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Effects of Palm Oil on the Serum Lipoprotein Profile of Rabbits Fed with Diets Supplemented with Egg and Crayfish

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Abstract:
The effect of palm oil on the serum lipoprotein profile of rabbits were investigated using diets supplemented with egg and crayfish. Twenty rabbits were divided into five groups of 4 and fed as follows: Gi (egg with oil), G2 (egg without oil), G3 (crayfish with oil), G4 (crayfish without oil), and G5 (oil without protein). The animals were acclimatized for two weeks on grower's mash, and then fed for four weeks on an experimental diet of corn starch (60 g), dried egg (20 g), crayfish (5 g), vitamin / mineral mix (5 g), saw dust for fibre (5 g) and the test palm oil (10 g). In those groups without oil, its corn starch was increased to 70 g. After the experimental feeding period, the animal were sacrificed, and blood collected and used to assay total cholesterol, triacyl glycerol (TAGS) and high density lipoprotein cholesterol (I-IDL-C) concentrations. It was observed that relative to the control group, rabbits fed with G3 (crayfish oil) and G5 (oil- protein) had reduced mean total cholesterol ($p < 0.05$), TAG ($P < 0.05$) and HDL-C ($P< 0.05$) while the non-HDL-C levels were also reduced for all the groups, though the difference was statistically insignificant.

Keywords: *Palm oil, Serum cholesterol-Triaclyglyceral, High density lipoprotein cholesterol*

1. Introduction

Palm oil is extracted from the palm fruit pulp (pericarp) which contains about 30-70 % fat depending on the variety of the palm. The predominant fatty acids of palm oil are saturated palmitic acid (C16:0) and the unsaturated oleic acid (C18: l). Palm oil also contains 0.2-0.8 % non acyglycerolunsaponifiable matter and consists of carotenoid, tocopherol, sterols, phosphatides and alcohol. Palm oil, when taken in diet is broken down y lipase enzyme to fatty acids. These fatty acids are energy-rich and serve as a major source of fuel for metabolic processes. Lipids are derived from food or are synthesized in the body, mostly in the liver and can be stored fat cells for future rise (Zilva et at., 1992). The two major lipids transported in the blood are cholesterol and triacylgycerol. Virtually all lipids in mammals are transported complexes with protein except for fatty acids, which are bound directly to albumin. The lipids are carried in special macromolecular complexes termed lipoproteins (Lehninger, 2000). Lipoproteins are classified by their densities which reflect their size (Voet and Voet, 1995). The major classes of lipoprotein in plasma are Chylornicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) so-called bad cholesterol and high density lipoprotein (1{DL) the so-called good cholesterol. Typically, cholesterol is a product of animal metabolism and therefore occurs in foods of animal origin.

Palm oil like other vegetable oil is cholesterol free and is made up of 50 % saturated fatty (palmitic and stearic) acids. Stearic and palmitic acids do not elevate blood cholesterol in normocholesterolemic individuals (Hayes and Khosla, 1986). The position of the saturated and unsaturated fatty acid chains in a triacglyceroI backbone of the fat molecule determines whether such fat will elevate cholesterol level in the blood (Krichvesky, 1996). In palm oil, 75% of the unsaturated fatty acid chains are found in position 2 of the carbon atom of the triacyglycerol backbone molecule. This could explain why palm oil is not cholesterol elevating. Vitamin E, particularly tocotrienols present in palm oil can suppress the synthesis of cholesterol in the liver. As a consequence, tocotrienols lower blood cholesterol levels. The purpose of this study is to compare the effect of palm oil diet and palm oil protein diet on the lipoprotein profile of rabbits and to assess the biochemical significance of the results obtained.

2. Materials and Methods

Reagents and Chemicals (concentrated sulphuric acid, picric acid) used in this study were of analytical grade, HDL-Cholesterol and Triacyiglycerol assay kits

Experimental Animals Twenty rabbits weighing between 850-1400g (Wistar strain) were obtained from Aduwawa market, along Benin - Auchi road, Benin City. The animals were divided into five experimental groups of four each and housed in stainless steel cages. Each animal was marked with picric acid for the purpose of identification on the head, back and legs respectively. The animals were acclimatized for two weeks with growers mash and at the end of the two weeks acclimatization, their weights were taken and blood samples collected through the ear veins and analyzed as control. The animals were assigned to each one of the dietary treatments for a four-week period to achieve a metabolic steady state prior to analyses. At the end of the feeding period the animals were weighed again and blood samples were collected for analysis. Each experimental group was fed with compounded diet and water ad libitum.

Diet Composition I Formulation and Administration

The animals were fed with different dietary reginlens Gi, G2, G3, G4 and G5 for four weeks (Table 1).

	GI	**G2**	**G3**	**G4**	**G5**
Corn starch	60.0	70.0	60.0	70.0	80.0
Dried egg	20.0	20.0	-	-	-
Crayfish	-	-	20.0	20.0	-
Vitamin/mineral mix	5.0	5.0	5.0	5.0	5.0
Sawdust	5.0	5.0	5.0	5.0	5.0
Palm oil	10.0	-	10.0	-	10.0
Total	100.0	100.0	100.0	100.0	100.0

Table 1: Composition of experimental diets for rabbits (g/100g)

2.1. Collection of Blood Sample

The experimental animals were starved for 12 hours prior to blood sample collection. They were then weighed. Blood samples were drawn through the ear veins after sterilization with cotton wool soaked in methylated spirit (70%alcohol) using 1ml needle into sterile anticoagulant containers. The blood samples were then centrifuged at 80,000 rpm for 5 minutes and the plasma was collected into plain bottles and stored in the refrigerator.

2.2. Assay Procedure

Ten micro litre of plasma was added into different dry test tubes and 100 µl of cholesterol reagent was added and the content of each tube was mixed thoroughly. The mixture was incubated for 5 minutes at 37^0C. Standard cholesterol and blank were at the same time treated as sample (Allain et. al, 1974). The absorbance of both sample and standard were measured against reagent blank at 500nrn with a spectrophotometer and the results obtained are as in Table 2. Table 3 and 4 are the assay procedures for triacyglycerol and HDL- cholesterol.

Test tubes	**Reagent blank(µL)**	**Standard (µL)**	**Sample (µL)**
Distilled water	10	-	-
Standard	-	10	-
Sample	-	-	10
Reagent	1000	1000	1000

Table 2: Reagent mix for determination of absorbance of cholesterol

Samples were assayed in duplicates. The concentrations of the plasma were calculated using the equation:

Conc. of cholesterol in sample (mmol/L) =

$$\frac{\text{Absorbance of sample x Conc. of standard.}}{\text{Absorbance of standard}}$$

	Reagent blank(µL)	**Standard (µL)**	**Sample (µL)**
Sample	-	-	10
Standard	-	10	-
Reagent	1000	1000	1000

Table 3: Absorbance of triacyglycerol in the sample

The mixture was incubated for 5 minutes at 37^0 C. Absorbance of sample was measured at 500nm against reagent blank in a 1cm cuvette.

2.3. Calculation of Concentration in Sample =

Absorbance of sample x Conc. of standard -0.11
Absorbance of standard

2.4. Cholesterol Determination

1. Precipitation (semi-Micro) 200µL of the sample and 500 µL of the diluted precipitant were thoroughly mixed and allowed to stand for 10 minutes at room temperature and then centrifuged for 15 minutes at 100,000 rpm. The clear supernatant was separated and cholesterol level determined by the CHOD-PAD method.

2. **Cholesterol assay**

The mixture was incubated for 5 minutes at 37°C. Absorbance of sample and standard were measured against blank within 60 minutes at 500nm in a 1cm cuvette.

2.5. Calculation

Concentration of HDL- Cholesterol =

Absorbance of sample x cone.of standard
Absorbance of standard

Test tubes	Reagent blank(µL)	Standard (µL)	Sample (µL)
Distilled water	100	-	-
Supernatant	-	-	100
standard	-	100	-
Reagent	1000	1000	1000

Table 4: Reagent mix for determination of absorbance due to cholesterol in the sample

2.6. Data Analysis

The data generated in this study were analyzed for level of significance using student T-test.

3. Results and Discussion

The results of total cholesterol, triacyglycerol and HDL- cholesterol concentrations in the plasma are shown in Tables 5, 6, and 7 respectively, while Table 8 shows the mean non-HDL-cholesterol concentration in all the plasma in the five study groups.

Group	GI	G2	G3	G4	G5
Before	0.75±0.01	0.83±0.03	0.82±0.08	0.82±0.08	0.82±0.15
After	0.83±0.06	1.66±0.20	0.40±0.07	0.74±0.05	0.58±0.07
Tcal	-4.00	-5.19	12.00*	2.67	4.8*

Table 5: Total cholesterol concentration in rabbit plasma (mmol/L)
** = significant at 5% level, Tcal= t-test calculated*

Group	GI	G2	G3	G4	G5
Before	1.93±0.05	1.97±0.06	1.96±0.07	1.95±0.09	1.94±0.05
After	1.94±0.07	2.17±0.01	1.49±0.04	2.02±0.09	1.37±0.07
Tcal	-0.50	-5.75	9.20*	-0.89	28.5*

Table 6: Triglycerol concentration in rabbit plasma (mmol/L)
** = significant at 5% level, Tcal= t-test calculated*

Group	GI	G2	G3	G4	G5
Before	0.72±0.01	0.74±0.06	0.70±0.05	0.69±0.05	0.68±0.03
After	0.71±0.01	1.01±0.04	0.35±0.02	0.72±0.02	0.53±0.04
Tcal	1.00	-4.80	11.67*	-1.00	3.75*

Table 7: HDL- cholesterol concentration in rabbit plasma (mmol/L)
** = significant at 5% level, Tcal= t-test calculated*

Group	GI	G2	G3	G4	G5
Before	0.30±0.89	0.90±0.07	0.12±0.04	0.13±0.11	0.14±0.29
After	0.12±0.06	0.06±0.20	0.12±0.09	0.02±0.09	0.05±0.06
Tcal	-1.41	-2.72	0.25	1.20	1.13

Table 8: Non-HDL cholesterol concentration in rabbit plasma (mmol/L)
Values in the columns are statistically insignificant (p>0.05)

A steadily increasing interest in the metabolic role played by dietary fat has stimulated studies with a wide variety of animals. The present study deals with the effect of the palm oil on the serum lipoprotein profile of rabbits fed diets supplemented with egg and crayfish. Lipid profiles are important because they are risk indicators of coronary heart disease and may be involved directly in the development of atherosclerosis.

The rabbits fed G3 (crayfish with oil) and G5 (oil alone) had a reduced total cholesterol concentrations. Palm oil has a lipid lowering effect despite its high concentration of saturated fatty acid (50% palmitic acid). Animals fed G4 (crayfish without oil) had reduced total cholesterol concentrations (0.74±0.05) than that of the control group. Dietary fibre, protein source, and vitamins also influence plasma cholesterol concentration. It is possible that the diet also contributed to the generally low cholesterol concentration. Rabbits fed with G2 (egg without oil) had a higher total cholesterol concentration because of influence of dietary cholesterol of egg. (Marzuki et al., 1992).

Triacyglycerol concentration in plasma of rabbits fed G3 (crayfish with oil) and G5 (oil alone) are lower than those fed with Gi (egg with oil). However serum triacyglycerol (TAG) levels in the animals not fed with oil (G2) and (G4) were elevated. This indicates an increase in TAG synthesis in liver. Although the difference was statistically insignificant, one may conclude that TAG is affected by changes in body weight. The high TAG level through, does not uniformly increase the risk for atherosclerosis or coronary artery disease. However, extraordinary high levels of TAG may lead to pancreatitis (Hajjar and Nicholson, 1995).

Rabbits fed G2 (egg without oil) and G4 (crayfish without oil) had a higher level of plasma HDL- cholesterol of 1.01±0.04 and 0.72±0.02 respectively compared to the baseline values (0.74±0.05 and 0.69±0.05 for G2 and G4 respectively), although the difference was not statistically significant. The high levels of HDL would be associated with effective transport of cholesterol from peripheral tissues, including arteries wall, to the liver for excretion in bile or conversion to bile acids. High circulating HDL concentrations, on the other hand appear to be beneficial in some studies (Miller and Miller. 1975), but not other (Pocock et al., 1986). However rabbits fed diets containing oil (Gi, G2, and G5) showed reduced HDL-cholesterol concentration. Low levels of HDL often reflect obesity, lack of exercise or impaired glucose tolerance. Genetic influences may also be responsible for low HDL level. Serum non-HDL cholesterol concentrations were reduced for all the groups but were statistically insignificant. It could be inferred that groups G1, G2, G3 and G4 had lowest risk of developing atherosclerosis, since atherosclerosis is particularly linked to high levels of LDL- bound cholesterol (Hajjar and Nicholson, 1995). Since dietary cholesterol and saturated fats are the principal dietary factors that increase serum cholesterol concentration and palm oil is free of cholesterol, being a vegetable oil, it can be concluded that palm oil is a safe source of dietary fat.

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