November 2013 – January 2014, Vol. 4, No. 1; 355-360. E- ISSN: 2249 –1929

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section A: Biological Sciences

CODEN (USA): JCBPAT Research Article

Palm Oil Inclusion in the Diets of Rabbits fed Cholesterol and its Effect on the Peroxidation of Lipids and the Activity of Glutathione Peroxidase

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Received: 18 November 2013; **Revised:** 19 December 2013; **Accepted:** 13 January 2013

Abstract: Palm oil inclusion (5%) in a cholesterol based diet (5%) was studied to determine its effect on the peroxidation of lipids and on the activity of glutathione peroxidase activity in the liver of rabbits. Enriched membrane fractions were used to measure lipid peroxidation in vitro using thiobarbituric acid (TBA) as an indicator of lipid peroxidation. Measurements of conjugated diene levels were also carried out. Palm oil supplementation significantly ($P<0.05$) reduced the rate of lipid peroxidation in the liver of rabbits fed the 5% cholesterol diet, compared to the rate of lipid peroxidation in the liver of rabbits fed the 5% cholesterol diet without palm oil inclusion (5%). On the other hand, glutathione peroxidase activity was significantly ($P<0.05$) increased in the liver of rabbits fed the 5% cholesterol diet with 5% palm oil inclusion when compared to the activity of glutathione peroxidase in rabbits fed the cholesterol diet without palm oil inclusion. This study suggests that palm oil inclusion in the diet of rabbits could prevent lipid peroxidation and increase glutathione peroxidase activity in the liver of rabbits fed cholesterol diets.

Key Words: Palm oil, cholesterol, peroxidation of lipids, glutathione peroxidase activity, enriched membrane fractions.

INTRODUCTION

Cholesterol feeding has often been used to raise serum cholesterol levels in studies of the etiology of hypercholesterolemia-related metabolic disturbances such as atherosclerosis in livestock. The metabolic changes associated with cholesterol feeding have received considerable attention in recent years. Cardiovascular disease (CVD) is one of the leading causes of death in the world. Elevated concentrations of total serum cholesterol and LDL- cholesterol have been shown to be amongst the major risk factors in the development of CVD[1]. It has been shown that diets high in cholesterol increased LDL-cholesterol, total serum cholesterol and in turn increases the risk of cardiovascular diseases[2]. Cholesterol feeding has also been observed to increase the activity of some enzymes involved in lipid metabolism. These enzymes include triglyceride lipase, lipoprotein lipase and lecithin-cholesterol transferase which together play a crucial role in the metabolism of HDL-cholesterol[3]. Feeding of cholesterol to rabbit's results in a rapid hepatic infiltration of lipids rich in tri-glycerides and much of this is usually accumulated in the esterified form[4] and is thus highly susceptible to peroxidation. Lipid peroxidation or oxidative degeneration is a measure of the peroxide value which is used to assess the stability of fats. Lipid peroxidation makes oil harmful for consumption as the free radicals generated are seen to be carcinogenic[5].

Palm oil is by far the highest oil producing plant with an average yield of 3.5 tons of oil/hectare/year[6] . Studies with livestock and humans have indicated that palm oil is quite different from other fats such as lard or coconut oil[7-9]. Palm oil is the major vegetable oil consumed in Nigeria; however information on the effect of palm oil to health is limited. Some papers have shown that palm oil could maintain normal growth of rabbits and could cause significant reduction in serum cholesterol in rats compared to soybean oil[10]. Several authors have also shown the beneficial effects of palm oil inclusion in the diets of livestock[11].

This study looks at the effect of palm oil inclusion as a supplement to cholesterol based diets on the peroxidation of lipids and glutathione peroxidase activity in rabbits. Glutathione peroxidase is a physiologically important lipid peroxide decomposing enzyme found in the liver of livestock.

MATERIALS AND METHODS

A total of 36 male Chinchilla rabbits with average weights of 122± 7.7g, were used for the study and were obtained from the Nigerian Institute of Veterinary Research Vom, Nigeria. The experiment took place in the rabbit unit of the Research and Teaching unit of the University of Ibadan (7.380 N and 3.93^0 E), Oyo State Nigeria. Strict compliance to the animal ethics of the World Rabbit Association was observed. The animals were housed in stainless steel cages with raised wire floor in a room with 12 hours light/dark cycle, the relative humidity was between 50-60% and room temperature was maintained at 30^o C. The animals had free access to food and water and were handled according to the Nigerian guidelines for the care and use of laboratory animals. The rabbits were acclimatized to the facilities for two weeks before the start of the experiments. The experimental animals were assigned to four different diets with nine rabbits per diet with each rabbit being used as a replicate. The composition of the diets was as follows: (a) Diet 1 was the control diet, composed of a normal maize diet for growing rabbits. (b) Diet 2 was a palm oil based diet. (c) Diet 3 was a cholesterol based diet and (d) Diet 4 was a mixture of cholesterol and palm oil as shown in **Table-1.**

The feed used was obtained from Top Feeds Limited, Sapele in Delta State, Nigeria. The chemicals used were of analytical grade and were products of British Drug House Chemicals Ltd, Poole, England unless otherwise stated. The palm oil used for feed formulation was obtained from the Nigerian Institute for Oil palm Research, Edo State. The experiment was conducted over a period of

forty eight (48) days. Before commencement of the experiment, the animals were starved overnight but allowed access to water ad lib. Random sampling was carried out and three rabbits in each group were sacrificed from which blood and liver samples were collected on the first day of the experiment to obtain initial readings. The rabbits had free access to their diets and were weighed weekly.

Table- 1: Composition of Diet (% weight)

Feed Composition	Diet 1	Diet 2	Diet 3	Diet 4
Maize	70	70	70	70
Fish meal	10	10	10	10
Ground nut cake	20	15	15	10
Cholesterol	-	-	5	5
Palm oil	-	5	-	5

Enriched membrane fractions were used to measure lipid peroxidation. This kind of preparation allows for a better coefficient of variation (4.8± 0.2%) than in other cell fractions 34.9± 6.5% in whole cells or 15.8± 2.1% in mitochondrial cells [12]. The method used for determination of lipid peroxidation in the liver was the thiobarbituric acid (TBA) method[13], with slight modifications. Approximately 1g of each liver from the sacrificed animals was quickly removed and weighed and then homogenized in 9ml of a 75mM potassium phosphate buffer solution (pH 7.0). Half of the homogenate was added to a 25ml incubation flask containing 3.5ml of the buffer solution. After thorough mixing, the mixture was transferred to a test tube containing 2.5ml of 8% trichloroacetic acid which was then used for the TBA test. Another portion of 0.05ml was used for the determination of conjugated diene levels according to modified methods of Pedro *et al* [14]. The remaining portion was then incubated under air at 37^0C for 2 hours and the experimental procedures repeated. 2ml of 0.67% TBA solution was added to each tube and boiled for 15 minutes. After centrifugation, color intensity of a portion of the supernatant was determined at 532nm using a digital colorimeter. TBA reacting compounds were expressed as micrograms malondialdehyde (MDA) per gram of liver. Samples containing known levels of MDA were treated in the same way and used to prepare standard curves. The Conjugated diene levels were expressed as 1 x 102 nmole per gram of liver based on an extinction coefficient of 2.2 x 104 (Pedro *et al*.[14]) The other half of the homogenate was centrifuged at 1000 x g for 10 minutes at 4^0C. The supernatant fraction was then carefully decanted for enzyme assays. Glutathione peroxidase (EC. 1.11.1.9) activity was determined according to the method of Little *et al* [10] with cumene hydroperoxide as substrate.

All data collected were subjected to one way analysis of variance in a completely randomized design. Probability was accepted at 5% level and means were separated using Duncan's multiple range procedure[15].

RESULTS AND DISCUSSION

The results of the experiment are shown in **Table 2**. There were no significant (P>0.05) differences in body weight between the rabbits fed the experimental diets, neither were there significant (P>0.05)

differences in the average daily feed intake of the rabbits fed the experimental diets. The liver size of rabbits fed Diets 3 and 4 were significantly (P<0.05) larger than the liver size of the rabbits fed Diets 1 and 2. Higher values were recorded by rabbits fed Diet 3. The rate of lipid peroxidation in the liver homogenates of rabbits fed Diets 3 and 4 based on both TBA tests and on conjugate diene measurements were significantly (P<0.05) higher than in rabbits fed Diets 1 and 2. The rate of lipid peroxidation was observed to be highest in the liver homogenate samples of rabbits fed Diet 3. Liver glutathione activity was significantly (P<0.05) lower in rabbits fed both Diets 3 and 4 when compared to rabbits fed Diets 1 and 2. This activity was also observed to be significantly (P<0.05) lower in rabbits fed Diet 3 than rabbits fed Diet 4.

Table- 2: Lipid peroxidation and glutathione peroxidase activity of rabbits fed cholesterol diets supplemented with palm oil

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Body Weight (g)				
Initial	122.0± 1.8^{a}	122.6±2.2^{a}	122.8±3.8^{a}353	122.2±3^{a}
Final	357.5±40^{a}	359.4±39.2^{a}	.9±27^{a}	354.4±4^{a}
Average daily feed intake (g)	20.8± 1.2^{a}	20.6± 1.4^{a}	20.5±1.0^{a}	20.7± 1.0^{a}
Liver weight (g/100g body wt)				
Initial	4.2±0.3^{a}	4.3±0.1^{a}	4.1±0.2^{a}	4.3±0.2^{a}
final	4.9±0.1^{b}	4.9±0.2^{b}	6.2±0.3^{a}	5.1±0.3^{ab}
Lipid peroxidation				
Baseline TBA values[1]: 0hrs	9.2±1.2^{a}	9.4±1.2^{a}	9.5±1.0^{a}	9.2±1.2^{a}
2hrs	13.7±1.3^{a}	13.6±1.3^{a}	13.9±1.2^{a}	13.6±1.3^{a}
Net change	4.5±0.1^{a}	4.2±0.1^{a}	4.4±0.2^{a}	4.4±0.1^{a}
Post diet TBA values[1]: 0hrs	17.4±4.5^{c}	17.8±5.2^{c}	41.7±5^{a}	30.6±3.3^{b}
2hrs	93.2±10.4^{c}	96.3±15.4^{c}	381.3±20^{a}	257±14.5^{b}
Net change	75.8±5.9^{c}	78.5±10.2^{c}	339.6±15^{a}	226.4±11.2^{b}
Conjugated diene[2]				
Baseline values: 0hrs	3.5±0.1^{a}	3.3±0.2^{a}	3.3±0.2^{a}	3.4±0.1^{a}
2hrs	3.8±0.2^{a}	3.7±0.3^{a}	3.8±0.3^{a}	3.8±0.2^{a}
Net change	0.3±0.1^{a}	0.4±0.1^{a}	0.5±0.1^{a}	0.4±0.1^{a}
Post diet values: 0hrs	8.2±1.0^{c}	8.8±1.3^{c}	40.8±5.9^{a}	27.1±3.3^{b}
2hrs	15.5±1.6^{c}	16.2±1.8^{c}	68.3±9.0^{a}	56.5±7.5^{b}
Net change	7.3±0.6^{c}	7.3±0.5^{c}	27.5±3.1^{b}	34.4±4.2^{a}
Glutathione peroxidase activity[3]				
Baseline values	2.3±0.2^{a}	2.3±0.1^{a}	2.1±0.1^{a}	2.3±0.2^{a}
Post diet values	7.1±1.3^{a}	6.9±1.0^{a}	4.0±1.1^{c}	5.8±1.2^{b}
Net change	4.8±1.1^{a}	4.6±0.9^{a}	1.9±1.0^{c}	3.5±1.0^{b}

^{a,b,c} Means with different superscripts within rows differ (P<0.05) significantly
Legend: [1]µg malondialdehyde/g liver; [2] 1x10^{2} nmoles/g liver; [3]µmoles NADPH

In the present studies, the concentration of TBA reacting compounds and the level of conjugated diene in the incubation of the liver homogenates were assumed to represent the peroxide activity of the livers. The observed effects of palm oil inclusion to a cholesterol based diet on lipid peroxidation and on glutathione peroxidase activities in the liver of rabbits are based on results which are expressed on a gram per liver basis. The results obtained in this present study indicate that the addition of palm

oil to a cholesterol based diet significantly (P<0.05) reduces lipid peroxidation in the liver when compared to the high rate of lipid peroxidation in the livers of rabbits fed cholesterol diets only. Elevated levels of liver cholesterol have been shown to be associated with increased rate of liver cholesterol degradation[16]. Lipid peroxidation has been suggested as a normal process in cholesterol metabolism[17-18]. The increased dietary intake of cholesterol may increase the rate of hepatic lipid peroxidation. The potential of palm oil to suppress the attendant rate of lipid observed in this study is of interest as several diseases including cardiovascular diseases usually involve free radical lipid peroxidation in biological membranes[19]. Results from the present studies show that the inclusion of palm oil in the diets of the rabbits significantly (P<0.05) increased the activity of glutathione peroxidase in cholesterol fed rabbits.

CONCLUSION

The role of palm oil in suppressing cholesterol induced lipid peroxidation in the liver of rabbits has been reported in this present study. The study also shows that palm oil at 5% inclusion in diet of rabbits fed a 5% cholesterol diet produces a significant increase in the enzyme activity in the liver of the rabbits. Glutathione peroxidase is an enzyme involved in the detoxification of lipid peroxides. A reduction in the activity of this enzyme could decrease the inherent protection of membrane tissues against lipid peroxidation.

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