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Serum Lipid Profile of Rats (*Rattus norvegicus*) Fed With Palm Oil and Palm Kernel Oil-containing Diets

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ABSTRACT

The serum lipid profiles of rats (*Rattus norvegicus*) fed with Palm Oil (PO) and Palm Kernel Oil (PKO)-containing diets were studied. Phytochemicals detected in the PO included tannins, flavonoids, saponins, cyanogenic glycosides and β-carotene while the PKO contained only tannins, flavonoids, cardiac glycosides and cyanogenic glycosides. The PO and PKO were acidic; with pH values of 5.54±0.01 and 5.85±0.01, respectively. The PO used was more ($p<0.05$) rancid and contained longer mono and polyunsaturated fatty acids than the PKO. Proximate compositions of the oils showed that they contained high sources of energy. Incorporating the oils at 10 mL per 100 g feed increased the lipid and energy contents of the feeds. Feeds were administered to the rats *ad libitum* for 35 days. The PO group (POG), PKO group (PKOG) and Control Group (CG) all drank distilled water as the only source of fluid. Consumption of the treated feeds reduced ($p<0.05$) daily feed intake and improved body weight gained and conversion of feed mass to body mass. The serum lipid profile of the rats showed that the POG had the highest ($p<0.05$) levels of triacylglycerol (TG) and Very Low Density Lipoprotein-cholesterol (VLDL-C). The PKOG had the lowest ($p<0.05$) high density Lipoprotein-chloesterol (HDL-C) compared to the CG and POG. It also had the highest ($p<0.05$) Total Cholesterol (TC): HDL-C ratio, low density lipoprotein-cholesterol (LDL-C) concentration and LDL-C: HDL-C ratio. While PO encouraged the formation of phospholipids as seen in the HDL-C, the PKO promoted the biosythensis of cholesterol as seen in the LDL-C. The study showed that PKO was more atherogenic because it was more saturated and contained fewer types of antioxidant phytochemicals.

Key words: *Rattus norvegicus,* lipid profile, palm oil, palm kernel, diets

INTRODUCTION

Palm Oil (PO) is a form of edible vegetable oil that is extracted from the fruit of the tropical palm tree *Elaeis guineensis* and has been used as a nutritious source of oil for thousands of years (Chandrasekharan *et al*., 2000; Mukherjee and Mitra, 2009). It may have now surpassed soybean oil as the most widely produced vegetable oil in the world (Chu-Sing, 2006; Mukherjee and Mitra, 2009). Whereas, PO is present in the fleshy mesocarp of palm fruit, Palm Kernel Oil (PKO) is sourced from the kernel or seed of the fruit. These two oils have different fatty acid compositions (Chow, 1992; Wardlaw and Kessel, 2002; Hayes and Khosla, 2007). While PO has greater use in food products, PKO may sometimes be used as the fatty substance in non-dietary coffee creamer, margarine and livestock feed (Hayes and Khosla, 2007).

The link between dietary fats, especially edible oils and health (Ye and Kwiterovich, 2000; Ighosotu and Tonukari, 2010) has necessitated a growing research interest in many valuable oils (Eidangbe *et al.*, 2010). For instance, apart from PO, other edible oils such as almond and avacado oil are good sources of Vitamin A and E (http://vitamins.lovetoknow.com /Foods_Containing_ Vitamin_E). Canola oil; obtained from rapeseeds, flax seed oil and soyabean oil contain high levels of omega-3 fatty acid which lowers the risk and predisposition to cardiovascular diseases (Arterburn *et al.*, 2008). But it has been generally agreed among nutritionist that edible oils such as coconut and peanut oils should be avioded due to high content of saturated fatty acids (Enig, 1996). Although edible oil such as PO contains approximately 50% saturated fatty acids (palmitic acid, stearic acid) with 50% unsaturated fatty acids (oleic acid, linoleic acid), it does not promote arteriosclerosis and arterial thrombosis (Pereira *et al.*, 1990; Edem *et al.*, 2002; Jian *et al.*, 1997). Similarly, PKO is composed vertually of esterified saturated fatty acid (approximately 80%) and there are contradictory research finding on the health implications as a result of the consumption of PKO and PO (Hornstra, 1990; Jian *et al.*, 1997; Vessby, 1994; Edem *et al.*, 2002; Steinberg, 2006; Adam *et al.*, 2008; Mukherjee and Mitra, 2009).

Previous reports have shown that different class of dietary lipids may promote beneficial or detrimental health conditions (Poveda *et al.*, 2005; Adam *et al.*, 2008; Mukherjee and Mitra, 2009) by their capacity to alter blood lipid profile. Therefore, measurement of blood lipid profile serves as readily available and reliable diagnostic parameter for establishing either condition. In the current study, we measured the effects of PO and PKO-containing diet on serum Total Cholesterol (TC), High Density Lipoprotein-Cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and Very Low Density Lipoprotein-Cholesterol (VLDL-C) concentrations in weanling male albino rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Sources of palm oil and palm kernel oil: Fresh PO samples were purchased from Nkwo-Ukwu Market, Ihiagwa, Nigeria while refined PKO samples were products of Camela Vegetable Oil Limited, Irete, Nigeria.

Analyses of palm oil and palm kernel oil: Hydrogen ion (H^+) concentration was measured with pH meter (Hanna pH/EC/TDS/Temp Meter w/pH Electrode Diagnostic Hi 9813-6 716815). Determinations of Acid Value, Iodine Value and Saponification Number were by standard method of analyses AOAC (1990). Phytochemical screening was carried out for the presence of tannins, flavonoids, saponins, alkaliods, cardiac and cyanogenic glycosides as described by Ayoola *et al.* (2008a,b). The presence of β-carotene was detected by the method of Evans (2003).

Proximate analyses of diet: Proximate analyses of the PO and PKO samples for crude fibre, ash and moisture by oven assay, lipid by soxhlet and protein were performed according to AOAC (1990) procedures. The level of carbohydrate was obtained by the difference method. The energy value of the diet was evaluated according to Edem *et al.* (1990). The proximate composition of the feed was as stated by the manufacturer while those of the treated feeds were calculated by adding the respective proximate compositions of the weights of oils used (10 mL 100 g feed), calculated using the density of PO as 0.89 g cm^{-3} and that of PKO as 0.952 g cm^{-3} to that of the feed. Energy values (kcal/100 g) were calculated by summing the values of protein and carbohydrate which were multiplied by 4 and that of lipid which was multiplied by 9.

Experimental animals: Twenty-four healthy and mature male white albino rats (200-250 g) were obtained from the Animal Unit, Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. The rats were individually housed in stainless steel cages at about 24°C and a 12 h light cycle.

Animal feeding and experimental design: Whereas, test diets were formulated by mixing separately 10 mL of PO and PKO with 100 g of rat mash, the control diet was the mash only. The rats (n = 24) of were allowed to acclimatize for four days prior to diet treatment. They were randomly divided into three groups (n = 8 rats/group). The diet groups included the control (CG), palm oil (POG) and Palm Kernel Oil Groups (PKOG). Distilled water was the only source of fluid. Fluid and feeds were provided *ad libitum* for the duration of the study (35 days). At the end of the study, each rat was re-weighed before being anaesthetized with chloroform vapour. Daily weight gained, total feeds consumed were estimated and daily feed intakes were calculated. Feed Conversion Ratio (FCR) was calculated as a ratio of daily feed intake to weight gained.

Collection of blood sample and serum preparation: Incisions were made into their thoracic cavities. Blood samples were collected by heart aorta puncture using a 10 mL hypodermic syringe and allowed to clot in sample vials. The samples were centrifuged at 3000 rpm for 5 min using the B. Bran Scientific and Instrument Company, England, centrifuge. The supernatant was harvested by simple aspiration with Pasteur pipette and stored in clean tube at -4°C until analysis.

Serum lipid profile: Diagonistic kits for the lipid profile (with the exception of very low density lipoprotein-cholesterol, VLDL-C) were purchased from BioSystems® (S.A Costa Brava of Barcelona, Spain). The assays were performed according to the manufacturer's instruction. VLDL-C concentrations were estimated using the methods of Burnstein and Sammaille (1960) where the value in mg dL^{-1} is based on the assumption that in fasting subjects, the VLDL-C to total plasma TG ratio is relatively fixed at 1:5.

Statistical analysis: The data were analyzed by the use of the students' t-test of significance and the one-way analysis of variance (ANOVA). Mean $p<0.05$ was considered significant.

RESULTS

Phytochemical analyses indicated the presence of phytochemicals in PO except cardiac glycosides. PKO was devoid of saponin and β-carotene. Both oils contained tannins, flavoniods and cyanogenic glycosides (Table 1).

Table 1: Phytochemical contents of PO and PKO

Parameters	PO	PKO
Tannins	+	+
Flavoniods	+	+
Cardiac glycosides	-	+
Saponins	+	-
Cyanogenic glycosides	+	+
β-Carotene	+	-

+: Present, -: Absent

Table 2: pH values of PO and PKO

Samples	pH
PO	5.54±0.01
PKO	5.85±0.01

The values are means (X) ±SD of three (n = 3) replicates

Table 3: Physiochemical properties of PO and PKO

Parameters	PO	PKO
Acid value (mgKOH/g fat)	108.00±0.04^a	76.80±0.02^b
Saponification number (mgKOH/g fat)	188.27±0.03^a	204.53±0.04^b
Iodine value (g/100 g fat)	81.22±0.03^a	40.32±0.02^b

The values are means (X) ±S.D of three (n = 3) replicates. Means in the rows with the same letter are not significantly different at p>0.05

Table 4: Proximate composition of oils, mash and treated mash

Parameters	PO (100 g)	10 mL PO‡ (8.9 g)	PKO (100 g)	10 mL PKO‡ (9.52 g)	Mash (%)*	POM (108.9 g)	PKOM (109.52 g)
Moisture	000.89±0.05^a	00.08	000.44±0.03^b	00.04	002.31	002.39	002.35
Ash	000.06±0.03^a	00.01	000.14±0.04^b	00.01	006.08	006.09	006.09
Protein	002.71±0.05^a	00.24	000.91±1.27^b	00.09	013.32	013.56	013.41
Lipid	094.98±0.07^a	08.45	096.83±0.13^b	09.22	006.58	015.03	015.80
Carbohydrate	001.38±0.02^a	00.12	001.69±0.15^b	00.16	071.71	071.83	071.87
Energy value (Kcal)	871.18±000	77.49	881.87±000	83.98	399.34	476.83	483.32

Means with standard deviations are for values of triplicate determinations. ‡: Based on density of PO: 0.89 g cm^{-3}; PKO: 0.952 g cm^{-3}. *: Manufacturer's value, POM: Palm Oil Treated Mash; PKOM: Palm Kernel Oil Treated mash, Means in the rows with the same superscript letter are not significantly different at p>0.05

The pH values presented in Table 2 showed that PO and PKO exhibited weak acidic property. Although, PO was more acidic than PKO, there was no significant difference (p>0.05) between the levels of acidity. Table 3 showed that the acid and iodine values of PO were significantly (p<0.05) higher than PKO, whereas the saponification value of PKO was significantly (p<0.05) higher than PO.

Table 4 showed that the moisture contents of PO, 10 mL PO, PKO, 10 mL PKO, POM and PKOM were 0.89, 0.08, 0.44, 0.04, 2.31, 2.39 and 2.35%, respectively. Their ash contents were 0.06, 0.01, 0.14, 0.01, 6.08, 6.09 and 6.09%, respectively. Their protein contents were 2.71, 0.24, 0.91, 0.09, 13.32, 13.56 and 13.41%, respectively. The lipid contents were 94.98, 8.45, 96.83, 9.22, 6.58, 15.03 and 15.80%, respectively. The carbohydrate contents were 1.38, 0.12, 1.69, 0.16, 71.71, 71.83 and 71.87%, respectively and their energy values were 871.18, 77.49, 881.87, 83.98, 399.34, 476.83 and 483.32 kcal/100 g, respectively. It showed that the lipid and energy contents of the feeds were significantly increased (p<0.05) when the oils were, respectively added to the feed.

Table 5 showed that the daily weight gained by the CG, POG and PKOG rats were 1.56, 1.50 and 1.67 g, respectively. Their daily feed intakes were 10.97, 7.58 and 8.3 g, respectively. Their FCRs were 7.03, 5.05 and 4.81, respectively. While the daily weight gained by rats in PKOG were significantly increased (p<0.05), those in POG were reduced (p<0.05). Their feed intakes and FCR were however significantly (p<0.05) reduced when PO and PKO were, respectively added to the feed.

Table 6 showed that PO and PKO-containing diets did not cause significant (p>0.05) increase in serum TC levels. Compared with CG, the increase in serum TC level represented 2.90 and 3.71%

Table 5: Effect of treated diets daily weight gained, daily feed intakes and Feed Conversion Ratios (FCR)

Diet	Daily weight gained (g)	Daily feed intakes (g)	Feed conversion ratios (FCR's)
CG	1.56±0.00^{b}	10.97±1.77^{a}	7.03
POG	1.50±0.02^{a}	7.58±0.22^{c}	5.05
PKOG	1.67±0.03^{c}	8.03±0.28^{b}	4.81

The values are means (X)±SD of eight (n = 8) replicates. Means in the column with the same letter are not significantly different at p>0.05

Table 6: Serum lipid profile

	Group values		
	CG	POG	PKOG
Parameter	(mg dL^{-1})		
TC	100.00±9.06^{a}	102.90±2.63^{a}	103.71±5.23^{a}
TG	53.84±11.54^{a}	76.79±11.58^{b}	56.74±12.68^{a}
VLDL-C	10.77±1.78^{a}	15.36±1.92^{b}	11.35±3.50^{a}
HDL-C	60.37±0.72^{a}	57.75±0.01^{b}	49.15±0.01^{c}
Ratio; TC: HDL-C	1.66	1.78	2.11
LDL-C	63.88±2.78^{a}	63.90±2.78^{a}	77.70±0.01^{b}
Ratio; LDL-C: HDL-C	1.06	1.11	1.58

The values are means (X) ±SD of eight (n = 8) replicates. Means in the rows with the same letter are not significantly different at p>0.05

in POG and PKOG, respectively. Although, serum levels of TG were raised in POG and PKOG, PKO-containing diet did not elicit significant (p>0.05) increase in serum TG concentration. PKOG showed marginal increase in serum VLDL -C level, whereas the rats fed with PO-containing diet presented significant (p<0.05) raised serum VLDL -C level. The CG exhibited comparative high serum HDL-C level. However, the two test groups showed progressive decreasing HDL-C levels in the order: PKOG<POG. Serum LDL-C level in CG was not significantly different (p>0.05) from POG. Raised level of LDL-C in PKOG was equivalent to 21.63% of CG serum LDL-C level. The ratios of TC: HDL-C and LDL-C: HDL-C in the three groups of rats were in the increasing order of CG>POG>PKOG. Furthermore, TC: HDL-C>LDL-C: HDL-C.

DISCUSSION

Phytochemical analysis showed that PO contained more types of antioxidants than the PKO (Table 1), though they were of comparable acidity (Table 2). Owu *et al.* (1998) also reported that oils from plants were acidic. Analysis also showed that the PO used was more rancid, contained longer chain mono- and polyunsaturated fatty acids (Table 3). Wardlaw and Kessel (2002) also reported that PO contained more unsaturated fatty acids than PKO.

When the respective oils were added to the feeds (Table 4), they caused more than two fold increase in the lipid contents; leading to between 19.40 to 20.03% increases in the energy contents (Table 4). This showed that the oils were concentrated sources of energy. This corroborated the findings of Edem and Akpanabiatu (2006). However, PKO seemed to have increased the energy content of the feed contrary to the report of Wardlaw and Kessel (2002). Incorporation of the oils into the feeds reduced significantly (p<0.05) the daily feed intake of the test rats (Table 5). This supported the findings of Church and Pond (1988) who reported that when the energy contents of diets were increased, feed consumption decreased. Table 5 also showed that while PKO caused more (p<0.05) daily body weight gained, PO inexplicably caused a lower (p<0.05) body weight gain than even the control rats. The reduction in daily feed intakes caused a reduction in the FCR

values. This showed that the oils improved the rats' efficiency in feed utilization, for instance, their ability to convert feed mass to body mass. This implied that more of the total feed consumed was used for gain and less for maintenance.

Incorporation of PO significantly ($p<0.05$) increased the serum TG and VLDL-C of the rats (Table 6). However, both PO and PKO reduced ($p<0.05$) the serum HDL-C concentrations in the rats; with PKO effecting the greater ($p<0.05$) reduction. PKO also increased the serum TC: HDL-C ratio, LDL-C and LDL-C: HDL-C ratio, indicating that it promoted the formation of more cholesterol than phospholipids. HDL-C contains more phospholipids than cholesterol while LDL-C contains more cholesterol than phospholipids (Nelson and Cox, 2000; Glew, 2006). These results may indicate that PKO could promote the formation of gallstones; since it engendered the formation of more cholesterol and less phospholipids. Glew (2006) also reported that phospholipids; especially phosphatidylcholine, had detergent properties in bile and aided in solubilizing cholesterol.

The weight gained by the test rats (Table 5) may have been due to the deposition of more fat than lean muscle mass as the oils caused between 37.69 to 45.55 percent increases in the serum lipid levels of the test rats (Table 6). The seeming non-atherogenic nature of PO may have been due to its greater array of antioxidants (Table 1) and its greater contents of long chained mono-and polyunsaturated fatty acids (Table 3). Saponins and β-carotene are antioxidants and unsaturated fatty acids are fluid at room temperatures. Pantazari and Ahmad (2004) reported that endogenous cholesterol levels were reduced by PO due to its known tocotrienol contents and the peculiar somatic position of its fatty acids.

In conclusion, present study showed that PKO was more atherogenic because it contained more short chained saturated fatty acids and fewer types of antioxidant phytochemicals.

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