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### Comparative Analysis of Tissues Phospholipids Contents, Organ and Total Body Weights of Rats Placed on Different Edible Vegetable Oils.

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#### ABSTRACT

Red palm oil, coconut oil, groundnut oil, sesame oil (Beniseed oil), soybean oil, palm kernel oil are some edible vegetable oils derived from seeds or fruits of different plants. These oils supply lipids in the diets, and useful for their distinct aromas, colours, palatability and availability. Vegetable oils are rich in essential nutrients such as vitamins, anti-oxidant compounds etc. The type of diet and in particular the nature of dietary fats may raise or lower the phospholipids in man. This study aimed to investigate the impact of the various edible vegetable oils on tissues phospholipids contents, organ and total body weights changes in rats. The phospholipids content was estimated using standard procedure. Results indicate that while body weight gain was observed in RPO>SSO, body weight loss was seen in PKO>CCO>SBO. The liver and kidney weights were also significant ( $p<0.05$ ) higher in RPO compared with other groups. The liver weight was significantly ( $p<0.05$ ) lower in PKO, while CCO oil had the least kidney weight compared other groups. The brain weights were lowest in RPO and CCO. The liver and kidney phospholipids were significantly ( $p<0.05$ ) higher in all vegetable oils fed rats compared with control. Small intestine phospholipid content of CCO>PKO>SSO were significantly ( $p<0.05$ ) higher compared with control, SBO had the least small intestine phospholipids content. The brain phospholipids were significantly ( $p<0.05$ ) higher in RPO>SBO>SSO compared with other groups. In conclusion, RPO increases total body weight, liver, kidney weights and phospholipids contents. PKO and CCO reduce total body weight.

**Key words:** Edible vegetable oils, organ, body weight and tissues phospholipids content.

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## INTRODUCTION

Vegetable oil is derived from seeds or fruits of plants which grow in different parts of the world. Several hundreds of varieties of plants are available and the dietary consumption of seeds and fruits has been on the increase in the past years, due to the growing importance of vegetable oil in human nutrition, there has also been an increase in the production and consumption of edible oil, although some of these vegetable oils are inedible, (example, linseed oil, fungi oil, castor oil) [1]. They are used for paints, as lubricants, pharmaceutical components and other industrial purposes. Edible oils include red palm oil, coconut oil, groundnut oil, sesame oil (Beniseed oil), soybean oil, palm kernel oil, etc [2].

These oils are consumed not only for their supply of lipids in the diets but for their distinct aromas, colours, palatability and availability. These oils are rich in essential nutrients such as vitamins and anti-oxidant compounds [3,4]. Dietary oils serve as the major source of lipid in the diet, the type and amount of dietary lipid has been strongly linked to the incidence of several degenerative diseases [5,6]. Polyunsaturated fats have been recommended to reduce coronary heart disease [7]. Epidemiological investigations have also shown that the frequency of coronary heart disease and blood cholesterol level are related to eating habits [8]. The type of diet and particularly the nature of dietary fat have been found to raise or lower the phospholipids in man.

The role-played by vegetable oils and the various fractions in atherosclerosis is not clear. Hierholzer [9], also has reported that palm oil was atherogenic in rabbits. Furthermore, the important physiological role played by the minor components present in palm oil is becoming apparent [10]. It is therefore necessary to continually evaluate the type of lipids that are eaten in our diets. This study was necessary as it would assess and ascertain the impact of the various vegetable oils available in the Nigerian markets on organ / body weights and tissues phospholipids contents.

## MATERIALS AND METHODS

### Chemicals and reagents

All the chemicals used in this study were of analytical grade. The chemical were obtained from the British Drug House (BDH) Chemical Limited Pole, England and Sigma Chemical Company, St. Louis, MD USA.

### Collection of experimental samples

Vital feed (palletized grower feed) were purchased from vital feed depot in Calabar, Cross River State Nigeria. Red palm oil, palm kernel oil and soybean oil were purchased from a local market (Watt Market) in Calabar South Local Government Area of Cross River State, Nigeria, while coconut oil and beniseed oil (sesame oil) were extracted from their fruits.

### Experimental animals / protocol

A total of 42 (forty two) albino rats of Wistar strain weighting between 200-240g were used for these studies. The animals were housed in the animal house of the Department of Biochemistry University of Calabar Faculty of Basic Medical Science. Six group of rats were randomly selected, n = 7, placed on a specific test diet for 90 days.

Group 1 (control) received normal palletized grower feed (reference diet). Group 2 took reference diet + 10% red palm oil. Group 3 had reference diet + 10% palm kernel oil. Group 4 received reference diet + 10% coconut oil. Group 5 was fed reference diet + 10% soybean oil, and Group 6 was fed reference diet + 10 percent sesame oil.

### Growth rate

The growth rate was calculated as the ratio of weight gain (experimental animals) to the number of days constituting the experimental period. It is presented as growth rate.

$$= \frac{\text{Final body weight} - \text{initial body weight}}{\text{Number of days}}$$

### Percentage weight increase

Total body weight change was calculated as difference between the final and the initial body weights, while the percentage body weight increase was calculated by dividing the weight change by the initial body weight and then multiplied by 100.

$$= \frac{\text{final body weight(g)} - \text{initial body weight(g)} \times 100}{\text{Initial body weight(g)}}$$

### Organ weights of experimental animals

The weight of the liver, kidney small intestine and were isolated from each animal and then weight with an electronic weighing scale and presented as percentage organ body weight as follows:

$$= \frac{\text{Organ weight} \times 100}{\text{Final body weight}}$$

### Estimation of total phospholipid of the organ:

The total phospholipid concentration of the different organs was estimated using a modified method of Goldenbery and Fernandez (1966).

## Procedure

1ml of lipid extract was pipetted into test tube and 1ml of conc.  $H_2SO_4$  was added to it, the test tube and the contents were heated at about  $60^\circ C$  for one hour using a heating block. The heat was increased strongly for another 1hour and then allowed to cool to about  $60^\circ C$ . one drop of 20percent  $H_2O_2$  was added and heated strongly until the mixture became colourless. The digest was made up to 5ml with distilled water. 2.5ml of ferrous sulphate and 1ml of TCA reagent was added to 1ml of ammonium molybdate was then added to the sample. Blank (distilled water), standard ( $KH_2PO_4$ ) respectively. These were allowed to stand for 20minute for proper colour development. Absorbance was then read at 660nm against reagent blank.

## Statistical Analysis

Results were expressed as mean values  $\pm$  SEM ( $n = 7$ ). Means of seven samples were compared by analysis of variance (ANOVA). Significant differences between means were determined by least significant difference (LSD post hoc test;  $p \leq 0.05$ ). The software used was SPSS version 17.0)

## RESULTS

### Total body and organ weights changes of the different experimental group

Table 1 and figure 1 show the initial and final body weights, percentage body weight changes, growth rates and organ (liver, kidney, brain and small intestine) weights of the different experimental groups.

The initial body weights of the different experimental animals ranged between  $148.00 \pm 4.20g$  and  $223.30 \pm 2.00g$ . Rat placed on RPO, SSO and SBO had body weight increases of  $22.70 \pm 1.50$ ,  $15.35 \pm 1.60$  and  $3.60 \pm 1.30$  percent respectively, while PKO and CCO exhibited decreased body weight of  $9.09 \pm 0.20$  and  $6.88 \pm 0.10$  percent ( $p < 0.05$ ) respectively.

However, RPO recorded the highest growth rate per week, followed by SSO and SBO, their growth rate were RPO,  $0.37 \pm 0.15$ ; SSO,  $0.32 \pm 0.50$  and SBO,  $0.08 \pm 1.2$  percent respectively. On the other hand, the experimental animals fed with PKO and CCO diet recorded a negative growth rate per week of  $-0.22 \pm 0.1$  and  $-0.13 \pm 0.12$  percent respectively.

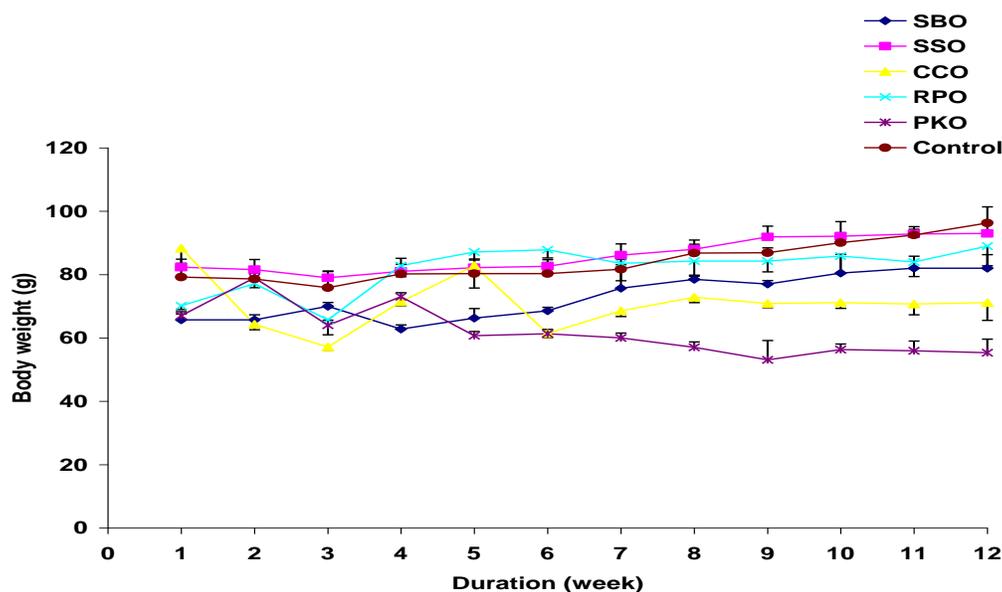
The mean liver weights was highest in RPO, followed by SBO, SSO CCO and then PKO with values of  $7.50 \pm 0.15g$ ,  $6.90 \pm 0.12g$ ,  $6.80 \pm 0.11$ ,  $6.70 \pm 0.12$  and  $5.90 \pm 0.10$  respectively. The kidney weight was significantly ( $p < 0.05$ ) higher in RPO ( $2.01 \pm 0.40g$ ) compared with control ( $1.96 \pm 1.50g$ ), while CCO and SBO had significantly lower kidney weights compared with control and RPO. The mean weight of the brain was lower in all the test rats compared with control. The weight of the small intestine did not differ significantly following feeding with the different experimental samples.

**Table 1: Body weight changes and organ weights of the different experimental groups**

Parameters	Control	RPO	PKO	CCO	SBO	SSO
Initial BW (g)	185.76 ±2.10	148.00 ±4.20	223.30 ±2.00	181.50 ±2.60	193.30 ±4.20	190.80 ±2.00
Final BW (g)	215.10 ±1.80	181.60 ±1.30	203.00 ±3.50	169.00 ±3.60	200.36 ±3.50	200.40 ±3.60
Change (g)	29.40 ±2.10	33.60 ±2.10	-20.30 ±0.20	-12.50 ±1.30	7.06 ±1.60	29.30 ±2.30
% Change	15.83 ±0.10	22.70 ±1.50	-9.09 ±0.20	-6.88 ±0.10	3.6 ±1.30	15.35 ±1.60
Growth rate (g/week)	0.32 ±0.11	0.37 ±0.15	-0.22 ±0.10	-0.13 ±0.12	0.08 ±0.12	0.32 ±0.05
Liver wt. (g)	6.80 ±0.11	7.50 ±0.15	5.90 ±0.10	6.70 ±0.12	6.90 ±0.12	6.80 ±0.05
Kidney wt. (g)	1.96 ±1.50	2.01 ±0.40	1.85 ±1.40	1.60 ±2.40	1.70 ±1.80	1.80 ±1.30
Brain wt. (g)	11.20 ±2.00	9.80 ±0.10	10.90 ±0.60	9.20 ±1.60	10.40 ±1.10	10.80 ±3.10
Small Intestine (g)	19.70 ±1.90	18.90 ±0.30	19.50 ±2.30	17.20 ±1.88	17.60 ±1.60	17.90 ±1.50

Values are expressed as mean ± SEM.

RPO = red palm oil, PKO = palm kernel oil, CCO = coconut oil, SBO = soybean oil, SSO = sesame oil, wt. = weight.



**Figure 1: Mean weekly body weight change in the different experimental groups. Values are expressed as mean ± SEM, n = 7.**

**Liver, intestine, kidney and brain phospholipid content (mg/g wt.)**

Table 2 shows the levels of total phospholipids in the liver, kidney and brain tissues of experimental animals.

The liver whole homogenate phospholipid contents (mg/g wt.) of the different experimental groups were control, 29.20 ±0.30; RPO, 30.80 ±0.20; PKO, 32.50 ±3.20; CCO, 32.80 ±1.50; SBO, 30.50 ±1.30 and SSO, 30.20±0.05. It was significantly (p<0.05) higher in all the test groups compared with the control.

The kidney phospholipid contents of all the test groups were also significantly (p<0.05) higher compared with the control. The control value was 25.10±0.60, while for the test groups: RPO, 27.50 ±0.40; PKO, 28.60 ±0.71; CCO, 26.90 ±1.20; SBO, 27.60 ±3.20 and SSO, 26.20 ±1.50mg/g.

The phospholipid content of small intestine were 36.90 ±0.90, 36.2 ±0.80, 37.50 ±0.20, 38.10 ±3.20, 33.60 ±0.30 and 34.60 ±0.40 for control, RPO, PKO, CCO, SBO and SSO respectively. It was significantly (p<0.05) higher in PKO and CCO, lower in SBO and SSO compared with control.

The brain phospholipid content of the experimental groups were 15.50 ±1.20, 18.60 ±1.20, 15.10 ±1.20, 15.90 ±0.49, 17.20 ±0.20 and 16.30 ±0.17, control, RPO, PKO, CCO, SBO and SSO respectively. It was significantly higher in RPO, SBO and SSO compared with control, PKO and CCO.

**Table 2: The result of tissues total phospholipids contents of the different experimental groups (mg/g wt)**

Parameters	Control	RPO	PKO	CCO	SBO	SSO
Liver	29.20 ±0.30	30.80 ±0.20*	32.50 ±0.20*	32.80 ±0.50*	30.50 ±0.30*	30.20 ±0.50*
Kidney	25.10 ±0.60	27.50 ±0.40*	28.60 ±0.71*	26.90 ±0.20*	27.60 ±0.20*	26.20 ±0.50
Small Intestine	36.90 ±0.90	36.21 ±0.80	37.50 ±0.20*	38.10 ±1.20*	33.60 ±0.30*	34.60 ±0.40*
Brain	15.50 ±1.20	18.60 ±0.20*	15.10 ±1.20	15.90 ±0.49	17.20 ±0.20*	16.30 ±0.17*

\*P<0.05 vs control, values are expressed as mean ± SEM.  
 RPO = red palm oil, PKO = palm kernel oil, CCO = coconut oil,  
 SBO = soybean oil, SSO = sesame oil.

**DISCUSSION**

The word vegetable oil is quite common and familiar to most people because oil is widely identified as an important ingredient for food preparation. But fat is not a common

terminology, although most oils are referred to as fat especially those of plant origin like red palm oil, soya bean oil, palm kernel oil, beniseed or sesame oil, as well as coconut oil are liquid at room temperature while the animal fats which are commonly referred to as fats are solid at room temperature, Fats and oil are collectively called lipids which are defined as water-insoluble organic bio-molecules found in plants and animal. There are simple and complex lipids. The simple lipids do not contain fatty acid and so are non-saponifiable. They are hydrocarbons which the body cannot absorb or metabolize and so are not edible. The complex lipids, which are fat and oil just as protein and carbohydrates are broken down by the body into simpler units (fatty acid) before they are utilized [7].

Phospholipids are major components of plasma membrane and organelle membranes that maintain the integrity of the cell or organelles by creating a semi-impermeable barrier from their outside environment. In normal cells, phospholipids are asymmetrically distributed in inner and outer leaflets of plasma membrane, with phosphatidylcholine (PC) and sphingomyelin (SM) predominantly in the outside leaflet and phosphatidylserine (PS) and phosphatidylethanolamine (PE) in the inner leaflet of plasma membrane. Phospholipid asymmetry is also seen with membrane organelles. More and more studies have indicated that phospholipid asymmetry may play critical roles in many important biological and cellular processes [11].

For example, phospholipid asymmetry helps target proteins to appropriate subcellular sites or organelles for specific cellular processes (e.g. organelle fusion/division or apoptosis), maintain biophysical properties of specific membranes, sustain cell shape, facilitate membrane vesicle trafficking/fusion/budding, regulate activities of membrane proteins, and transduce intracellular signals [12,13].

On the other hand, alteration of phospholipid asymmetry (for example, the externalization of PS by a cell) can also play important roles in activating cellular or biological processes such as blood coagulation, recognition and removal of apoptotic cells, cytokinesis, and cell fusion [14].

Our study shows that RPO increases total body weight, liver and kidney weight more than other vegetable oils we investigated. The increase in body weight observed in rat placed on RPO could be that their meal was more delicious compared with other diets, hence the animals consumed more of the RPO diet. Fat contributes to food palatability and also enhances satiety [15,16] since fatty foods remain in the stomach for longer periods of time than do foods containing protein and carbohydrate. The 10% RPO diets were well accepted by the rats. When moderate amounts of fat are added to the diet, caloric consumption is more frequently increased than depressed [17].

The inclusion of high oils diets had been reported to have a negative effect on rat growth probably because of the decreased food (caloric) intake [3]. Food intake is seemingly depressed when high amounts of fat are added to the diet. Another possible reason for the disparity in BWG between the groups fed the different vegetable oil diets may be the protein

imbalance imposed through dilution of nutrients especially protein with increasing amounts of fats.

On the other hand, red palm oil had been shown to have low levels of these saturated fatty acid hence would cause less problems to the body. Red palm oil for instance has been reported as a potent anti-cancer, anti-atherogenic and blood pressure stabilizing agent [18].

PKO, CCO and SBO were the only oils that cause reductions in body weights compared with other vegetable oils. SSO did not have any profound effect on the total body weight of the rats. All the oils were observed to elevate the liver and kidney phospholipids contents. Although, all the experimental groups recorded organ phospholipids contents which were higher than controls, the levels were within ranges that were normal and comparable. This result suggests that the oils did not adversely influence the distribution of phospholipids in the liver, kidney, small intestine and the brain of animals and may not adversely affect physiological functions in these organs.

The dramatic increase in soybean oil sales is largely credited to the Food and Drug Administration's (FAD-2001) approval of soybean oil as an official cholesterol lowering food; along with other heart and health benefits. A 2006 literature review argued that these health benefits were poorly supported by the available evidence, and noted that disturbing data on soy's effect on the cognitive function of the elderly exists [19].

In conclusion, RPO increases total body and organ weights, while PKO and CCO reduce body weights. Liver and kidney phospholipids were increased by the different vegetable oils. PKO and CCO may be recommended for obese patients.

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