. Changes in nutritive value. Raezniki Panstwowego Zadkladu Higieny 33 (3): 162-170.

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