THE RELATIONSHIPS BETWEEN FEEDING GRADED LEVELS OF DIETARY PALM OIL AND LIPID DEPOSITIONS IN TISSUES, BODY AND ORGAN WEIGHTS OF RABBITS

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INTRODUCTION

Although palm oil is a major source of dietary fat in the tropical and sub-tropical countries, it has high content of saturated fatty acids, principally palmitic acid (Khosla and Hayes, 1994; Wai, 1994). Studies have shown that lauric, myristic and palmitic acids are the three principal cholesterol-raising saturated fatty acids (Cottrel, 1991; Khosla and Hayes, 1994; Wai 1994). Previously, dietary palm oil has been found to be atherogenic in the rooster (OriuwarI et al., 1993), in the rabbit (OriuwarI et al., 1998a), as indicated by the linear increase in plasma cholesterol and also in the linear increase in plasma very low-density lipoproteins (VLDL) + low-density lipoproteins (LDL) cholesterol in the test animals (OriuwarI et al., 1998b). The liver is the major organ responsible for the production (Kane, 1983) and degradation (Carew et al., 1982; Pittman et al., 1982) of apolipoprotein (apo) B 100-containing lipoproteins. The type of dietary fatty acid consumed influences the hepatic levels of cholesterol esters, which affect plasma levels of LDL (Woollett et al., 1989). Since individuals with elevated low-density lipoproteins (LDL) and cholesterol have a higher than normal incidence of atherosclerosis, whereas individuals with elevated high-density lipoproteins (HDL) have a lower incidence, the HDL: LDL ratio was suggested to be a better index of the risk of the disease than cholesterol or LDL alone (Eisenberg, 1984). In this study, the rabbit was selected as the animal model because its liver is the primary site for lipogenesis, as in the human (Davis and Hui, 2001). Lipid measurements comprising liver cholesterol, triacylglycerol, low-density lipoproteins (LDL), high-density lipoproteins (HDL); and total lipids of the liver, heart and spleen from male and female rabbits were made, in rabbits fed graded levels (6.4, 8, 12%) of oil palm in diets. Body and organ (liver, heart and spleen) weights were also taken. Results showed that liver cholesterol and triacylglycerol increased linearly ($P < 0.05$) with incremental levels of palm oil. The lipoproteins, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were found linear to the dietary treatments, with the LDL increasing while the HDL decreased as the palm oil levels increased. Liver total lipid decreased linearly and the total heart lipid increased linearly, whereas the total spleen lipid was quadratic in both sexes. Upon application of the concept of HDL: LDL ratio, and the consideration of the results of the lipid measurements of the liver, it was found that the 12% palm oil treatment tended to be atherogenic, and that the tendency was higher in the females (60%) than the males (40%).

KEY WORDS: Cholesterol, Triacylglycerol, LDL, HDL, Atherogenesis

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MATERIALS AND METHODS

Four isonitrogenous diets different in digestible energy (DE 3144, 3394, 3658 and 3879) due to the addition of graded levels (0, 4, 8 and 12%) of dietary palm oil (crude red palm oil) formed the dietary treatments. Treatment A was the control basal rabbit diet formulated without the addition of palm oil. On weight basis, corn in the basal diet was replaced by palm oil to form dietary treatments B, C, and D representing 4, 8 and 12% palm oil, respectively (Table 1). The choice of palm oil as the fat source was because of its common usage in Nigerian households, and because it is a saturated fat. Its fatty acid analysis according to the method of Cottrel (1991) was: Lauric 0.8; myristic 1.2; palmitic 46.8; stearic 5.2; oleic 37.3; linoleic 9.1%.

| TABLE 1: Composition of Experimental Diets |
|-----------------|-------|-------|-------|-------|
| Ingredients     | 0%    | 4%    | 8%    | 12%   |
| Palm Oil        | 0     | 4.00  | 8.00  | 12.00 |
| Corn            | 15.00 | 11.00 | 7.00  | 3.0   |
| Palm Kernel cake| 30.00 | 35.00 | 42.00 | 44.00 |
| Soyabean meal   | 5.00  | 6.25  | 7.00  | 8.50  |
| Wheat bran      | 48.25 | 42.00 | 34.25 | 30.75 |
| Bone meal       | 1.00  | 1.00  | 1.00  | 1.00  |
| Salt            | 0.50  | 0.50  | 0.50  | 0.50  |
| 1Vitamin/mineral premix  | 0.25  | 0.25  | 0.25  | 0.25  |

Vitamin/mineral premix provided per kg diet, Vitamin A palmitate, 2000IU; Vitamin D3, 300IU; DL - α - tocopherol acetate, 20UI; menadione sodium bisulphite, 1mg; riboflavin, 3.6mg; pantothenic acid, 10mg; niacin, 27mg; vitamin B12, 0.009mg; cholin, 900mg; folic acid, 0.55mg; thiamin, 1.8mg; biotin, 0.4mg; ethoxyquin (66%), 200mg; 400mg MnSO4·H2O; 400mg CuSO4·5H2O; 31.15mg K103; 0.30mg NaSeO3.

Thirty two adult rabbits (24 weeks old) comprising 16 males and 16 females of the Chinchilla breed were used to test the four dietary treatments to which four males and four females were randomly allocated in a randomized block design (RBD). Sex was hereby considered as the nuisance variable (Block), and the model being:

\[ Y_{ij} = U + T_i + B_j + E(ij); \]
\[ Y_{ij} = \text{The random sampling variable} \]
\[ U = \text{True mean (constant)} \]
\[ T_i = \text{Fixed effect on the } i^{\text{th}} \text{treatment} \]
\[ i = 1, 2, 3, 4 \text{levels of palm oil} \]
\[ B_j = \text{Fixed effect of the } j^{\text{th}} \text{block} \]
\[ j = 1, 2 \text{types of sexes} \]
\[ E(ij) = \text{Random experimental error} \]

The rabbits were preconditioned for two weeks on the basal diet. They were individually caged in standard rabbit hutch, each rabbit representing a replicate, forming eight rabbits per treatment. Each rabbit was fed 150g of the test diet daily. They were weighed individually at the start of the experiment and at seven days intervals. Feed offered and residues were weighed daily. At the termination of the experiment, the animals were euthanized and the weights of the liver, heart and spleen from each animal was obtained using an electronic balance. The liver, heart and spleen excised from each sampled rabbit were preserved in a 10% formal saline (Baker et al., 1968) until analyses.

Liver, heart and spleen lipids were extracted using the method of Folch et al. (1957). The
extracted organ lipid was evaporated to dryness, weighed using an electronic balance and 2 ml of heptane added for the analyses of cholesterol and triacylglycerol by the method of Briggs et al. (1975), and for low-density lipoproteins (LDL) and high-density lipoproteins (HDL) fractions (Whitehead and Griffin, 1992), all measured in duplicate (mmol/l). Differences between treatment means were identified by orthogonal polynomial contrast after subjecting the data to analysis of variance (ANOVA) by a randomized block design (Gill, 1978).

RESULTS AND DISCUSSION

Mean body and organ weights of the rabbits are presented in Table 1. In both males and females significant treatment differences (P < 0.05) were observed in body weight, liver, heart and spleen weights, but it was only in body and spleen weights that significant sex differences were observed. Body weight was quadratic in both males and females, whereas liver weight was linearly depressed in both sexes. Heart weight was quadratic in both sexes. Spleen weights, on the other hand, were quadratic to treatments in both sexes. Mean lipid measurements in the rabbits are presented in Table II. Total heart lipid was linear whereas total spleen lipid was quadratic (P < 0.05) with incremental levels of palm oil. Total liver lipid decreased linearly in both male and female rabbits. Liver cholesterol and triacylglycerol (TG) were found linear (P < 0.05) to the incremental levels of palm oil in both sexes, whereas in the case of HDL and LDL (mmol/l), although they were all found linear in the male and female, HDL decreased while LDL increased with incremental levels of palm oil. Sex differences (P < 0.05) were found in total heart lipid, liver cholesterol, TG; HDL and LDL but not in total liver and spleen lipids (Table III). The HDL: LDL ratios were higher in the males than in the females on the dietary treatments.

### TABLE I: Body and organ weights of rabbits fed varying levels of dietary palm oil (Mean ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Level of palm oil (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Male 0</td>
<td>Male 4</td>
<td>Male 8</td>
<td>Male 12</td>
<td>Female 0</td>
<td>Female 4</td>
<td>Female 8</td>
<td>Female 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td>2.05° 0.04</td>
<td>2.27° 0.17</td>
<td>2.22° 0.17</td>
<td>2.05° 0.13</td>
<td>1.87° 0.17</td>
<td>2.19° 0.10</td>
<td>2.22° 0.04</td>
<td>2.15° 0.07</td>
<td></td>
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<tr>
<td>Liver weight (g/100g body)</td>
<td></td>
<td>2.36° 0.19</td>
<td>1.97° 0.04</td>
<td>1.74° 0.04</td>
<td>1.63° 0.07</td>
<td>2.32° 0.08</td>
<td>1.87° 0.07</td>
<td>1.78° 0.06</td>
<td>1.66° 0.04</td>
<td></td>
<td></td>
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<tr>
<td>Heart weight (mg/100mg body weight)</td>
<td></td>
<td>165.85° 8.1</td>
<td>167.78° 3.1</td>
<td>171.25° 6.5</td>
<td>180.81° 3.3</td>
<td>202° 7.4</td>
<td>142.22° 5.7</td>
<td>157.22° 5.7</td>
<td>167.5° 1.3</td>
<td></td>
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<tr>
<td>Spleen weight (mg/100mg body weight)</td>
<td></td>
<td>24.39° 0.41</td>
<td>27.60° 0.37</td>
<td>49.76° 0.35</td>
<td>48.76° 0.39</td>
<td>48.13° 0.39</td>
<td>42.81° 0.93</td>
<td>37.98° 0.66</td>
<td>28.5° 0.67</td>
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</tbody>
</table>

*Means ± SEM of 4 replicates of 8 rabbits each
a, b, c and d significant treatment difference in males (P < 0.05) within rows
A, B, C and D significant treatment difference in females (P < 0.05) within rows.

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TABLE II: Lipid measurements in the liver, heart and spleen of rabbits fed varying levels of dietary palm oil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level of palm oil (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total liver lipid (g/100 g liver)</td>
<td>1.64±1.8</td>
<td>1.04±0.3</td>
</tr>
<tr>
<td>Liver cholesterol (mmol/L)</td>
<td>1.70±0.8</td>
<td>2.95±1.4</td>
</tr>
<tr>
<td>Liver triacylglycerol (mmol/L)</td>
<td>1.97±0.07</td>
<td>1.12±0.08</td>
</tr>
<tr>
<td>Liver HDL (mmol/L)</td>
<td>1.75±1.0</td>
<td>1.6±0.9</td>
</tr>
<tr>
<td>Liver LDL (mmol/L)</td>
<td>1.75±0.11</td>
<td>1.84±0.62</td>
</tr>
<tr>
<td>Total heart lipid (g/100g of heart)</td>
<td>0.51±0.01</td>
<td>1.74±0.06</td>
</tr>
<tr>
<td>Total Spleen lipid (g/100g of spleen)</td>
<td>1.16±0.02</td>
<td>2.07±0.07</td>
</tr>
</tbody>
</table>

Notes: ±SEM of 4 replicates of 8 rabbits each.
A, B, C, and D significant treatment difference in males (P < 0.05) within rows.
A, B, C, and D significant treatment difference in females (P < 0.05) within rows.

The observed quadratic final body weight in both the male and female rabbits clearly indicated that the best digestible energy to crude protein ratio (E: CP) was met at the 8% palm oil treatment. Above this level of palm oil, body weight decreased in all sexes. In this study, diets were isonitrogenous but had increasing DE levels due to the incremental palm oil levels. This result agrees with the finding that for optimal growth to be achieved, increase in dietary energy requires corresponding increase in crude protein (Payne and Lewis, 1963). The sex differences observed in body weight could be explained by the finding that female individuals have greater feed efficiency than males (Jackson et al., 1992).

The results of the liver weight showed that as the dietary palm oil increased, liver weight in the both sexes decreased linearly (P < 0.05). Thus, this study supports the phenomenon that feeding high dietary fat reduces lipogenesis as well as decreases liver weight (Liu and Donaldson, 1973). Contrary to the liver results, the heart weights of both sexes were quadratic, corroborating the finding of Berne (1964).

The quadratic pattern observed in this study also agrees with previous findings (Abati and McGrath, 1993; Phetteplace and Watkins, 1989; 1990), with the decrease occurring after the 8% palm oil treatment (Table 2). The weights of spleen were measured because of their tendency to decrease arterial inflow of blood, increase venous outflow, and decrease of splenic weight upon stimulation of its nerve fibres (Green et al., 1960). In this study, the observed linearly increased spleen weight of the males and the linearly reduced weights in the females appeared to show that palm oil activated the splenic nerve of the male and female rabbits differently. However, the observed quadratic total lipid of the spleen tissues suggested that empying of the spleen occurred at the 12% palm oil treatment. On the other hand, a linearly increased total heart lipid was observed in this study, without visible heart parlor of fat (Owuari et al., 1993), indicating that the heart tissues of the rabbit efficiently metabolized the tested levels of palm oil.

Most importantly, it has been reported that when high-fat diets were fed, free fatty acids were the main sources of TG fatty acids in the liver and in plasma lipoproteins because lipogenesis from acetyl CoA derived mainly from carbohydrate was depressed (Tijburg et al., 1989). Previously,
it has been confirmed that one factor that enhances both the synthesis of TG and the secretion of VLDL by the liver is feeding high fat which caused high levels of circulating free fatty acids (Davis and Hui, 2002). Accordingly, in this study, emphasis was on the measurements of atherogenic liver lipids because the liver has been found to produce bile which facilitates the digestion and absorption of lipids (Russell and Setchell, 1992). It has active enzyme systems for synthesizing and oxidizing fatty acids (Reddy and Mannaerts, 1994) and for synthesizing TG and phospholipids (Tijburg et al., 1989). It also plays an integral part in the synthesis and metabolism of plasma lipoproteins (Brewer, 1988) and above all it synthesize apoB-100, which acts as ligand for interaction with lipoprotein receptors in tissues (Kane, 1983).

The liver is the major organ responsible for the production and degradation of apoB-containing lipoproteins (Carew et al., 1982; Pittman et al., 1982). The observed increased liver TG and cholesterol in this study confirms the finding of Davis and Hui (2001); thus indicating that their increased levels in both sexes were caused by the incremental levels of dietary palm oil fed to the rabbits (Table III). The levels of TG at all dietary treatments were lower than those of cholesterol because TG does not normally accumulate in the liver, but must be transported from the liver in VLDL as rapidly as it is synthesized and that the synthesis of apo B-100 is not rate-limiting (Reddy and Mannaerts, 1994). However, the synthesis of TG provides the immediate stimulus for the formation and secretion of VLDL and subsequently LDL while hepatic triacylglycerols are the immediate precursors of triacylglycerols contained in plasma VLDL (Brewer, 1988). Considering the observed linear increase of TG and LDL in the liver, which forms apo B 100 containing lipoproteins that are responsible for atherogenesis, this study confirms that atherosclerosis is a liver disease of the heart (Davies and Hui, 2001). In this study, liver LDL and HDL were measured because the liver is responsible for the synthesis and uptake of these lipoproteins, as remnant lipoproteins from the circulation when VLDL is hydrolysed to LDL and when HDL has scavenged (Tijburg et al., 1989).

The results demonstrated that in both sexes LDL and HDL were significantly linear to the dietary treatments. However, HDL decreased while LDL increased with each incremental level of palm oil. This relationship between liver HDL and LDL observed in the present study agreed with the phenomenon that there is an inverse relationship between plasma HDL concentration and coronary atherosclerosis, and that the most predisposing relationship is HDL/LDL cholesterol ratio (Khosla and Hayes, 1994; Wai, 1994; Davis and Hui, 2001). This relationship is explainable in terms of the proposed roles of LDL in transporting cholesterol from the liver to the extrahepatic tissues and of HDL acting as the scavenger of cholesterol from tissue to the liver, a process known as reverse cholesterol transport (Brewer, 1988; Davis and Hui 2001). The liver is potentiates because of the presence of LDL receptors, the LDL (apo B-100, E). The HDL performs this function because it acts as a repository for apo C and apo E which are required in the metabolism of chylomicrons and VLDL (Eisenberg, 1984; Brewer, 1988; Russell and Setchell, 1992, Davis and Hui 2001).

The observed sex difference in the liver lipid measurements in this study supports that found in the study of Oruwari et al. (1998), where plasma cholesterol and TGs in female rabbits were significantly higher than those in males. The finding was contrary to that of Polin and Wolford (1977).

Furthermore, applying the concept of HDL: LDL ratio (Eisenberg, 1984) those calculated in this study (3.4:1; 0.9:1; 0.6:1; 0.3:1) for the 0, 4, 8, 12% palm oil treatments in the males, and 1.9:1; 0.5:1; 0.4:1; 0.2:1) for the 0, 4, 8, 12% palm oil treatments in the females, respectively, demonstrated that 12% palm oil treatment tended to be atherogenic. The ratios obtained
also showed that the females (60%) were more predisposed to the incidence of the disease than the males (40%) in this study.

CONCLUSION

The study showed that total liver lipid decreased while total heart lipid increased as the tested levels of dietary palm oil increased in both male and female rabbits. However, that of spleen was quadratic for both sexes. Liver cholesterol and TG increased with the treatment. In the case of liver lipoproteins, HDL decreased and LDL increased with incremental levels of palm oil with the highest amount found in the females fed the 12% palm oil diet.

REFERENCES


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