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Effect of Trans Fatty Acids Consumption on Some Haematological Indices in Albino Wistar Rats

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Abstract: This study was carried out to investigate the effect of trans fatty acids on haematological indices. This was done by supplementing the diets fed to the albino Wistar rats with different concentrations of thermally oxidized palm oil and margarine as sources of trans fatty acids. Fifty albino Wistar rats were used for this study and were randomly selected into five groups of ten rats. Group 1 rats serving as the control received only the stock diet. Group 2 received 85% rat pellet supplemented with 15% margarine. Group 3 was fed with 75% rat pellet and 25% margarine. The fourth group was fed with 85% rat pellet supplemented with 15% thermally oxidized palm oil. Group 4 was fed with 75% rat pellet supplemented with 25% thermally oxidized palm oil. The feeding experiment lasted for six weeks at the end of which rats were sacrificed for determination of haematological indices. Results showed significant ($p<0.05$) decrease in Red Blood Cell (RBC) count, White Blood Cell (WBC) and platelet counts, Haemoglobin (Hb) concentration and Packed Cell Volume (PCV) in all test groups. This probably suggests that trans fatty acids may adversely affect the health of an individual and should be reduced in diet.

Key words: Haematological indices, margarine, trans fatty acids, thermally oxidized palm oil

INTRODUCTION

Considerable attention has been focused on the potential adverse effects of *trans* fatty acids produced by the method of partial hydrogenation of vegetable oils or marine oils, which may decrease their essential fatty acid contents and raise the saturated fatty acid content. This change in geometric configuration from *cis* to *trans* isomers have been reported to play critical role in the development of several health problems including coronary heart disease and fetal and infant neurodevelopment and growth and childhood allergies (Masanori, 2002). Trans fatty acids are unsaturated fatty acid with at least one double bond in trans configuration. It occurs naturally in dairy and other natural fats. It has long been known that the rumen and faeces of animals and humans contain relatively high amount of trans fatty acids (Mensink and Katan, 1990). Metabolic studies have shown that trans fatty acids have adverse effects on blood lipid level; increasing LDL-cholesterol (bad cholesterol) and decreasing HDL-cholesterol(good cholesterol). This combined effect on the ratio of LDL-cholesterol to HDL-cholesterol is doubles that of saturated fatty acids (Mensink and Katan, 1990 and Khosla and Hajiri, 1997). Trans fats have been implicated in increased risk of coronary heart disease in epidemiologic studies (Willet and Ascherio, 1993). Trans fatty acids have been shown to decrease the body's ability to produce natural anti-inflammatory prostaglandins (Chardigny *et al*., 1995). However, this work focused on the impact of trans fatty acids on some haematological parameters in albino Wistar rats.

MATERIALS AND METHODS

Fifty albino wistar rats weighing between 70 and 140 gram were obtained from animal house of the department of Biochemistry, University of Calabar and used for this study. These animals were kept in plastic cages with wire mesh top under standard conditions (28°C ambient temperature and 46% relative humidity) with a 12 h light-dark cycle. The animal room was adequately ventilated and the animals were fed with commercial rat chow and water *ad libitum* throughout the experimental period. The animals were assigned into five groups of ten (10) rats. Each of these groups was given a compounded diet of different composition. The first group being the control was fed with normal stock diet (rat pellet). The second group was fed with 15% trans fatty acid (lower concentration of margarine) supplemented with 85% rat pellet. The third group was fed with 75% rat pellet supplemented with 25% trans fatty acid (higher concentration of margarine). The fourth group was fed with 85% rat pellet supplemented with 15% trans fatty acids (lower concentration of thermally oxidized palm oil). The fifth group was fed with 75% rat pellet supplemented with 25% trans fatty acids (higher concentration of thermally oxidized palm oil). Weight of the various experimental groups were taken on weekly basis during the course of the experiment. These animals in the test groups (1-5) were fed for six weeks with different compounded diets. On the last day of the feeding experiment; feed was withdrawn from the rats twelve hours to the time of sacrifice. The rats were

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euthanized with chloroform and blood collected into sample tubes by cardiac puncture for some haematological indices assessment.

Haematological assay

Determination of haematocrit-packed cell volume (Dacie and Lewis, 1991): Capillary tube method as described by Dacie and Lewis (1991) was used to estimate packed cell volume. The method permits the determination of the volume occupied by the Red Blood Cells (RBC) in the blood. A capillary tube is filled with whole blood and spun in a centrifuge to pack the red blood cells. The haematocrit value (the ratio of the height of cells over the total height of the fluid in the tube is read and converted from a microhaematocrit reading device Whole blood was gently swirled to give an even distribution. Capillary tubes were then filled by capillary action to the mark with whole blood. The bottom end of the capillary tube was sealed with plasticine and then placed in a digital micro haematocrit centrifuge for centrifugation which lasted for 5 min. The percentage haematocrit was then read on the graduated scale positioning the capillary tube containing the packed cells and plasma in the slot of the haematocrit reader so that the baseline of the reader intersects the base of the cells.

Estimation of haemoglobin concentration in whole blood (Crosby *et al*., 1954; Pla and Fritz, 1971): Blood haemoglobin determination was carried out by the cyanomethaemoglobin method of Crosby *et al*., 1954) as modified by Pla and Fritz (1971).

The ferricyanide oxidizes the haemoglobin to methaemoglobin to form a non toxic cyanomethaemoglobin. Sodium bicarbonate is used in stabilizing the products.

Estimation of total Red Blood Cell (RBC) counts: Red blood cell pipette was used to suck whole blood up to 0.5 mark and excess blood on the tip of the pipette was wiped off. The RBC diluting fluid (Hayem's fluid) sucked up to 101 mark. The pipette was rolled on the palm and kept for 5 min. The cover slip was fixed on the counting chamber; the chamber was charged and viewed under the microscope using high power magnification. The total number of RBCs in one cubic mm of blood = 10000 n where n is the number of cells (Dacie and Lewis, 1991).

Estimation of total white blood cell count: Blood was sucked up to 0.5 mark using White Blood Cell (WBC) pipette and the excess blood was wiped off from the tip of the pipette. Turk's fluid was sucked up to 101 mark. The pipette was gently rolled on the palm to allow the sample mix properly. The mixture was kept for sometime and the chamber charged. It was viewed under the microscope using higher magnification. The total number of white blood cells in one cubic mm of blood = 5,000n where n is the number of cells (Dacie and Lewis, 1991).

Estimation of platelet count (Wu and Hoak, 1974): Blood was sucked up to 0.5 mark using red blood cell pipette. The excess blood was wiped off from the tip of the pipette. The Rees and Ecker solution sucked up to 101 mark. The pipette was rolled on the palm and kept for five minutes before the chamber was charged with the mixture, this was viewed under the microscope and the cells counted. The number of platelets present in one cubic mm of blood = 5,000 n where n is the number of cells.

Statistical analysis: The results were analyzed for statistical significance by one way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data are expressed as Mean±SEM. P values < 0.05 are considered significant.

RESULTS

The results obtained for the weight gain/feed efficiency ratio and some haematological parameters are shown in Table 2 and 3.

DISCUSSION

The test groups show significant weight gain ($p<0.05$) except group 3 (higher margarine). The increases may be attributed to incorporation of additional fatty acids in the formulated diet and adequate utilization by Wistar rats.

There were decreases in RBC counts in all treatment groups relative to controls which showed that the trans fatty acids fed to the different experimental animal groups had adverse effect on hematopoietic status. The least value was found in the group fed with higher concentration of margarine. At ($p<0.05$) there was significant variation. The likely explanation for the effect of trans fatty acids on RBC may probably be due to destruction of the membrane structure of RBC. Infact, the work of Alexander (1978) suggested that some constituents of thermally oxidized palm oil alter electrical conductivity of cell membrane. The kidney has been reputed to be a target site of toxicity of oxidized palm oil diets. The kidney elaborates erythropoietin which is produced by intestinal peritubular cells (Davidson, 1990). Failure of erythropoietin may have caused the decrease RBC count in the groups fed with test diets. Oxidized palm oil has been implicated in kidney tissue damage especially partial tubular atrophy (Osim *et al*., 1992). There were decreases ($p<0.05$) in the levels of platelet counts in all test groups. Moreso, there was decrease in WBC in the treatment groups compared to

Table 1: Experimental design

---------- Group ----------		Number of rats	% Feed composition ratio	Weight of feed and oil in gram	Percentage (%)
I	(Control)	10	Rat chow only	840	100
II	Lower margarine	10	Rat chow(85%)	768	85
			Margarine(15%)	72	15
III	111 (Higher margarine)	10	Rat chow(75%)	678	75
			Margarine (25%)	162	25
IV	1V (Lower thermally oxidized palm oil	10	Rat chow (85%)	768	85
			Thermally oxidized palm oil	72	15
V	V (Higher thermally oxidized palm oil	10	Rat chow (75%)	678	75
			Thermally oxidized palm oil (25%)	162	25

Table 2: Weight gain and feed efficiency ratio for the experimental albino wistar rats

Group	Average initial weight (g)	Average final weight (g)	Weight gain (g)	Total feed consumption (g)	Feed efficiency ratio
(Control)	134.5	172	38	42 x 12 = 504	0.0753
(Lower margarine)	122.3	180	57.7	42 x 12 = 504	0.11
(Higher margarine)	124.5	160	36.0	42 x11.5 = 483	0.0745
(Lower thermally oxidized palm oil)	131.5	175.5	44	42 x 12 = 504	0.087
(Higher thermallypalm oil oxidized palm oil)	124	169	45	42 x 11 = 462	0.097

Mean±SE, n = 10, p<0.05

Table 3: RBC, WBC, Platelet counts, Packed cell volume and haemoglobin concentration in albino wistar rats

---------- Group ----------		RBC count ($x10^3$ cell/dl)	WBC count ($x10^3$ cell/dl)	Platelet count ($x10^3$ cell/dl)	PCV (%)	Mean cell haemoglobin concentration	Hb (g/dl)
I	Control	555.75±129	11.94±0.6474	328.50±8.97	29.5±4.5	0.35	11.665±0.153
II	Lower margarine	439.75±9.54	10.86±0.4588	222.75±4.57	26.5±1.97	0.32	8.825±0.2645
III	Higher margarine	387.5±15.55	8.9975±0.8978	210.25±7.02	24±1.83	0.29	6.75±0.4690
IV	Lower thermally oxidized palm oil	413.25±16.40	8.2±0.4637	215.5±9.34	26±2.16	0.28	7.4±0.4690
V	Higher thermally oxidized palm oil	402±16.66	9.1325±0.8924	210.95±14.26	25±2.16	0.29	7.175±0.9

Mean±SE, n = 10, p<0.05

controls. The decrease was significant and results suggest suppression of white blood cell production.

The packed cell volume showed decreases compared to controls The decrease in PCV observed followed similar trend with RBC count. Packed cell volume represents the percentage of the red blood cell in whole blood (Dacie and Lewis, 1991). There were significant (p<0.050) decreases in haemoglobin concentrations in all the test groups compared to controls. The decrease in haemoglobin concentrations may be due to decreased storage of iron in the liver as a result of damage to liver which strores iron as ferritin and haemosiderin (Murray *et al*.,1988). This damage to the liver may have caused depleted amount of iron stored and subsequently lower haemoglobin concentration.

Conclusion: Trans fatty acid consumption has been on the increase because of the changing life style and this increased consumption is from fast foods and other culinary practice of using abused oils (repetitive frying). However, findings from our present study show possible anaemic condition induced by consumption of trans fatty acids in Wistar rats. We conclude that oxidized palm oil diet induced toxicity on haematopoietic status of rats and hence detrimental to health. We conclude that oxidized palm oil diet adversely affect the haematopoeitic status of rats and hence detrimental to health.

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