THE EFFECT OF CHRONIC CONSUMPTION OF PALM OIL DIET ON THE SMALL INTESTINE, LIVER AND KIDNEY OF ALBINO RATS

BY

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DECLARATION

I, IBEGBU, AUGUSTINE OSELOKA, hereby declare, that I carried out the research reported in this thesis by myself under the supervision of Professor J. O. Hambolu, Professor S. A. Ojo and Dr. E. M. Sheriff.

That wherever references were made of the published work of other investigators and other sources of information used, these were properly acknowledged and referenced.

That no part of this thesis has been submitted or accepted elsewhere for a higher degree or diploma.

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CERTIFICATION

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DEDICATION

This work is dedicated to Him who is "The Fountain of all Hopes"

and

To all lovers of scientific knowledge.

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I am specifically grateful to Almighty God for His continual Love, guidance and protection.

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ABSTRACT

The study of the effect of chronic consumption of palm oil diet on the small intestine, liver and kidney of Albino rat was undertaken using thirty Albino rats which were randomly separated into three groups (A, B and C) of ten rats each. At the beginning of the experiment, the mean body weight of rats in the three groups was not significantly different from each other. The first group (A) was fed on thermoxidized palm oil diet made up of 15gm of thermoxidized palm oil mixed with 85gm of rat feed (15% W/W), while the second group (B) was fed on fresh palm oil diet comprising 15gm of fresh palm oil mixed with 85gm of rat feed (15% W/W). The third group (C) was used as control and were fed normal rat ration only.

At the end of the thirty-two weeks of feeding, the mean gain in body weight of the control was significantly greater than those fed both thermoxidized and fresh palm oil diets ($P<0.05$) while the mean change in weight between the two test groups was not significant. The mean change in body weight of the animals were 52.7 ± 15.7gm, 65.0 ± 19.3gm and 88.8 ± 23.7gm for groups A, B and C respectively.

The daily food consumption was significantly different between the control and the test groups (A and B) ($P<0.05$) and between the group A and B ($P<0.05$). The mean daily food intake per rat was 9.39 ± 1.91gm, 10.52 ± 1.33gm and 13.27 ± 0.86 for groups A, B and C respectively.

The surface amplification factor due to the jejunal villi was 3 to 4, 3 to 4 and 5 to 6 for groups A, B and C respectively, of which the level of difference was significant between the control group C and the test groups A and B only, while the

mean villous length was 382.50 ± 44.50um; 442.50 ± 65.27um and 487.5 ± 38.75um for groups A, B and C respectively.

Histological examination of the small intestinal mucosa, and submucosa, the liver and the kidney showed that they were affected in rats fed thermoxidized and fresh palm oil diets when compared to the control. In rats placed on thermoxidized palm oil diet, the mucosa and the villi of the small intestine were shown to be wearing away and reduced in height while the submucosa was enlarged and eroded. The rats fed fresh palm oil diet showed enlarged villi, stroma and submucosa.

The liver of rats fed both thermoxidized and fresh palm oil diet showed various accumulation of degenerative fatty vacoules and areas of necrosis, which was more pronounced in the thermoxidized group than the fresh palm oil group while the control groups showed normal histology of the liver.

The kidney of rats fed both thermoxidized and fresh palm oil diets showed proliferated lacis cells (Polkissen) and proteinacious cast in the lumen of some proximal and distal tubules. The proliferated cells and the deposited materials were more in group A animals than group B while the control showed normal histology. Also the blood vessels in the liver and kidney of the test animals (A and B) were congested when compared to the control.

This study therefore shows that chronic consumption of both thermoxidized and fresh palm oil diets are hazardous to the tissues of the small intestine, liver and kidney, due to the peroxides that are present in the oils.

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CHAPTER ONE

INTRODUCTION

Palm Oil is derived from the fruits of the palm tree (Elaeis guineensis) which is one of the most predominant cash crop in the Southern part of Nigeria and found in many countries in West Africa. Several products can be obtained from palm tree. These products include palm kernel from which we have kernel oil, kernel cake, creams and candles. Other products like brooms, baskets and wood can be produced from the different parts of the tree (Loncin, 1970; Bender, 1973).

Palm oil as a source of lipid and other nutrients like vitamins (Murray, et al., 1988) is produced and used extensively by majority of Nigerians. Palm oil is liquid at room temperature. It is processed by both traditional and industrial methods. The traditional method involves the pounding of the boiled fruits from which the oil is extracted by squeezing the fibres of the pounded fruits using both hands, while the industrial method involves the use of processing and extraction machines. The industrial method gives better yield compared to the traditional method (Formo, et al., 1979).

Freshly prepared palm oil contains about 50% free fatty acids when obtained by local means while the level of free fatty acid is increased with better methods employed in the industries. The free fatty acids in palm oil have approximately equal amounts of saturated and unsaturated fatty acids. The major fatty acids present in palm oil include, palmitic acid, stearic and oleic acids while the minor ones include, Myristic, Linoleic and Linolenic acids (Formo, et al., 1979; Alfin-Slater and Aftergood, 1980).

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The functions and uses of palm oil are numerous, ranging from direct consumption as a direct source of fatty acid (Murray et al., 1988) to its the use as raw material in the production of many industrial products. Directly, it is used in the cooking and frying of foods. In industries, it is used extensively in the manufacturing of margarine, cosmetics, waxes, lubricants and tooth pastes.

Palm oil as a source of lipid is a heterogenous group of compounds containing fatty acids. They have the common property of being relatively insoluble in water and soluble in non-polar solvents such as ether, chloroform, benzene, alcohol and other hydrocarbons (Murray, et al., 1988).

Lipids are important dietary constituents not only because of their high energy value but also their content of fat soluble vitamins A and E which are of physiological importance as well as the essential fatty acids contained in the fats of natural foods. In the body, fat serves as an efficient source of energy both directly and potentially when stored in adipose tissues. It serves as thermal insulator in the subcutaneous tissues and around certain organs like the kidney. The non-polar lipids acts as electrical insulator allowing rapid propagation of depolarization waves along myelinated nerves (Wills, 1971). Combinations of fats and protein forming lipoprotein are important cellular constituents occurring in the unit membrane of the cell and the organelles within the cytoplasm. It also serves as a means of transporting lipids in the blood for cellular metabolism (Murray, et al., 1988).

Palm oil, according to Umoh, et al., (1983) is used as a traditional antidote to cyanide toxicity. The oil is also used as antiseptic in the treatment of boils and wounds in many localities in the country.

However, palm oil while being used is subjected to different harsh conditions like frying and boiling to very high temperatures. It has been shown that oils get oxidized when heated to a high temperature in the open air. Oils and lipids have been established to be susceptible to oxidation from both external and internal sources (Frankel, 1980). The external sources include heat and radiation in the presence of oxygen, enzymes, metal ions, photo-sensitisers or even water (Isong, 1988), while internal sources relate to the presence of pro-oxidative substances in the oil that precipitates oxidation.

It has been shown that oxidized oils are injurious to health when the temperature the oil is subjected to exceeds 180°C (Lang, 1974). Repeated frying of palm oil as is done by many families and food vendors in Nigeria, subjects the oil to temperatures above 180°C.

This work is aimed at investigating the effect of chronic consumption of both thermoxidized and fresh palm oil diets on the liver, small intestine and kidney of Albino rats.

CHAPTER TWO

LITERATURE REVIEW

2.1. BRIEF REVIEW OF LIPIDS:

Palm oil is the most widely used cooking oil in Nigeria. Lipids which are the major constituent of palm oil can be classified based on the glycerol content. The glycerol based lipids include the simple lipids and the compound lipids. The simple lipids are esters of fatty acids with various alcohol, for example fats and oils. The compound lipids are esters of fatty acids containing groups in addition to an alcohol and a fatty acid, like glycolipids and phospholipids. The example of phospholipids are cephalins and lecithins. Non-glycerol based lipids include waxes, cerebrosides, steroids, and sphingomyelins (Murray, et al., 1988). The energy concentration of the lipids is very high due to their hydrated and low density properties (Ganong, 1983). Controlled oxidation occurring in vivo allows the release of these tremendous amount of energy entrapped in these biomolecules (Wills, 1971). When oxidation becomes unprecedented and uncontrolled, peroxidation results which is the type that occur during heating of oil to very high temperatures.

In this situation, the fatty acid of the oil, mostly, the polyunsaturated fatty acids are oxidized to form peroxides which may lead to the formation of hydro-carbons, cyclic and polycyclic acids that are hydroxylated while some, being volatile products, may escape from the oil (Lake and Water-Worth, 1980). The peroxides and other products formed are known to be very reactive and destructive compounds in the body (Frankel, 1980). These products have regrettably deteriorative reverberation owing to the fact that it proceeds by free radical generation. These

free radicals are harmful and highly reactive species containing one or more unpaired electrons (Halliwall and Gutteridge, 1985). They cause a chain of conjugation, polymerisation and fragmentation reaction, utterly disrupting the biochemical and physiological functions of the body by altering its histology. Many of these oxidative products are cytotoxic (Ziombski, 1982). While the actual consequences of ingested thermoxidized oil are subjected to different opinions (Kaunitz, et al., 1960; Shue, et al., 1968; Alexander, 1978; Artman, 1969; Barret and Henry, 1966). One fact that stands out is that the effect depends on the degree of heating which in-turn determines the extent of peroxidation and the amount of the thermoxidized oil consumed (Isong, 1988).

The relevance of malnutrition in this context is pertinent in that Hermans, et al., (1973) had reported that the results of thermoxidized oil experiments depended greatly on the dietary protein level. They indicated that adverse effects arising from oxidized fat ingestions were progressively ameliorated by increasing levels of protein. This shows why animals on low protein diets have been shown to be more predisposed to toxicant effects (Hermans, et al., 1973) than those on high protein diets. Different research materials available has been shown that thermoxidized oil is shown to affect virtually all the different organs and tissues of the body though the degree and type of effect varies (Isong, 1988).

The changes in some of the physical qualities of the oil associated with over-heating include changes in the colour, odour and flavour (Artman, 1969; Barret and Henry. 1966). These changes show deterioration of the oil and moreover extremely deteriorated oils are unpalatable (Gurr and James, 1975) and should not be used.

The provision of essential fatty acids as seen in the composition of palm oil helps in the synthesis of other important physiological compounds. For example, arachidonic acid or its precursor linoleic acid, is the most abundant precursor of prostaglandins (Ramwell, 1981). Lipids also play a very important role as structural components of membrane and constitute the very essence of biomembrane microarchitecture and functions, blood clotting, compartmentalization, selective transport and fusogenic activities (Lucy, 1971). Linoleic acid is needed in normal spermatogenesis (Cox and Marell, 1981).

2.2. OXIDATION OF OILS

Oxidation of lipids generally in the body is controlled by many factors like enzymes and oxygen. This allows for the release of the tremendous amount of energy entrapped in these biomolecules (Wills, 1971) but once oxidation becomes unprecedented and uncontrolled, peroxidation results. The oxidation of fats can occur both in vitro and in vivo (Artman, 1969; Murray et al., 1988).

In vivo, the lipids of the biomembranes are continuously being oxidized as a result of exposure to radicals, superoxides, hydroxy and peroxy radicals (Izaki, et al., 1984). This oxidation can be caused by enzymatic and non-enzymatic systems (Artman, 1969; Gurr and James, 1975; Lokesh, et al., 1981). These radicals cause the autoxidation of lipids and other biomolecules such as vitamins and catecholamines (Glavind, 1972).

In vitro oxidation results from the exposure of fats to external energy sources with or without a catalyst. This can be exposure to heat or radiation in the presence

of oxygen, enzymes, metal ions, photosensitisers or water (Isong, 1988). Oils are prone to thermal oxidation and polyunsaturated fatty acids are more susceptible (Formo, et al., 1979). Physical and chemical changes occur during thermoxidation of palm oil and some of these changes include odour, colour, elevated acid value, increase percentage free fatty acid level and reduced palatability (Artman, 1969; Barret and Henry, 1966). Other changes include, the formation of peroxides and other compounds. The nature and proportion of these products formed during thermoxidation depends on the following factors; duration of heating, period of time of keeping the oil hot, the surface area of the oil, volume ratio during oxidative exposure, extent of intermittent processing, storage time, natural pro-oxidant and antioxidant levels, irradiative intensity and temperature level (Artman, 1969).

During thermoxidation, the oxygen present combine with the free fatty acid to form allytic hydroperoxides which later transform to peroxides. These peroxides later decompose to form aldehydes and ketones as their concentrations increase (Artman, 1969; Ziombski, 1982). The presence of these products are responsible for the reversion of odour and flavour in oils that have been thermoxidized and concentrated amount of peroxides are also toxic to the body (Plea, 1975).

2.3. MOLECULAR AND PHYSIOLOGICAL EFFECTS OF INGESTED THERMOXIDIZED OILS

Experiments have confirmed the biotoxicity caused by peroxides present in thermally oxidized oils (Isong, 1988). The toxicity can be demonstrated when concentrated amounts of peroxides are administered orally. Carbontetrachloride induced peroxidation have been shown to potentiate hepatotoxicity and altered

cellular membrane (Plea, 1975). The permeability of mitochondria and activities of membrane-bound proteins were altered thus resulting in uncoupling oxidative phosphorylation. Other effects noted include alterations in endoplasmic reticulum with the cisternae of the rough endoplasmic reticulum becoming dilated and degranulated while RNA content was doubled showing an inhibition of protein synthesis (Plea, 1975). Also the activities of Glucose-6-phosphatase and mixed function oxidase are reduced (Hermans, et al., 1973). The loss of Glucose-6-phosphatase activity was due to distortion of endoplasmic reticulum membrane microarchitecture essential for its activity (Wills, 1971). There are morphological changes in endoplasmic reticulum and triacylglyceride which accumulate resulting from defective lipoprotein (Plea, 1975). There is such toxic responses as transient influx of calcium ions into liver parenchymal cells and elevation of transaminases, lactate-dehydrogenase, isocitrate dehydrogenase and aldolase levels (Plea, 1975). Red blood cell membrane fluidity is altered by phenylhydrazine induced peroxidation of membrane lipids resulting in haemolysis (Rice-Evans and Hochstein, 1981).

Peroxidation also increases membrane permeability for ions like H^+, K^+ and Na^+. The electrical conductivity of these membranes and the oxidation of sulphahydryl groups are also amplified (Alexander, 1978). The effects of ingestion of whole oxidized oils range from loss of appetite, poor feed efficiency, reduced gut absorption, steatorrhaea, growth depressions, to enlarged livers, kidneys and lungs, fatty hepatic necrosis, haemolysis, calciferous myocardial lesions and even death (Alexandar, 1978). However, toxicity varies with age, sex, organs, species and nutritional status of the individual (Isong, 1988).

Altered hepatic and renal functions, exaggerated responses of the central nervous system to stimuli, spontaneous movement, intensified capillary permeability, changes in motor and secretory functions, thyroid gland changes and kidney deposits are other symptoms reported by Kaunitz, et al., (1960) as arising from the ingestion of oxidized oil. Shue, et al., (1968) observed rapid decline in body temperature in addition to fatty livers in animals feed oxidized oil. Lipoxidation have been found to follow the depletion in vitamins A and E (Formo, et al., 1979). There are remarkable similarity in the symptomatic presentation emanating from these secondary vitamin deficiencies and oxidized oil ingestion. Vitamin E which protects rat brain DNA alterations are lost from the oil by peroxidation (Jayanthi-Bai, et al., 1982). However, in gross peroxide accumulation vitamin E is destroyed, membrane damage results and carcinogenic activity is triggered (Alexander, 1978). Cataractogenesis have also been suggested by Jav-Bock, (1975).

Cyril and Eric, (1964) have shown that vitamin A deficiency as in oxidized oils may lead to renal stones in rats but there is no evidence that it plays any part in human calculus formation. They also showed that young rats fed on a diet complete in all respects except for the absence of vitamin A may cause growth to cease, loss of weight and finally death. Pathological changes are found in the eyes, intestine and respiratory tract. The mucous secreting cells of the intestine become atrophied and the tips of the villi necrosed (Cyril and Eric, 1964). The deficiency of vitamin E may cause degeneration of the germinal epithelia of the testis of lower animals, resorption of the foetus after conception, malformation of the foetus, muscular dystrophy and prevents normal growth in man (Cyril and Eric, 1964).

The peroxides derived from oxidized lipids can polymerise and denature biological proteins such as enzymes and membrane proteins. Such membrane damage increases membrane permeability and cell leakage often results. (Plea, 1975). This poses serious danger with respect to lysosomes which may be eroded (Gurr and James, 1975; Bessis, 1970). Blood cholesterol level is elevated due to the unsaturation of major fatty acids and is accompanied by a decreased phospholipid level in the body (Hamilton, et al., 1980).

The toxicity of these oxidative products on the liver are more pronounced than any other organ (Isong, 1988). Cyril and Eric, (1964) have shown that the toxicity on the liver manifests mainly in fatty liver which shows a diffuse hepatic fibrosis called Cirrhosis while gross chronic fatty liver leads to death of many liver cells and their replacement by scar tissue.

Zimmerman (1982) explained that the toxic agents lead to injury to the plasma membrane which permits intrahepatic accumulation of calcium ion. The high concentration of calcium ion in-turn enhances plasma membrane injury permitting even higher intracellular content of the ion which leads to necrosis. The role of injury to the plasma membrane is the pathogenesis of necrosis. It leads to rapid loss of intracellular K^+, electrolytes enzymes and coenzymes and entry of extracellular Ca^{++} electrolytes. Thus the entry and intracellular accumulation of Ca^{++} has been considered by Zimmerman, (1982) to be responsible for triggering of necrosis. Wood, (1965) has reported mitochondrial enlargement in the liver of rats intoxicated with ethionine, in the hepatocytes of diabetic spiny mice, of mice treated with bis-cyclohexanoneoxaldihydrazone and of fatty-acid-deficient mice. Wilson and Leduc

(1963) showed that the essential fatty acid deficiency reduces the fatty acid composition of the lipids in the mitochondrial membranes. Also within the mitochondria of hepatocytes of rats intoxicated with ethioine are present amorphous masses as in Gilbert's disease or carbon tetrachloride poisoning (Wood, 1965; Rynold, 1965).

Cyril and Eric, (1964) have shown that fats accumulate in the intima of blood vessels from the circulating lipoproteins forming artheroma. They showed rabbits that are fed excessive amounts of cholesterol develop yellow athermatous plaques while in rats atheroma is much more difficult to produce by cholesterol feeding and human cases of atheroma may have an abnormal lipid pattern in their blood.

Most available literatures on the effect of oxidized oil on animals show that reduced weight or stunted growth is one of the visible manifestations (Kaunitz, et al., 1960). Some workers attributed this to reduced consumption due to unpalatibility of such diet as well as reduced gut absorption and steatorrhae. Isong (1988) observed some toxic manifestations in the reproductive systems and features of embryo-foetal toxicity like depressed foetal yield, neonatal birthweight, infertility and delayed sexual maturation in the rats. However, these effects are sex dependent, being more pronounced in the females than males, less severe in the offsprings than their parents. There is the presence of retinal degeneration due to the absence of vitamin A, atherosclerosis, tissue necrosis and fibrosis (Nafastad, 1974; Alexander, 1978).

2.4. DIGESTION AND ABSORPTION OF FAT

The small intestine is the main organ responsible for digestion and absorption

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of ingested fat. However, a small amount of short chain triglycerides of butter fat origin is digested in the stomach by gastric lipase, but the amount of digestion is so small that it is unimportant (Guyton, 1986).

After the food in the stomach has been thoroughly mixed with gastric juice, it becomes known as chyme and is ready to enter the small intestine. The small intestine is very important in that it produces and secretes intestinal juice or succus entericus (Guyton, 1986 and Alleyne, et al., 1978). The small intestine also secretes two other fluids known as the pancreatic juice from the pancreas and bile from the liver. The two secretions enter the duodenum through the spinctea oddi (Ganong, 1983; Guyton, 1986; Kajimoto and Mukai, 1970).

The first step in fat digestion is to break the globules into smaller sizes so that the water-soluble digestive enzymes can act on the globule surface. This process is called emulsification of fat and is achieved under the influence of bile secreted by the liver (Ganong, 1983; Guyton, 1986). Cyril and Eric, (1964) have shown that the bile do not contain any digestive enzyme but rather contain large quantity of bile salts in the form of ionized sodium salts responsible for the emulsification of fats. The carboxyl and other polar parts of the bile salt molecule are highly soluble in water, (hydrophilic), whereas most of the sterol portion of the bile salt is highly soluble in fat, (hydrophobic) (Cyril, and Eric, 1964; Guyton, 1986). Therefore, the fat soluble portion of the bile salt dissolves in the surface layer of the fat globule with the polar end of the salt projecting outward and are soluble in the surrounding fluid. This greatly decreases the surface tension of the fat (Ganong, 1983; Guyton, 1986).

The most important enzyme for fat digestion is the pancreatic lipase in the

pancreatic juice (Cyril and Eric, 1964; Ganong, 1983). Also the epithelial cells of the small intestine contain a minute quantity of lipase known as enteric lipase (Guyton, 1986). Both help in the hydrolysis of fat. During fat digestion, the triglycerides of the diet are split into free fatty acids and 2-monoglycerides while small portions are left undigested (Ganong, 1983). Guyton, (1986) has shown that the hydrolysis of triglycerides is a highly reversible process and that the accumulation of monoglycerides and free fatty acids in the vicinity of digesting fats very quickly blocks further digestion. The bile salts play an important role in removing the monoglycerides and free fatty acids from the vicinity of the digesting fat globules almost as rapidly as these end products of fat digestion are formed (Cyril and Eric, 1964; Guyton, 1986; Causeret, et al., 1978; Klaasen, 1975).

The end absorbable products of fat digestion, monoglycerides, fatty acids and glycerol have the propensity to combine with bile salts to form micelles (Alleyne, et al., 1978; Guyton, 1986) which entrap the individual products and prevent their recombination. The micelles carry the fatty products to the brush borders of the cells, lining the small intestine for their absorption (Cyril and Eric, 1964; Murray, et al., 1988; Guyton, 1986). Since these end products are fat and membrane soluble (Miller, et al., 1977), they easily diffuse into the epithelial cells where they reform triglycerides (Guyton, 1986; Murray, et al., 1988). These triglycerides within the cells, complex with phospholipids and cholesterol and become enclosed in a protein coat forming chylomicrons (Murray, et al., 1988). The chylomicrons diffuse out of the epithelial cells and enter the central lacteals of the villi (Alleyne, et al., 1978, Guyton, 1986, Murray, et al., 1988). From there, they are propelled along with lymph by the

lymphatic pump upward through the thoracic duct to be emptied into the great veins of the neck (Cyril and Eric, 1964; Guyton, 1986; Murray, et al., 1988; Combe,et al., 1978; Combe et al., 1981; Palay and Revel, 1964).

2.5. THE HISTOLOGY OF THE SMALL INTESTINE

The small intestine is a coiled tube extending from the pylorus of the stomach to the ileocaecal junction where it joins the large intestine in mammals. Comparatively, the small intestine measures about 6 - 7 meters long in man, 2 - 5 meters in dogs, 1.30 meters in cats and about 0.5 meters in rats (Copenhaver and Pehaneer, 1971; Dellman and Brown, 1981; Lucy, 1975; Nickel, et al., 1973). However, the small intestine is longer after death due to the loss of muscle tone (Copenhaver and Pehaneer, 1971). The small intestines of the dog, cat and rat, unlike those of the other mammalian species have the simple arrangement found in man (Nickel, et al., 1973; Lucy, 1975). The small intestine of man occupies the central and lower parts of the abdominal cavity, usually within the colonic loop and is related in front to the greater omentum and the abdominal wall (Reith and Ross, 1977). The small intestine is divided into three parts; duodenum, jejunum and ileum. The duodenum can be distinguished from the jejunum and ileum by the presence of glands in the submucosa. The jejunum and ileum are not distinguished by any clear cut difference (Reith and Ross, 1977; Leeson and Leeson, 1976; Dellman and Brown 1981).

The inner surface of the small intestine contains permanent circular folds called plicae circularis, valvulae conniventes, valves of kerckring which form ridges, most of which are roughly at right angles to the long axis of the tube (Copenhaver

and Pehaneer, 1971; Leeson and Leeson, 1976). These folds appear as a series of regular elevations. However, some of these ridges branch and may travel in longitudinal directions for short distances. The plicae circularis begin in the duodenum and are the tallest and most numerous in the jejunum while they become shorter and less numerous in the ileum (Copenhaver and Pehaneer, 1971; Leeson and Leeson, 1976; Lucy, 1975; Ham, 1974; Spencer, 1960).

Isolated nodules of lymphoid tissue are located in the proximal end of the intestinal canal (Reith and Ross, 1977). In the distal part, they appear as increasing large aggregates, so that in the ileum aggregates of lymph nodules called peyer's patches are regularly found. These nodules are found opposite mesentric attachment (Copenhaver and Pehaneer, 1971; Reith and Ross, 1977). There is also the interruption in the muscular layer where blood vessels are passing between the mesentery and the submucosa.

In routine hamatoxylin and eosin sections, the four layers of the intestinal wall can be distinguished as follows according to Copenhaver and Pehaneer, (1971); Reith and Ross, (1977); Leeson, and Leeson, (1976); Lee and Toner (1980).

The mucosa which appears dark due to large numbers of epithelial cells and the numerous cells in the lamina propria. It contains both villi and intestinal glands. The glands are called crypts of Lieberkuhn. The villi project above the surface into the lumen and the glands dip into the supporting connective tissue (Reith and Ross, 1977; Dellman and Brown, 1981).

Three kinds of cells are present on the surface of the villi and are named, columnar absorptive cells, globet cells and argentaffin cells (Copenhaver and

Pehaneer, 1971). Leeson and Leeson, (1976) included Paneth cells, caveolated cells and migrating cells. The nuclei of the absorptive cells are elongated and located at the basal region of the cells. The absorptive cells of the small intestine possess a striated border and can easily be identified while the argentaffin cells are in contact with the basement membrane and require special stain for their demonstration (Copenhaver and Pehaneer, 1971; Leblond and Stevens, 1948; Palay and Karlin 1957).

The submucosa is a light staining layer under the mucosa. It forms the core of the plica circularis and contain blood vessels.

The muscularis externa is external to the submucosa. It is disposed into two distinct muscular layers namely, an inner circular layer and an outer longitudinal layer.

The serosa is the light staining region external to the muscularis externa. Where the intestine has no serosa as in the visceral peritoneum, the layer external to the muscularis externa would be referred to as the adventitia (Leeson and Leeson, 1976). For example, the duodenum is adherent to the posterior abdominal wall and therefore has much of its wall as an adventitia rather than a serosa.

Whereas the plicae circularis have submucosa as their central core, the villi are mucosal projections and have lamina propria as their central core (Leeson and Leeson, 1976; Copenhaver and Pehaneer, 1971). Each villus contains a centrally located lacteal which is a lymphatic capillary which begins in the villus (Miller, et al., 1977; Reith and Ross, 1977). In addition to the lacteal, the lamina propria contain small blood vessels and smooth muscle cells (Copenhaver and Pehaneer, 1971; Reith

and Ross, 1977). The lacteals are lined by extremely flat endothelial cells with elongated dark staining nuclei (Copenhaver and Pehaneer, 1971; and Reith and Ross, 1977; Palay and Revel, 1964).

2.6. THE HISTOLOGY OF THE LIVER

The liver is the largest and the heaviest gland in the body and comparatively weighs about 1.5kg in man, 127gms to 1.35kg in dogs, 68.5gms in cats and 5 to 30gms in rats (Nickel, et al., 1973; Lucy, 1975; Leeson and Leeson, 1976). The size and weight of the liver vary greatly because it stores fats and glycogen. It weighs more in well-fed animals than in emaciated ones. The weight of the liver decreases with age (Nickel, et al., 1973). The colour of the liver depends on the amount of blood it contains, the species, the age of the animal and the nutritional status of the animal. It is reddish brown or dark red in fresh condition because of the very rich blood supply (Copenhaver and Pehaneer, 1971; Leeson and Leeson, 1976; Reith and Ross, 1977). It receives all the food materials absorbed from the intestinal tract except lipids (Guyton, 1986). In addition to the digested and absorbed materials which are assimilated and stored in the liver, the portal blood carries to the liver various materials which are detoxified and excreted by the liver (Leeson and Leeson, 1976; Ganong, 1983; Guyton, 1986; Klaasen, 1975). Bile from the liver drains via duct system into the duodenum (Cyril and Eric, 1964; Leeson and Leeson, 1976).

The portal vein and hepatic artery enter and hepatic ducts leave the liver at the *porta hepatis* which is a transverse fissure on the inferior surface. The entire surface of the liver is covered by a fibro-connective tissue capsule called capsule of

Glisson from which thin connective tissue septa enter the substance of the liver at the *porta hepatis* to divide it into lobes and lobules (Cyril and Eric, 1964; Leeson and Leeson, 1976; Reith and Ross, 1977). The capsule is covered by a peritoneum except in an area making direct contact with the diaphragm and viscera of the posterior abdominal wall called the `Bare area' of the liver (Leeson and Leeson, 1976).

The liver is seen under low magnification of the microscope as a mass of epithelial cells arranged as branching plates which form a three dimensional lattice (Leeson and Leeson, 1976; Miller, et al., 1977).

Leeson and Leeson, (1976) have recorded that between these plates are sinusoidal blood spaces and portal areas or portal canals and within these portal areas are found; branches of portal vein, the hepatic artery, bile or hepatic duct, lymphatic vessels and little connective tissue. They showed that the portal area are arranged to separate lobules of liver tissue and these lobules are called hepatic or classical lobules. The lobules are made up of ramifying columns of hepatic cells of which the cell outlines are often indistinct so that the columns form a syncytium (Cyril and Eric, 1964). Within each lobule are found many portal canals at its periphery, central vein in the centre from which radiates plates of parenchymal cells (Leeson and Leeson, 1976; Reith and Ross 1977). The portal vein and the hepatic artery found at the periphery form the afferent vessels through the sinusoidal channels between plates of liver cells (Leeson and Leeson, 1976; Copenhaver and Pehaneer, 1971).

On higher magnification, each plate of liver parenchyma is made up of one or two rows of liver cells. Between the rows are found bile canaliculi which drain to

the bile ducts. The canaliculi are spaces between adjacent liver cells and have no epithelium. The sinusoidal spaces between liver plates are lined by reticuloendothelial (Kupffer) cells in the meshwork of reticular fibres (Leeson and Leeson, 1976; Copenhaver and Pehaneer, 1971). Within a liver lobule are found two types of cells namely parenchymal or hepatic cells found on the walls of sinusoids and blood cells in the lumen of the sinusoids. The functional unit of the liver is the liver acinus while the structural unit of the liver is the hepatic lobule (Copenhaver and Pehaneer, 1971; Leeson and Leeson, 1976; Reith and Ross, 1977).

2.7. THE HISTOLOGY OF THE KIDNEY

The kidneys are a pair of bean-shaped organs situated in the posterior part of the upper abdomen one on each side of the upper lumbar vertebrae in man while it lies in the lumbar area to the right and left of the median plane in other mammalian species. Each kidney is enclosed in a thin fibrous connective tissue capsule which may be stripped easily (Leeson and Leeson, 1976; Miller, et al., 1977). There is a depression on the medial aspect called the hilum through which the blood vessels enter and leave, while the ureter leaves the kidney (Leeson and Leeson, 1976; Miller, et al., 1977). The upper part of the ureter is expanded forming the pelvis which is subdivided into the major and minor calyces of which each minor calyx envelops a conical protrusion of renal substance called the renal papilla perforated by collecting ducts (Copenhaver and Pehaneer, 1971; Leeson and Leeson, 1976; Reith and Ross, 1977). Vertical hemisection of the kidney shows that each papilla is the lip of a pyramidal area extending from the hilum towards the capsule. This area is

called the medullary pyramid which contains straight tubules and parallel blood vessels. The medullary materials which extend into the cortex form the medullary rays. The medula can be divided grossly into inner and outer zones. Between adjacent medullary pyramids, cortical materials extend between the pyramids to separate them and form the renal columns (Leeson and Leeson, 1976; Reith and Ross, 1977). The granular appearance of the cortex is due to the presence of spherical bodies called the renal corpuscles and convoluted uriniferous tubules (Cyril and Eric, 1964; Leeson and Leeson, 1976).

Each pyramid with its associated cortex is called a lobe and hence the kidney is multilobar in man while unilobar in rat and rabbit (Leeson and Leeson, 1976). While Cyril and Eric, (1964) showed that the functional unit of the kidney is the nephron which consists of Bowman's capsule and the renal tubule proper, Leeson and Leeson (1976) showed that a lobule of the kidney is a smaller functional unit comprising a medullary ray, the kidney units or the nephrons which drain into it and the continuation of the ray in a medullary pyramid. Each human kidney contains one million nephrons which drain via the collecting tubules into the renal pelvis and hence into the ureter (Cyril and Eric, 1964 and Guyton, 1986). The nephron is a long, epithelial lined tube which starts blindly and terminates by joining an excretory duct but the nephron are so tortuous and intermingled that histological section of the kidney give no clear idea (Leeson and Leeson, 1976; Reith and Ross, 1977).

The Bowman's capsule is the terminal dilatation of each uriniferous tubule and contains a tuft of capillary vessels, the glomerulus. The Bowman's capsule and the glomerulus together constitute the malpighian or renal corpuscle (Cyril and Eric,

1964; Ganong, 1983; Guyton, 1986).

The glomerulus contains a simple squamous epithelium called glomerular endothelial cells of which the major part of the cells is a thin layer of cytoplasm which forms the lining of the capillary while the nucleus bulges into the capillary lumen (Cyril and Eric, 1964; Leeson and Leeson, 1976). The epithelial cells which form the visceral layer of Bowman's capsule are separated from the capillary membrane by a basement membrane which is the only intact membrane in the filtering surface of the glomerulus (Cyril and Eric, 1964; Leeson and Leeson, 1976; Guyton, 1986). The epithelial cell layer does not provide a continous covering for the basement membrane and capillaries, being connected with the basement membrane only by a series of processes called pedicels. These epithelial cells are called podocytes (Cyril and Eric, 1964; Leeson and Leeson, 1976; Reith and Ross, 1977; Dellman and Brown, 1981).

CHAPTER THREE

METHODOLOGY

3.1. THERMOXIDATION OF PALM OIL

MATERIALS:

Fresh palm oil, stainless steel pot, cooking stove, thermometer and plastic containers.

METHODS:

Hundred litres of Fresh palm oil was purchased from an accredited distributor of palm oil in Samaru Market. The oil was divided into two parts each containing fifty litres. One part was thermoxidized while the other part was given to the animals in the fresh form.

The method of thermoxidation involved heating the oil in a stainless steel pot over a cooking stove intermittently for five rounds of heating and opening the pot to allow for more air into the pot. Each round lasted for twenty minutes. No condiments were added to the oil sample. The heating temperature of the oil was between 200 - 220°C. Both forms of oil were stored in black, air-tight containers and kept in the refrigerator to prevent further oxidation of the oil component according to the recommendations of Isong, (1988) and Osim, et al., (1992). The temperature was measured using the 45mm Immersion Quickfit MF31/0/360 range thermometer made by ZECOL of England.

3.2. PREPARATION OF THE EXPERIMENTAL DIETS

MATERIALS:

Commercial animal feed, fresh and thermoxidized palm oil, weighing balance and mixing container.

METHOD:

The commercial animal feed was bought from a local dealer of animal feed in Samaru. The animal feed was the grower's mash brand of the Pfizer Livestock feeds Plc Kaduna and the composition as appeared on each 25kg bag is shown in Appendix A.

During the preparation of the diets, 15gm of the fresh palm oil was mixed in a mixing container with 85gm of the animal feed to make up 15% fresh oil level diet. Also 15gm of the thermoxidized oil was mixed with 85gm of the animal feed to make up 15% thermoxidized oil level diet. The two diets were each mixed thoroughly to allow homogeneity. The diet with 15% thermoxidized oil level was given as the first test diet, group A while the one with 15% fresh oil level was given as the second test diet, group B. The control group were given the normal rat ration, group C.

3.3. ANIMAL EXPERIMENTS

The animals used in this study were the Albino rats of Wistar breed bought from the animal house of the Department of Pharmacology of the Ahmadu Bello University, Zaria. These rats, thirty in number were young growing rats between the ages of eight and ten weeks. The rats made up of 15 males and 15 females, weighing between 100 and 140 gms were kept in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria.

The animals were grouped into three groups of ten rats made up of five males and five females in each group. The animals in the first group (A) were used as the first test animals and was placed on diet mixed with thermoxidized palm oil at 15% level. The second group of animals (B) were given diet mixed with 15% level of fresh palm oil. The third group of animals were used as the primary control group (C) and were placed on normal ration. The rats were fed with these diets for a period of thirty-two weeks. The rats were weighed with a beam balance. The initial weight was recorded before the commencement of feeding on the first day. The rats were fed with the normal feed for one week duration before the actual feeding with the experimental diets in order to normalise their feeding habit. These rats were subsequently weighed at one week interval throughout the period of feeding. The average food consumption was also recorded daily. The Beam balance for all the measurements was the Mettler P4400, Delta Range.

Perspex cage with stainless steel, mesh floor and top for facilitated cross-ventilation and measuring 45x28x12cm was to house each group of five rats of the same sex. Each group of ten rats were separated into 5 males and 5 females. This separation was done to discourage breeding which will affect the physiology of the test animals. Plastic water bottles with stainless steel nozzles were used to provide water. The food was provided in porcelain bowls to reduce spillage. The cages were cleaned daily and washed weekly.

3.4. SAMPLING OF TISSUES FROM RATS

The animals were anaesthetized at the end of the feeding period by making them inhale a reasonable amount of chloroform until they were unconscious. Their

abdomen was opened by a midline incision and part of the small intestine, the liver and the kidney of all the three groups were excised and placed in cold normal saline until excision of all the tissues were completed. The idea being to normalize the temperature of the tissues before they are introduced into the fixatives.

3.5. PREPARATION OF TISSUES FOR MICROSCOPIC EXAMINATION

MATERIALS:

Normal saline, 10% Neutral formalin, 15% Formalin, Bouins fluid, Absolute alcohol, 95% Alcohol, 70% alcohol, Xylene, Paraffin wax, Egg albumen, Oven, Microtome, Cryostat, Slides and Microscope.

METHOD:

The process of preparation of various tissues and organs for histological examinations has been differentiated into a number of stages. These stages include Fixation, Tissue processing, Sectioning, Staining and Photomicrography. This permanent tissue preparations were made using routine and special techniques as outlined by Gurr, (1962); Culling, (1963); Drury and Wallington, (1973); Bradbury, (1977).

3.5.1. FIXATION

The excised tissues; the small intestine, the liver and the kidney were trimmed and washed with the normal saline solution and immediately transferred to the fixatives. The fixatives were the 10% Neutral formalin, 15% formalin and Bouins fluid. These solutions were contained in a small air-tight bottles. This was done separately for each of the tissues from the three groups.

3.5.2. TISSUE PROCESSING

Both manual and automatic tissue processing techniques were used. The manual processing techniques used are as outlined by Gurr, (1962); Culling, (1963); Drury and Wallington, (1973); Bradbury, (1977). Some of the tissues were processed using the Histokinette bench model automatic tissue processing machine obtained from the Department of Human Anatomy, Ahmadu Bello University, Zaria.

3.5.3. SECTIONING AND MOUNTING

Sections were cut using the Rotary Microtome and the sizes were between 5 - 8 microns. The cut sections were floated on hot water bath and were picked on clean slides precoated with egg albumen for staining.

3.5.4. STAINING

Different staining techniques were employed for both general or routine staining and special staining. The general or routine staining techniques used were, the Haematoxylin and Eosin (H and E) method and the Haematoxylin and Light Green method called Rafferty's stain. They were used to demonstrate the general structures of the tissues.

The special staining techniques used were the Periodic Acid Schiff (PAS) method for glycogen and the Highman's Congo Red method for tissue fat deposits. These methods were used as outlined by Gurr, (1962); Culling, (1963); Drury and Wallington, (1973); Bradbury, (1977).

3.6. MORPHOMETRIC ANALYSIS OF SMALL INTESTINE

Sixteen villi from each of the 3 groups A, B and C animals were selected at random. The length of the villi, the mucosal length and the stromal dimensions were

measured using a Leitz Dialux Microscope at the magnification of x 100 and both stage and eye-piece micrometers, according to the specification of Culling, (1963); Abbas, et al., (1989).

Estimates of the Surface area of Villi over the Surface area of the Primary Mucosa; S(V)/S(PM) obtained from each group of animals were used to calculate group means and their coefficients of variation according to Mayhew, (1988) through which the surface amplification due to villi was determined for each group of animals.

3.7. STATISTICAL ANALYSIS

The growth rate, rate of food consumption and Amplification factors from the three groups of animals were compared using their mean and standard deviation (SD). The student's-t-test was used for testing the level of significance. A, P-value less than 0.05 was considered significant. One-Way Analysis of Variance (ANOVA) was used for comparing the means, while the Multiple Range test was used to find the Least Significant Difference (LSD) between the groups, according to Campbell, (1967).

CHAPTER FOUR

4.0. RESULTS

4.1. GENERAL OBSERVATIONS

At the end of the feeding period, there was marked loss of hairs in animals in groups A and B, with those of group A more prominent while the remaining hairs was scanty and clumped, as shown in Plates 1, 2 and 3. Also observed was increased sensitivity to noise and increased nervousness of animals in group A than those in group B while none of the above observations were recorded for animals in group C. Also observed were yellow pigmentations on the skin of animals of the two test groups.

4.2. GROWTH RATE

The effect of thermoxidized and fresh palm oil supplemented diets on the mean change in body weight in the three groups of animals is shown in figures 1 and 2.

The results in fig. 1, show the mean change in weight of rats following the administration of the three diets. The group fed on control diet (Group C), had a mean change in body weight greater than those fed on fresh palm oil diet (Group B) which in turn had a mean change in body weight greater than those fed on thermoxidized palm oil diet (Group A). The mean change in body weight are 52.7 ± 17.6, 65.7 ± 23.1 and 88.6 ± 32.0 for groups A, B and C respectively. While the results in fig. 2, show the mean change in body weight of each rat throughout the feeding period. The rats on control diet (Group C) had a mean change in body

weight greater than the two test groups, (B and A). The mean change in body weight are 52.7 ± 15.7, 65.0 ± 19.3 and 88.6 ± 22.3 for groups A, B and C respectively.

The mean change in body weight of the control group was significantly greater than those of the fresh palm oil group ($P<0.05$) and the thermoxidized palm oil group ($P<0.05$). The mean change in body weight of the two test groups were not significantly different. At the beginning of the feeding experiments, the body weight of rats in the three group were not significantly different from each other.

4.3. DAILY FOOD CONSUMPTION

The mean weight of food consumed per group are shown in fig. 3. The result showed that the total food consumed by the animals in group C was more than that consumed by animals of group B which in turn was more than that consumed by group A animals. Rats on thermally oxidized palm oil diet (Group A), consumed their food at the rate of 9.39 ± 0.91 gm per rat daily. Rats fed on fresh palm oil diet consumed their food at the rate of 10.52 ± 1.33 gm per rat daily, while rats on control diet consumed their food at the rate of 13.27 ± 0.86 per rat daily. The rate of consumption of food in group C was significantly different from groups B and A ($P<0.05$). While the rate of food consumption between groups B and A was significantly different ($P<0.05$).

4.4. SMALL INTESTINAL MORPHOMETRY

The surface amplification factors due to jejunal villi estimated by transverse sectioning approach for the three groups are shown in table 4.1 and fig. 4. The result showed that the surface amplification factors are 3.59 ± 0.42, 3.86 ± 0.62 and 5.50 ±

0.60 for groups A, B and C respectively. This means that the jejunal villi amplify the mucosal surface three to four times, three to four times and five to six times for groups A, B and C animals respectively. The amplification factor differs significantly between the control and the test groups ($P<0.05$) while the difference was not significant between the test groups.

The result of the effect of treatments on the villous length, crypt length and stromal dimension are shown in table 4.1. The villous length are 382.5 ± 44.5 um, 442.5 ± 65.27 um and 487.5 ± 38.75 um for groups A, B and C respectively while crypt height are 136.88 ± 18.87 um, 148.13 ± 25.62 um and 114.38 ± 16.32 um for groups A, B and C respectively. The stromal cross section for group A, B and C are 82.5 ± 30 um, 101.25 ± 41.17 um and 79.69 ± 40.52 um respectively.

The length of the villi differs significantly between the three groups ($P<0.05$). The difference in the crypt height was significant between groups C and A, and C and B ($P<0.05$), while the difference between groups A and B was not significant. The difference between the stromal cross-section of the three groups was not significant.

4.5. MICROSCOPIC EXAMINATION OF TISSUES:

4.5.1. THE SMALL INTESTINE

The result of the microscopic examination showed the jejunal mucosa with normal, tall slender villi and stroma in group C animals as shown in plate 4. The stroma and the villi were enlarged in the jejunum of group B animals while the villi were reduced in height as shown in plate 5. The jejunal mucosa and villi of group A animals were wearing away and the villi reduced in height as in plate 6, which

showed the sloughing of the apical part of the villi and the wearing away of the villi of group A.

Plate 7, showed submucosa of group C animals showing normal submucosa between the muscular layer and the mucous layer. The submucosa of group B animals were highly enlarged as in plate 8 while the submucosa of group A animals were enlarged and some parts became eroded as in plate 9.

4.5.2. **THE LIVER**

The microscopic examination of the liver showed the normal liver architecture with normal cord arrangement of cells from group C animals as shown in plates 10 and 11. Animals in group B, showed fatty vacuoles in some hepatocytes as in plate 12, while some in addition to fatty vacuoles contained area of necrosis as shown in plate 13.

The group A animals showed degenerative fatty infiltrations in form of fat vacuoles in some hepatocytes as in plate 14 while plate 15, showed fat vacuoles with focal areas of necrosis. Some liver tissues from both group A and B animals, showed both univacoular and multivacuolar fatty infiltrated hepatocytes, as shown in plate 16 for group B animals and plate 17 for group A animals.

Also present were enlarged hydropic cells in group A as shown in plate 18. The liver of the test animals showed areas of diffuse nonsuppurative necrosis of the hepatocytes which were more severe in group A animals than group B animals as shown in plates 19 and 20, while in addition to that, the liver of group A animals showed deposits of bile pigments as shown in plate 21. The blood vessels of the test

animals were congested as shown in plates 22 for group B animals and 23 for group A animals.

4.5.3. **THE KIDNEY**

The microscopic examination of the kidney showed normal kidney glomerulus for control animals (Group C) as in plate 24 while plate 25 showed normal renal tubules as shown from group C animals. The kidney of the test animals (group B and A), showed proliferated lacis cells (Polkissen) lying between the macula densa and glomerular hilum as shown in plate 26 and 27 respectively. This also showed the proliferated lacis cells, renal deposit in the distal tubule and sloughing of the epithelium into the lumen of the tubules of both groups B and A animals.

Plate 28 shows renal tubular deposits, seen as proteinacious materials in group A animals while plate 29 shows that of group B animals. Also present in the test group (A) are congested blood vessel as in plate 30.

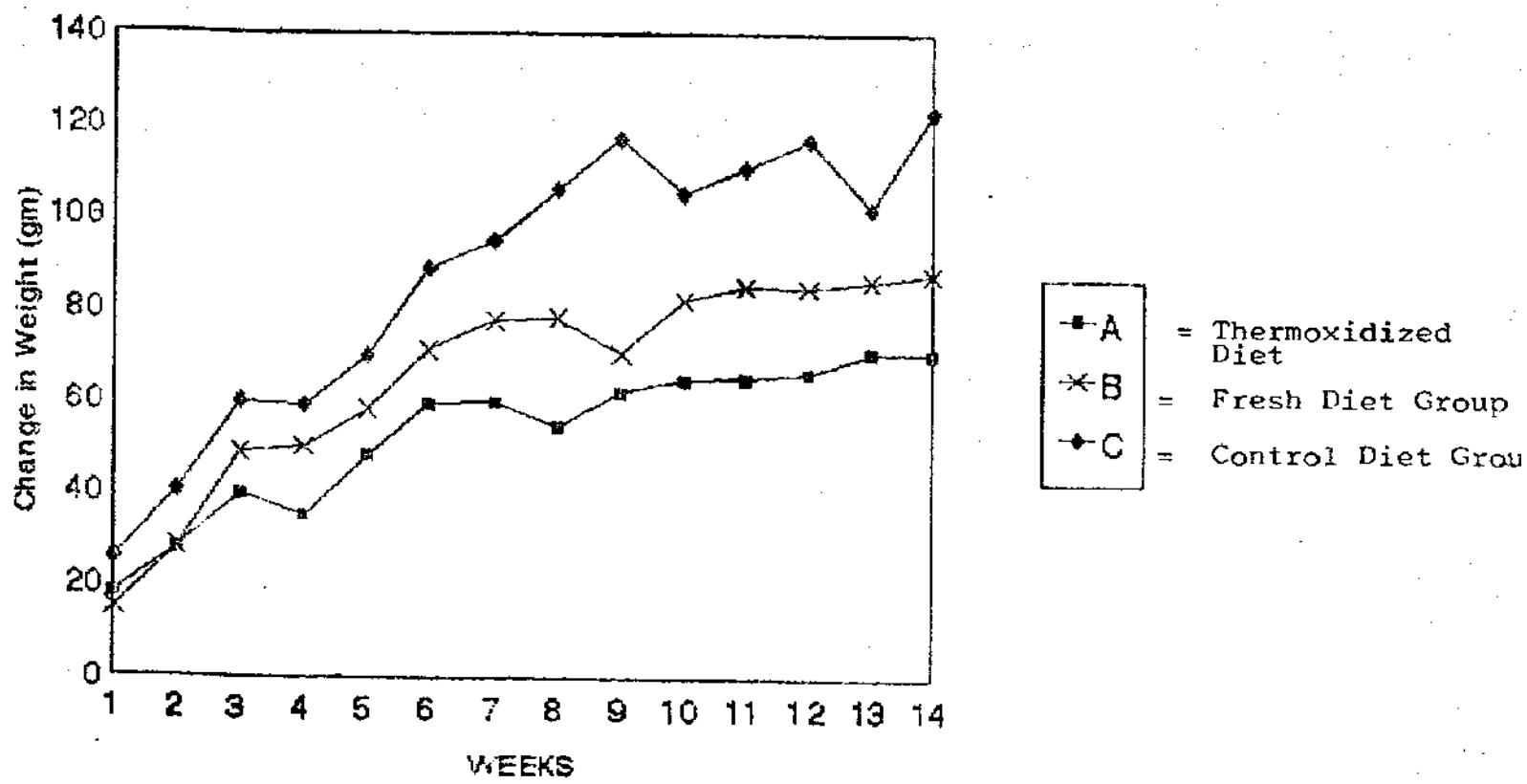


Fig. 1: CHANGE IN WEIGHT FOLLOWING CHRONIC CONSUMPTION OF THERMOXIDIZED, FRESH AND CONTROL DIETS

A = Thermoxidized Palm Oil Diet Group

B = Fresh Palm Oil Diet Group

C = Control Diet Group

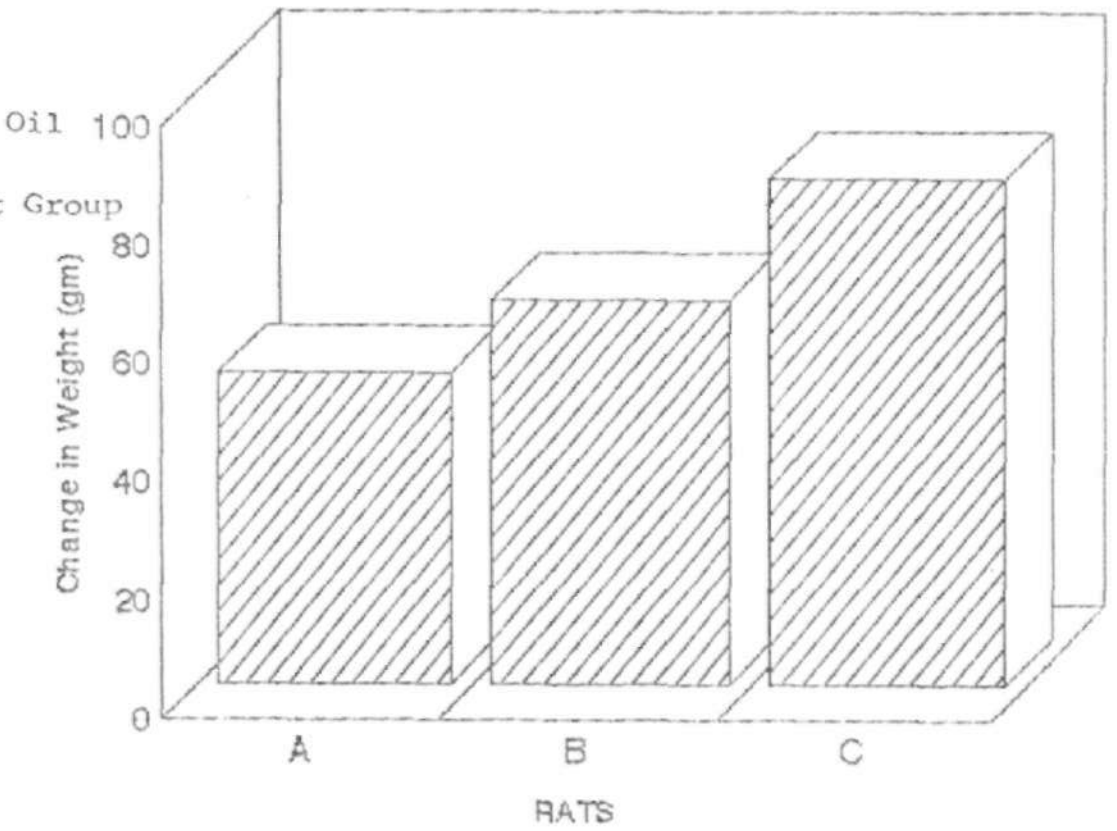


Fig. 2: HISTOGRAM OF MEAN CHANGE IN WEIGHT PER RAT

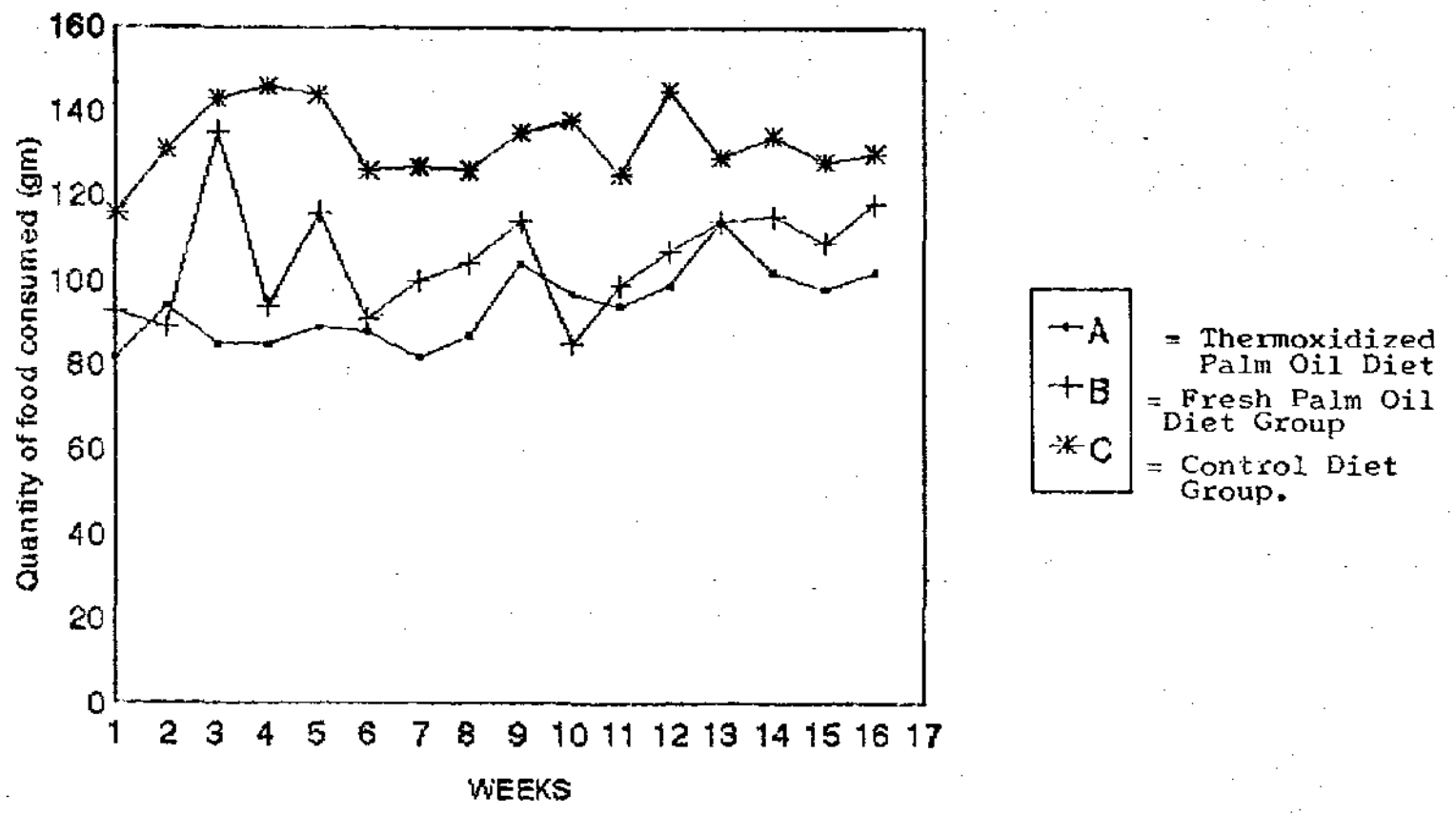


Fig. 3: RATES OF FOOD CONSUMPTION IN THE THREE GROUPS, A, B AND C.

A = Thermoxidized Palm Oil Diet Group

B = Fresh Palm Oil Diet Group

C = Control Diet Group

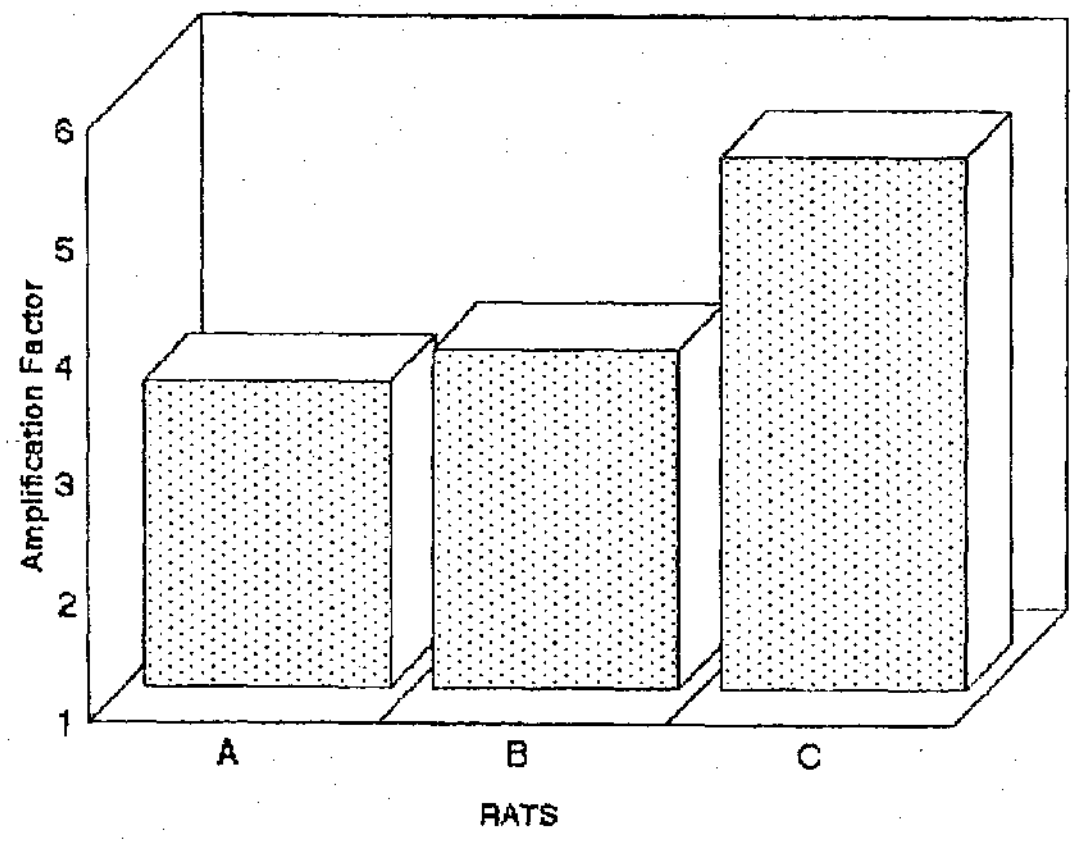


Fig. 4: SURFACE AMPLIFICATION FACTOR DUE TO THE VILLI IN GROUPS A, B AND C RATS.

Table 4.1. Small Intestinal Mucosal Measurements (Mean, S.D. and Range) of the Thermoxidized Palm Oil Diet (Group A) Fresh Palm Oil Diet (Group B) and Control Diet (Group C) (n = 16).

Variable.	Group A	Group B	Group C
1. Villous Length (um)	382.50	442.50	487.50
(Mean)			
S. D.	± 44.50	± 65.27	± 38.75
Range	(300-450)	(300 - 540)	(420 - 570)
2. Crypt Length (um)	136.88	148.13	114.38
(Mean)			
S. D.	± 18.87	± 25.62	± 16.32
Range	(120 - 180)	(120 - 180)	(90 - 150)
Total Mucosal Height (um)	519.38	590.63	601.88
3. Stromal Dimension (um)	82.50	101.25	79.69
(Mean)			
S. D.	± 30.00	± 41.17	± 40.52
Range	(60 - 150)	(30 - 180)	(30 - 180)
4. Amplification Factor	57.45	61.78	87.98
(Mean)	3.59	3.86	5.50
S. D.	0.42	0.62	0.60
Range	(3 - 4)	(3 - 4)	(5 - 6)



Plate 1: Photograph of rat fed thermoxidized palm oil diet (group A); with focal areas of Alopecia (A).



Plate 2: Photograph of rat fed fresh palm oil diet (Group B), with hair losses and scanty hairs.



Plate 3: Photograph of rat fed control diet (group C) with normal appearance of the hairs.

4.5. MICROSCOPIC EXAMINATION OF TISSUES

4.5.1. THE SMALL INTESTINE

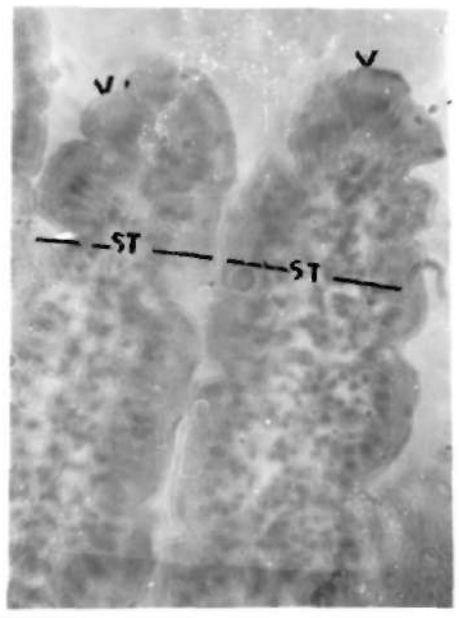
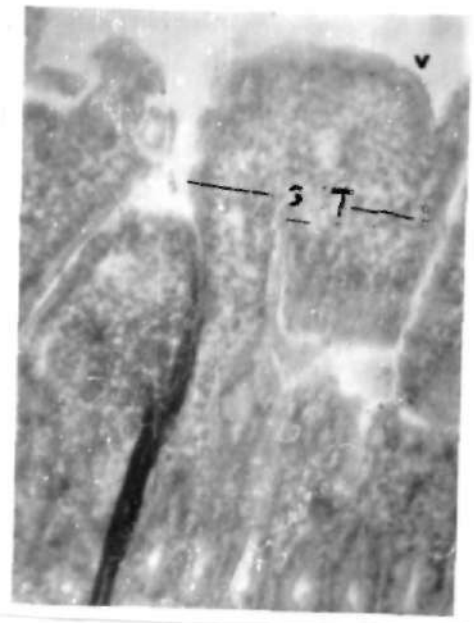


Plate 4: A section of jejunal mucosa from the control animals, group C. Showing normal tall slender villi (v) occupying most of the total mucosal height. The stromal area (ST) is also shown to be normal. H and E x 100.

Plate 5: A section of jejunal mucosa from group B, animals fed fresh palm oil diet, showing (v) enlarged villi which were also observed to be reduced in height. The stroma (ST) shown to be enlarged. H and E x 100.



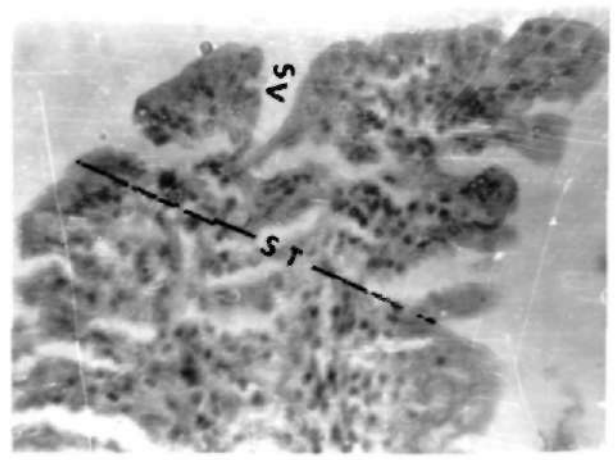


Plate 6: Section of the jejunal villous of group A, animals fed thermoxidized palm oil diet, showing sloughing of the apical parts of the villus (SV) and wearing away of the villus. The stroma (ST) is enlarged. H and E x 250.

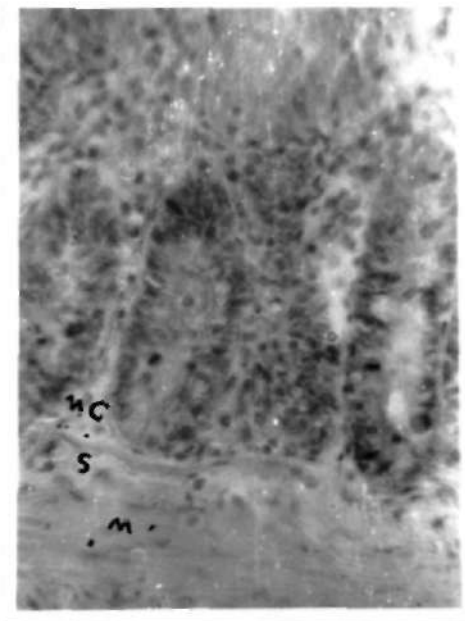


Plate 7: Section of the jejunal submucosa (s) of Group C, control animals, showing normal submucosa between the muscular (m) and the mucous (mc) coats. H and E x 250.

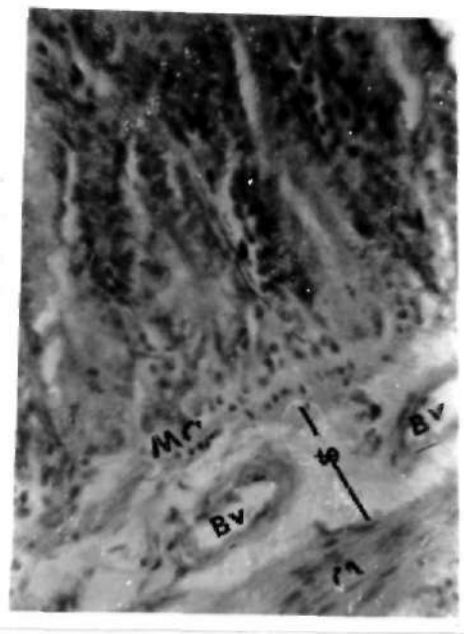


Plate 8: Submucosa of the jejunum of Group B, showing highly enlarged submucosa (s), containing blood vessels (BV). H and E x 250.

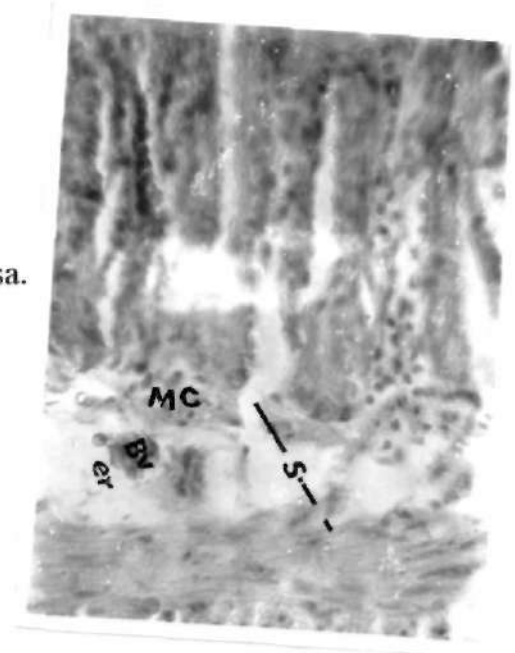


Plate 9: Submucosa of the jejunum of Group A, showing enlarged submucosa (s) and erosion (er) of some part of the submucosa. H and E x 250.

4.5.2. **THE LIVER**

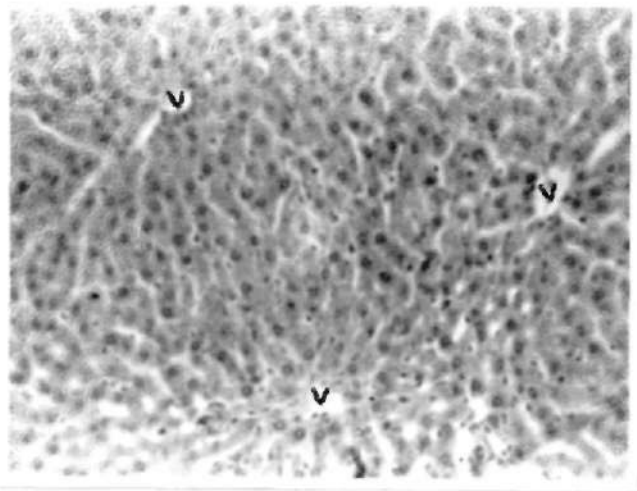
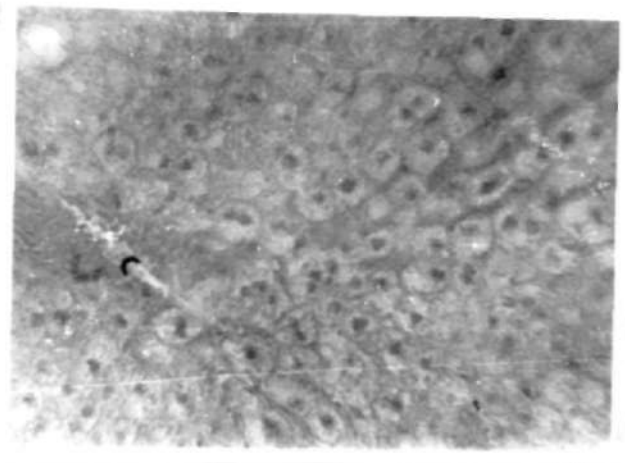


Plate 10: Normal liver architecture, showing radiations of the cord from the central vein (v) group C. Light Green x 100.

Plate 11: Higher mag. of liver from group C, Control animals, showing normal hepatocytes around centrolobular area (c). The cellular components are highly PAS positive indicating high glycogen storage. PAS x 250.



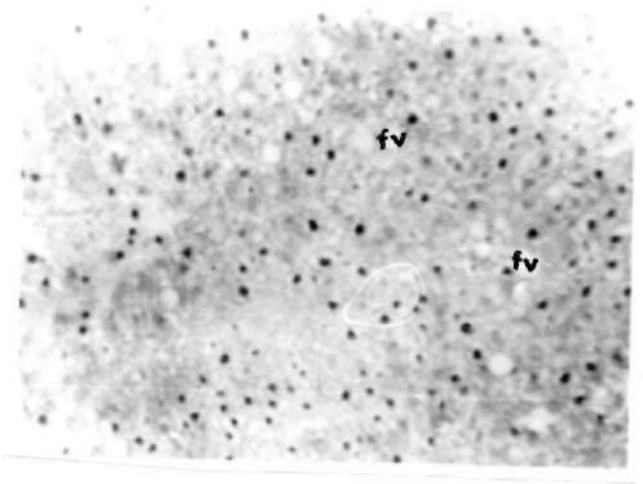


Plate 12: Liver of group B, showing fat vacuoles in some of the hepatocytes (fv). L/G x 100.

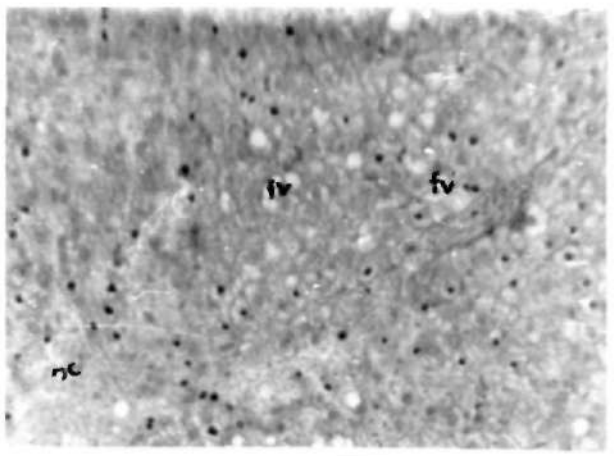


Plate 13: Liver of group B, showing fat vacoules (fv) and small area of necrosis (nc). H and E x 100.

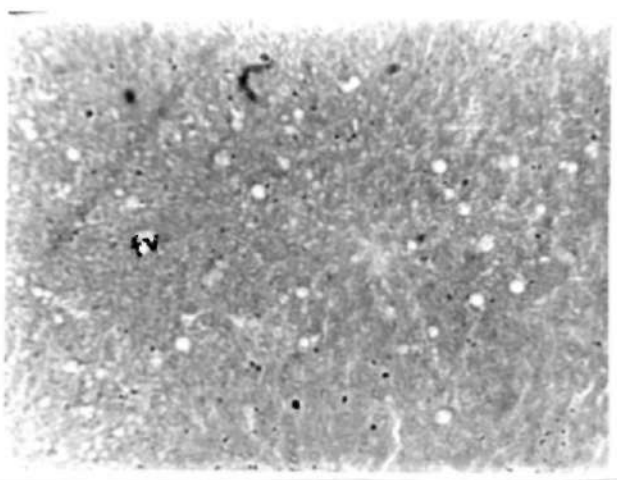


Plate 14: Liver of group A with numerous scattered fat vacoules (fv), which are PAS negative indicating decreased glycogen concentration in the cells. PAS x 100.

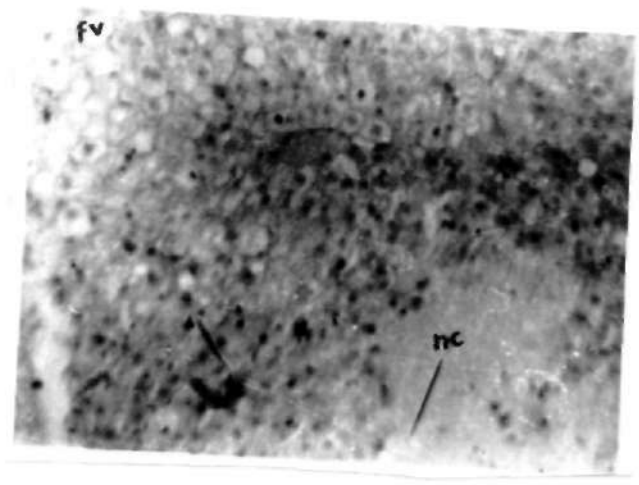


Plate 15: Liver of group A with numerous fat vacoules (fv) in some hepatocytes and small area of cell necrosis (c). Congo red x 100.

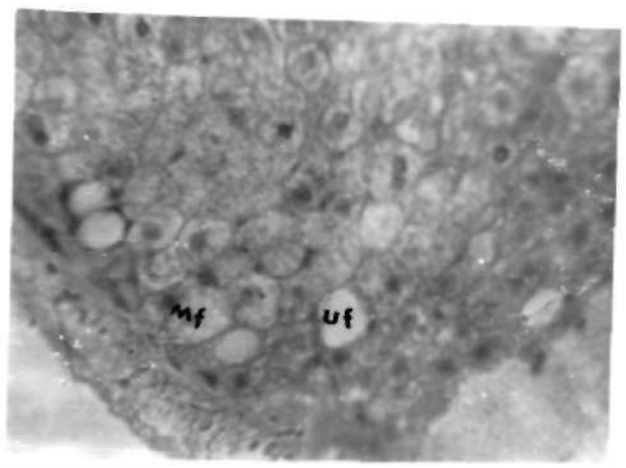


Plate 16: Liver from group B showing enlarged univacoular fatty infiltration (uf) and some multivacoular fatty infiltration (mf) in some hepatocytes, with few PAS positive materials in the cells indicating reduced glycogen concentration in the cells. PAS x 250.

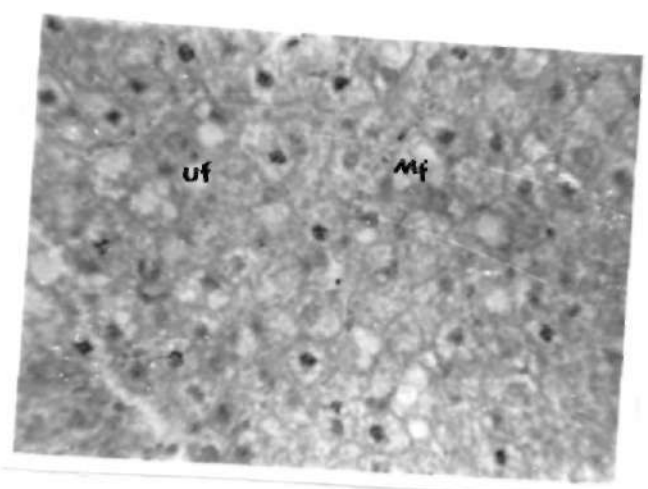


Plate 17: Liver of group A showing multivacoular fatty degeneration (mf) and few univacoular fatty (uf) cells. H and E x 250.

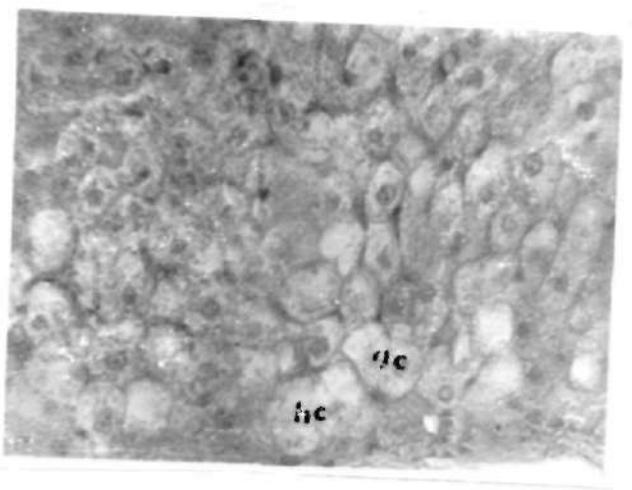


Plate 18: Liver of group A animals showing enlarged hydropic hepatocytes (hc), with few PAS positive materials indicating reduced glycogen storage and degeneration of cells. PAS x 250.

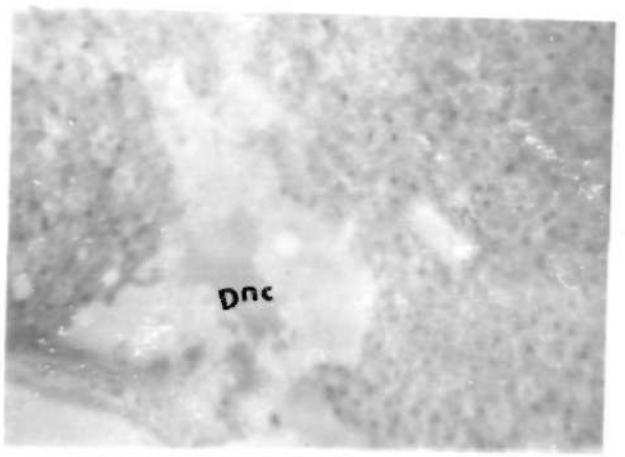


Plate 19: Liver of group A, showing area of diffuse nonsupurative necrosis (Dnc) of the hepatocytes H and E x 100.

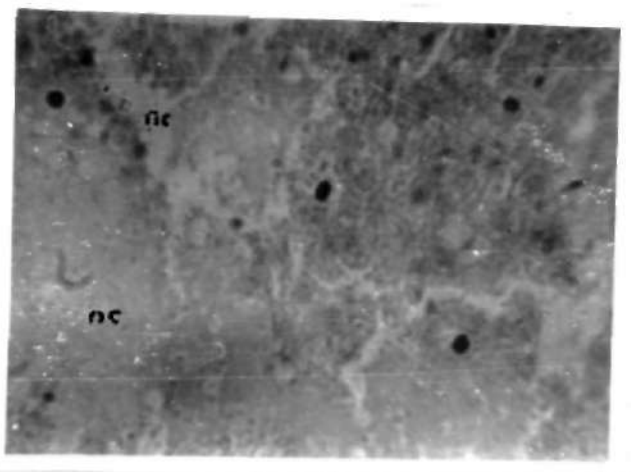


Plate 20: Liver of group B showing area of necrosis of the hepatocytes (*nc*). H and E x 100.

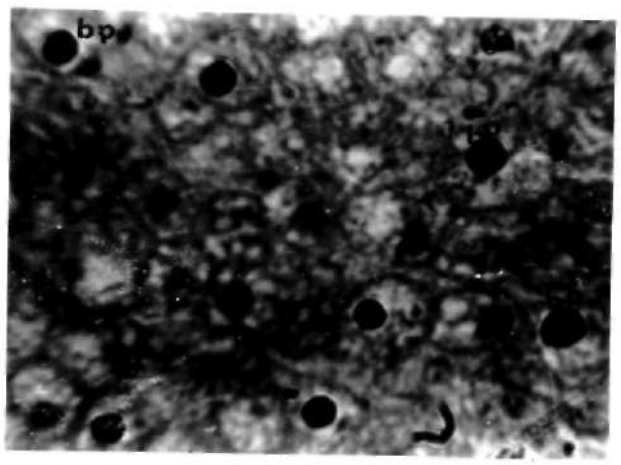
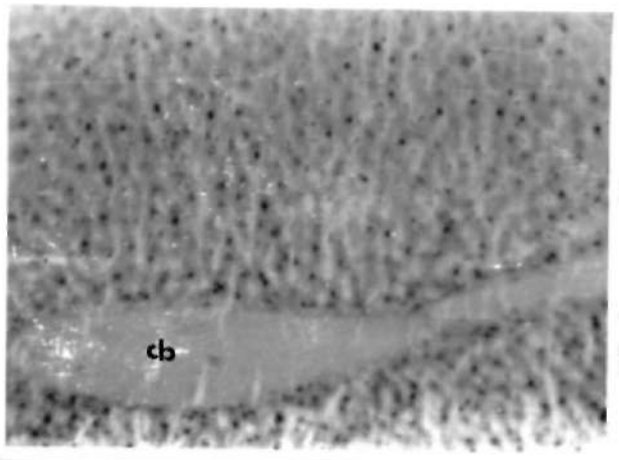


Plate 21: Liver of group A showing deposits of bile pigments (bp) in the tissue. Congo-red x 400.

Plate 22: Liver of group B showing congested blood vessel (cb). H. and E x 100.



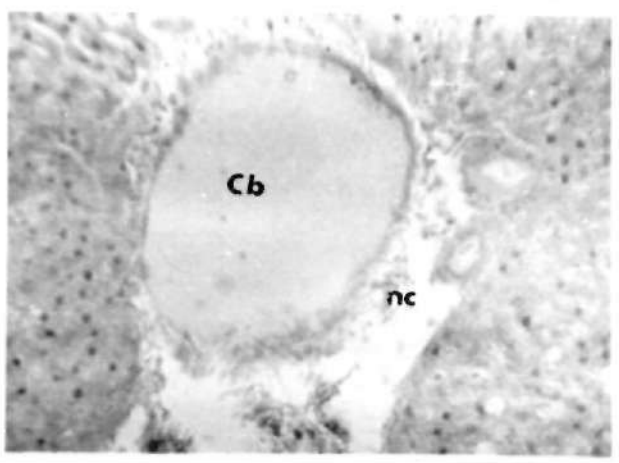


Plate 23: Liver of group A showing congested blood vessel (cb) and necrosis (nc) around blood vessel. H and E x 100.

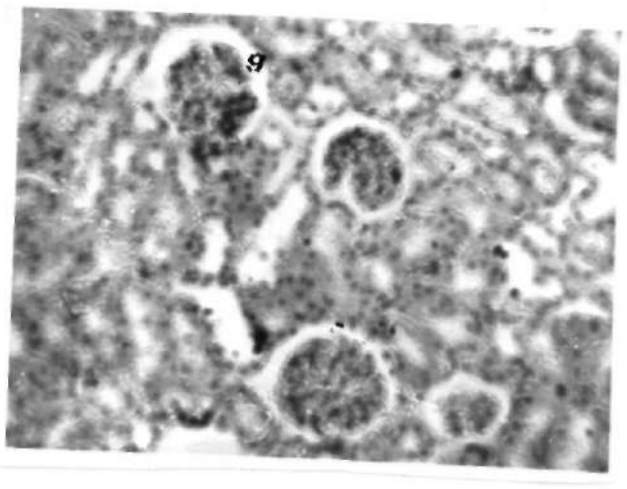


Plate 24: Kidney of group C, showing normal glomerulus (g). H and E x 100.

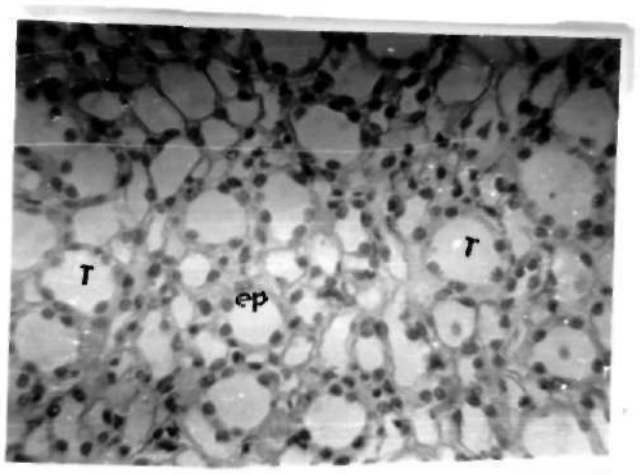


Plate 25: Kidney tubules of group C, showing normal orientation of the tubules (T) and epithelium (ep) H and E x 100.

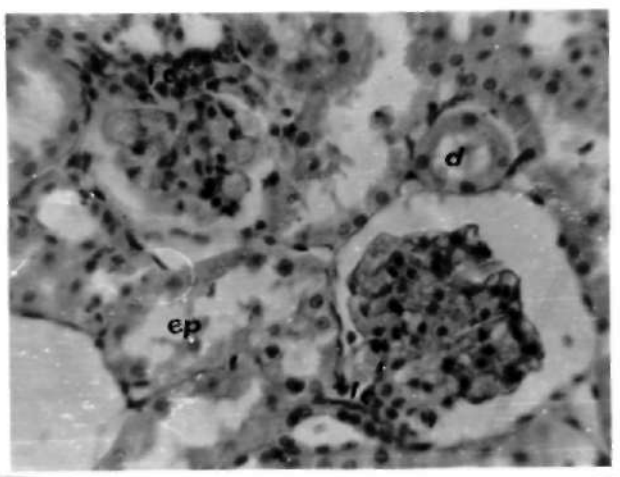
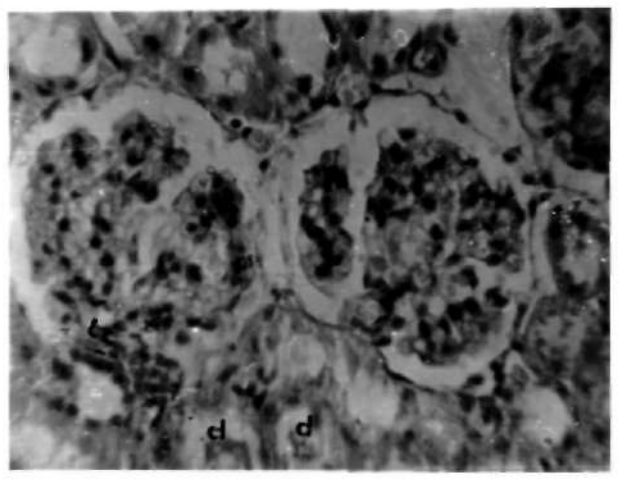


Plate 26: Kidney of group B, showing the proliferated lacis (Polkissen) cells (lc) and proteinacious deposit (d) in the distal tubule and a sloughing of epith (ep) into the lumens. PAS x 250.

Plate 27: Kidney of group A, showing the proliferated lacis (Polkissen) cells (lc) and tubular deposit (d) in the lumen of distal tubules. PAS x 250.



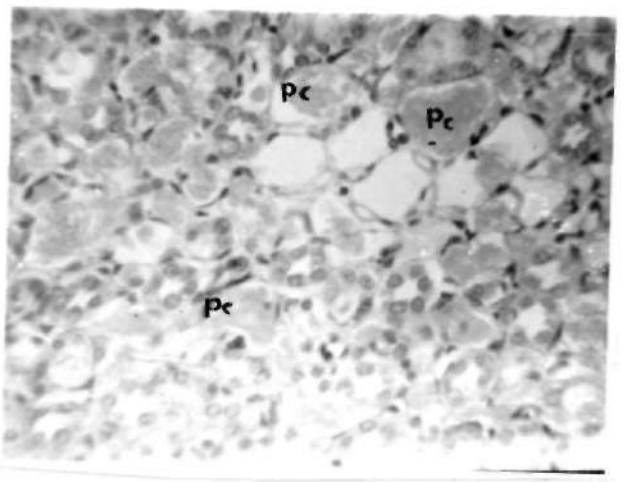
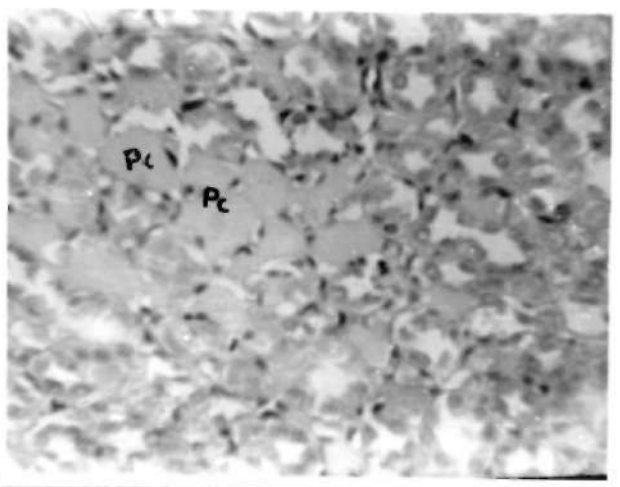


Plate 28: Kidney tubules of group A, showing deposit of protenacious materials (pc) in the lumen of some proximal and distal tubules. H and E x 250.

Plate 29: Kidney tubules of group B, showing protenacious materials (cast) (pc) in the lumen of some proximal and distal tubules H and E x 100.



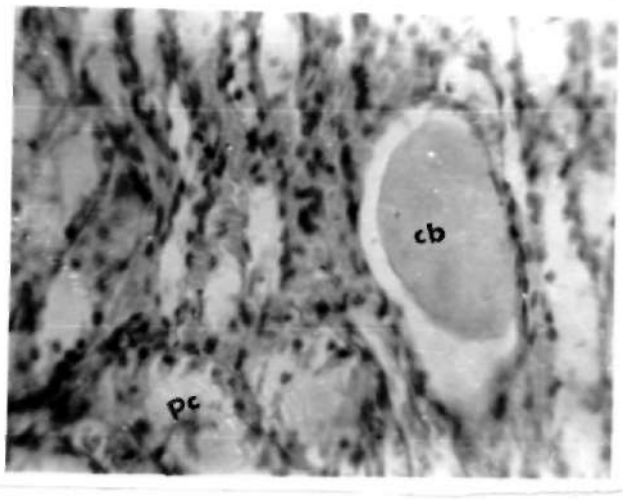


Plate 30: Kidney of group A, showing congested blood vessels (cb) and deposit of materials (pc) in the lumen of a tubule. Congo-Red x 250.

CHAPTER FIVE

5.0. DISCUSSION

The marked loss of hairs on rats in the thermoxidized oil diet (Group A) as well as the remaining hairs having a scanty and clumped appearance with yellow pigmentation could be due to the oxidative toxicity of the thermoxidized oil. Isong (1988) had confirmed the biotoxicity caused by peroxides present in thermally oxidized oils. This toxicity was demonstrated when concentrated amount of peroxides was administered orally in rats. These peroxides might have led to the formation of hydrocarbons, cyclic and polycyclic acids which, when hydroxylated became very reactive and destructive compounds in the body (Lake and Water-Worth, 1980; Frankel, 1980; Nolen, et al., 1967.

The rats fed with fresh palm oil diet showed lesser degree of pigmentation and loss of hairs than those fed with thermoxidized palm oil diet. This showed that the fresh palm oil could have undergone some measure of autoxidation which resulted in the formation of small quantities of cytotoxic materials. This explained the slight pigmentation of the group fed with fresh palm oil diet.

The hair losses as evidenced in the two test groups may indicate the effort of the body to conserve protein, because the lipid supplemented diets could have imposed a protein imbalance in the diet by dilution of the existing protein. The cytotoxic materials in the oil might have evoked an extra demand for dietary protein and even water for the excretion of the cytotoxic materials from the body (Shue, et al., 1968; Alleyne, et al., 1978; Isong, 1988; Lea, et al., 1964).

The ability of the control group to selectively absorb more protein than the

test groups was seen in their more rapid growth rate. However, the fresh palm oil diet group showed a greater increase in growth than the thermoxidized group for the same reason. That is, the fresh palm oil diet group were able to make use of more protein. The demand for protein by the test animals was to aid anabolism and tissue regeneration (Combe, 1980; Isong, 1988) which resulted in reduced body growth of groups A and B.

The reduced body growth could also be a direct result of non-palatability of the food due to the formation of flavour reversion compounds and decreased food consumption. The reduced weight or stunted growth of the animals was one of the most visible manifestation of peroxide toxicity (Kaunitz, et al., 1960). The effects of ingestion of whole oxidized oil ranges from loss of appetite, poor feed efficiency, reduced gut absorption, steatorrhaea, growth depression, enlarged livers, kidneys and lungs, fatty hepatic necrosis, haemolysis and calciferous myocardial lesions (Alexander, 1978; Gabriel, et al., 1977; Billek, et al., 1979).

The reduced quantity of food consumed and body growth could also be attributed to the inability of the small intestine of the test animals for effective nutrient absorption which might be caused by oxidative toxicity of the mucosa of the small intestine bearing the villi which form the main organ responsible for the absorption of the nutrients. Results obtained from photomicrographs of the small intestinal mucosa and villi of the tests and control rats support this. The mucosa, villi, stroma and submucosa of the test animals showed some changes in comparison to the control animals. The higher growth rate of the control group showed that their effective nutrient absorption are normal (Alleyne, et al., 1978) which was

followed by those of the fresh palm oil group because of small amount of cytotoxic materials generated by autoxidation. The thermoxidized palm oil group showed the least food consumption ability and retarded growth due to the accumulation of peroxides in the oil causing more damage to the mucosa, villi, stroma and the submucosa.

The inability of the test animals to effectively absorb more protein from the small intestine to meet increased demands was established by Alleyne, et al., (1978); Shue, et al., (1968); Alexander, (1978); Cutter and Haywood, (1974). They showed that the relative ability to absorb protein was defective and concluded that irreparable damage might have been inflicted upon the intestinal morphology of the test animals with those of the thermoxidized group being more pronounced.

Dietary lipids were only maximally absorbed by the test groups as was shown by Combe, et al., (1978) and Combe, et al., (1981), where they demonstrated the effective absorption of lipids and oxidative products in animals fed oxidized oils. This increased lipid absorption by the test animals could be collaborated by the increased size of the stroma of the villi of the small intestine while the length of the villi are reduced due to the sloughing or excoriation of the apical part caused by the cytotoxic agents in the oxidized oil.

Also the reduced quantity of food consumed and the reduced body growth of the test animals might be attributed to the reduction of surface amplifications of the intestinal mucosa due to the reduction in the length of the villi. The study showed decreased length of the villi in the two test groups which might result in decreased rate of absorption because of reduced surface area of the intestine. This finding is

in agreement with Mayhew, (1988) which showed that the villi are relatively fixed in number during life but the amplification factor changes during experimental fasting and diabetes.

It has been demonstrated that cyclic dihydroxyl esters, peroxides and cyclic fatty acids are present in oxidized oils (Artman, 1969). Thermoxidized oils contain more of these radicals than the fresh oils (Frankel, 1980). It has been shown that these compounds were toxic to tissues (Shue, et al., 1968; Alleyne, et al., 1978; Alexander, 1978; Isong, 1988). It was likely that the damage done to the intestinal mucosa, villi, stroma and submucosa by the test diets was due to these toxic products. These effects might also be associated with the influence of fat soluble vitamins which in group A animals might be deficient while group B animals might be having hypervitaminosis. It has been shown that hypervitaminosis A leads to skin pigmentation, generalized pruritus, changes in the horny structures of the skin and loss of hairs while deficiency of vitamin A led to stunted growth, pathological changes in the intestine and necrosis of the tips of the villi (Cyril and Eric, 1964; Guyton, 1986; Hills, 1976; Ganong, 1983). This is true because lipoxidation follows depletion of vitamin A and E (Formo, et al., 1979) while in gross peroxide accumulation, vitamin E is destroyed, membrane damage results and carcinogenic activity is triggered (Alexander, 1978; Giani, et al., 1985; Billek, et al., 1979).

The liver of the rats in groups A and B showed degenerative fat deposits in the form of fatty vacoules which may be as single vacoules termed univacoular fatty infiltration or numerous vacoules in a cells called multivacoular fatty infiltration. It has been shown that some agents like cyclohexim inhibit protein synthesis, thereby

producing fatty liver while others like orotic acid do not inhibit hepatic synthesis of protein but lead to fatty liver by interfering with egress of lipid (Zimmerman, 1982). Damage to the plasma membrane may contribute to the accumulation of fat in that in gross peroxide accumulation membrane damage results (Alexander, 1978). These degenerative fatty changes in the liver may not be unconnected with the cytotoxic agents in the test diets like the cyclic dihydroxyl esters, peroxides and cyclic fatty acids of which these radicals were more in the thermoxidized oils (Artman, 1969; Frankel, 1980; Shue, et al., 1968). These toxic radicals might have affected the plasma membrane which led to the interference with the movement of the very low density lipoprotein out of the cell and their effect on the synthesis of very low density lipoprotein might be responsible for the accumulation of lipid (Grundmann, 1980; Zimmerman, 1982). Also manifested were necrosis of liver cells and tissues in both test groups. This supports the finding of Isong (1988) that the toxicity of these oxidative products on the liver were more pronounced than any other organ. Cyril and Eric, (1964) have shown that the toxicity on the liver manifested mainly in fatty liver which showed diffuse hepatic fibrosis called Cirrhosis while gross chronic fatty liver led to death of many liver cells and their replacement by scar tissue. Zimmerman (1982) explained that the toxic agents led to injury to the plasma membrane which permitted intrahepatic accumulation of calcium ion. The high concentration of calcium ion in turn enhanced plasma membrane injury permitting even higher intracellular content of the ion which led to necrosis. The role of the injury to the plasma membrane was the pathogenesis of the necrosis. This led to rapid loss of intracellular electrolytes, enzymes and coenzymes and entry of

extracellular electrolytes. Thus the entry and intracellular accumulation of calcium ion have been considered by Ziemmerman, (1982) to be responsible for triggering of necrosis. It has also been shown that the inability to synthesize protein that have been destroyed by the toxic agent might conceivably contribute to necrogenesis (Grundmann, 1980; Zimmerman, 1982; Scheuer, 1973). The toxic agents in the oxidized oil group (A) might be responsible for the initiation of necrogenesis as well as the degenerative fatty infiltration present in the test groups. However, Grundmann (1980) has shown that in all the cases, the nature of cytotoxic agent is less influential on the causation of liver injuries than personal and individual susceptibility. Some hepatocytes showed hydropic changes, referred to toxic swelling of cells and this might be due to the abnormal storage of glycogen due to degradation or a considerable dilatation of the endoplasmic reticulum cisterns resembling the pattern found in chronic alcoholism (Grundmann, 1980). Plea (1975) has shown that carbon tetrachloride-induced peroxidation can potentiate hepatotoxicity, altered cellular membranes, and the permeability of mitochondria. Activities of membrane-bound proteins were altered thus resulting in uncoupling oxidative phosphorylation. Other effects noted include alterations in endoplasmic reticulum with the cisternae of the rough endoplasmic reticulum becoming dilated and degranulated while RNA content was doubled showing an inhibition of protein synthesis (Plea, 1975). Hermans, et al., (1973) have shown that the activities of glycose-6-phosphatase and mixed function oxidase were reduced. The loss of Glucose-6-phosphatase activity was due to distortion of endoplasmic reticulum membrane microarchitecture essential for its activity (Wills, 1971). This might be a

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situation resulting in hydropic hepatocyctes in the test groups.

The bile deposits found in the test groups might be due to the effect of the toxic agents present. This might occur due to injury to the membrane or formation of insoluble precipitates in or around the canaliculi and injury to bile ducts. It could also occur due to abnormal reabsorption or secretion of water and electrolytes along the course of the ductule (Zimmerman, 1982). Several agents, (of which the cyclic dihydroxyl esters, peroxides and cyclic fatty acids present in oxidized oil could be part of), appear to produce canalicular membrane injury (Artman, 1969; Zimmerman, 1982; Wepler and Wildhirt, 1972), which might lead to egress of bile from the membrane of bile canliculi into the liver tissue. It has been shown that distortion of molecules of the membrane or alkylation of other macromolecules of the cells could lead to defective membrane function (Grundmann, 1980; Zimmerman, 1982; Boyd, 1955; Baron, 1980).

The blood vessels of the test groups were shown to be congested. This is in agreement with the observation made by Grundmann, (1980) that some hepatotoxic agents lead to congestion which might also lead to edematous impregnation of the walls in preceding veins. The tunica intima of the hepatic vessels of the rats fed thermoxidized palm oil diet might have undergone ulceration or become worn away due to peroxidation caused by the oxidative toxicity of the test oils. Plea, (1975) has recorded that the permeability of mitochondria and the activities of the membrane-bound proteins were altered while Wills (1971) showed that the loss of Glucose-6-phosphatase activity was due to the distortion of endoplasmic reticulum membrane caused by oxidative toxicity of the oxidized oils. Lipid peroxides polymerise and

denature biological proteins like enzymes and membrane proteins and lipids. Membrane damage, increased permeability and cell leakage results, posing a serious danger to lysosomes (Gurr and James, 1975). When the endothelium of the intima became ulcerated or worn away, vascular damage sets-in which might result in the aggregation of platelets to prevent deleterious effect of blood loss from the vessels. Platelet aggregation might be due to the damage to the endothelial wall of the tunica intima of the blood vessels by oxidized oils which contained peroxides since platelet aggregation and thrombogenesis could be secondary responses to blood vessel wall damage (Nafastad, 1974; Osim, et al., 1992). Aggregation of platelets represent an early event in the complex chain of events leading to the formation of thrombus and other cardiovascular diseases (Halmilton, et al., 1980; Osim, et al., 1992; Mckay, 1976). Thrombosis due to platelet aggregation results in the occlusion of blood vessels and eventually formation of plug (Guyton, 1986) which might be the cause of congestion of blood vessels of the liver and the kidney. Thrombosis can also be caused by sluggish flow of blood through blood vessels and roughened endothelial surface of a vessel due to hypertension (Guyton, 1986). The congestion of the blood vessels of rats fed with fresh oil might be due to the thickening with lipid deposits of varying extent in the endothelium of the tunica intima. The blood cholesterol level has been shown to be elevated due to the unsaturation of major fatty acids and was accompanied by a decreased phospholipid level in the body (Hamilton, et al., 1980). The deposition of fats on the intima of the blood vessels could have caused blood flow to be sluggish and the endothelium to be roughened thus causing aggregation of platelets which might have resulted in thrombosis and then congestion.

Fatty acids contribute to both platelet aggregation and thrombosis since they are precursors of two important clinical compounds; prostaglandin and thromboxane produced from unsaturated fatty acids (Murray, et al., 1988). Hyperlipidemia and saturated fatty acids have been implicated by Lowe, et al., (1979); Turner, (1979); Shue, et al., (1968); Murray, et al., (1988) in the development of platelet aggregation, atherosclerosis and ischemic heart diseases. Turner, (1979); Osim, et al., (1992) have proved that saturated fats increase plasma cholesterol level and platelet stickiness. The association of dietary saturated and unsaturated fatty acids and cholesterol levels with thrombosis was because of the contribution of aggregation to the development of coronary heart diseases, pulmonary embolism and other cardiovascular diseases (Turner, 1979; Murray, et al., 1988) of which the congestion of the blood vessels of the test groups A and B in the liver and kidney might be part of.

The proliferation of lacis cells (Polkissen) found in the test groups, A and B with the hyperplasia in group A is more pronounced than those of group B. This is in agreement with the observations of Meadows, (1973) which showed that, the polkissen are frequently increased in number in a variety of renal diseases even though their function is usually not very clear. This could be true in the sense that the oxidative agents in the test diets might have induced pathological conditions in the kidney thus resulting in the hyperplasia of the polkissen.

Also found in the kidney of the test groups are proteinacious cast in the lumen of some proximal and distal tubules. Meadows, (1973) have shown that casts consisting of homogenous, eosinophilic materials are extremely common in a wide variety of renal diseases. He stated that casts are associated with trophic changes in

the involved tubules. The renal disease condition might have been evoked by the cytotoxic agents in both test diets. Duve, (1970) have shown lysosomes to participate in the production of many pathological disorders. This is because the lipid soluble toxic agents might have been incorporated in the lysosomal membrane rendering it more fragile. Also big hyaline droplets of the nephrotic kidney which represent over loaded lysosomes and are filled with protein arise because lysosomal proteolysis cannot keep pace with the rate of protein reabsorption by the kidney tubule cells (Duve, 1970; Meadows, 1973; Grundmann, 1980). This might represent vitamin A deficiency in group A rats because Cyril and Eric, (1964) have shown that deficiency of vitamin A might lead to renal stones in rats while it plays no such role in human calculus formation. They showed calculus as a mass of precipitated material derived from a secretion and deposited in the kidney duct. Those in group B rats might be due to the secretion and precipitation of cholesterol present in the palm oil of the diet. The sloughing of the epithelium into the tubular lumen might not be unconnected with the toxic agents in the test diets in that Cyril and Eric, (1964) have shown that in vitamin A deficiency, infection of the damaged epithelial structures often occur in the kidney.

CHAPTER SIX

SUMMARY AND CONCLUSION

The effect of chronic consumption of both thermoxidized and fresh palm oil supplemented diets on the small intestine, liver and kidney of the Albino rat was investigated. The morphology of the mucosa, villi, crypt and villous stroma was found to be affected by the test diets. The differences in the parameters were significant ($P<0.05$) except for the stromal cross-section in the 3-groups. A significant ($P<0.05$) value was observed on the mean change in body weight between the control and the test animals.

The liver of the test groups showed degenerative fatty changes resembling those found in alcoholic livers and degenerative changes in form of necrosis. The blood vessels of the test groups are congestive in the liver and kidney of the test animals. The kidney of the test groups showed deposits within some proximal and distal tubules with hyperplasia of lacis cells.

In conclusion, the chronic consumption of thermally oxidized palm oil diet and fresh palm oil diet may predispose to damages of the small intestinal mucosa with consequent reduction in the rate of absorption and rate of growth due to malabsorption of nutrients. It may also predispose to liver and kidney diseases, intravascular thrombosis and other cardiovascular diseases.

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APPENDIX A

COMPOSITION OF THE RAT FEED

(Pfizer Livestock Feeds PLC: Grower's Mash)

(a) Composition:

Maize

Sorghum

Wheat Middlings

Brewer's Dried grains

Maize Bran

Soya Bean Meal

Groundnut Cake

Bone Meal

Oyster Shell

Mineral premix

Antibiotic

Antioxidant

(b) Analysis of the Feed:

Content	Percentage
Protein	14.0
Fats	3.5
Fibre	7.5
Calcium	0.9
Phosphorus	0.35
Energy	2,300kcal/kg.

APPENDIX B1: Weekly Change in Body Weight of Rats, following Chronic Consumption of Thermoxidized Palm Oil Diet (Group A).

S/No	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Man Change in Weight	S.D	S.E
1.	7.0	18.5	20.6	27.0	10.0	13.0	6.5	11.5	-1.5	11.0	12.36	8.1	0.8
2.	19.5	22.0	63.5	43.5	23.0	27.5	21.0	31.0	15.5	13.0	28.0	15.2	1.5
3.	31.0	25.0	42.3	51.5	34.0	52.0	31.0	65.0	50.6	15.5	39.8	15.1	1.5
4.	36.0	29.0	38.0	50.5	39.0	82.5	30.0	33.0	3.5	21.0	36.3	20.4	2.0
5.	40.0	30.0	42.0	55.0	65.0	85.0	50.0	85.0	0.0	30.0	48.2	26.1	2.6
6.	58.5	65.5	59.5	85.0	81.0	46.0	56.0	97.5	1.0	43.5	59.4	26.8	2.7
7.	71.0	86.5	52.5	97.0	89.0	31.0	69.5	42.5	8.5	50.0	59.8	28.0	2.8
8.	64.0	69.0	49.5	85.5	80.0	45.0	61.0	37.0	12.0	42.5	54.6	21.9	2.2
9.	72.5	72.0	65.0	92.0	90.5	33.0	78.5	28.0	35.0	54.5	62.1	23.5	2.4
10.	70.0	70.0	72.0	94.0	88.0	38.0	73.0	36.5	50.0	56.5	64.8	19.4	1.9
11.	80.0	67.0	75.0	95.0	91.0	41.5	74.0	38.0	30.0	59.0	65.1	22.4	2.2
12.	85.0	70.5	80.2	91.2	94.0	45.0	78.0	42.0	42.0	35.0	66.3	22.8	2.3
13.	95.0	75.2	87.0	99.5	103.0	48.0	75.0	41.0	43.0	36.5	70.3	26.0	2.6
14.	96.5	74.6	88.7	101.0	101.0	46.0	76.0	42.5	41.0	38.0	70.5	26.3	2.6
Mean Change in Weight	59.0	55.3	59.7	76.3	70.6	45.3	55.7	45.0	23.6	36.1	76.02		
S.D.	28.1	24.2	20.0	25.0	31.0	19.2	24.2	22.8	20.0	16.2	84.3		
S.E.	2.0	1.7	1.4	1.8	2.2	1.4	1.7	1.6	1.4	1.1			

APPENDIX B2: Weekly Change in Body Weight of Rats, following Consumption of Fresh Palm Oil Diet (Group B)

S/No	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	Man Change in Weight	S.D	S.E
1.	7.0	11.5	22.5	18.9	20.0	21.5	12.0	11.5	5.5	21.0	15.1	6.3	0.6
2.	27.0	26.5	51.0	18.9	35.0	03.0	25.5	17.5	30.5	48.0	28.3	14.2	1.4
3.	29.0	57.6	61.5	37.4	54.0	31.5	45.2	34.0	49.0	89.5	48.9	18.2	1.8
4.	35.0	34.0	60.5	53.4	58.0	41.0	45.2	29.5	38.0	46.0	50.0	17.0	1.7
5.	40.0	50.0	72.0	64.4	64.0	50.0	50.0	35.5	52.0	55.0	53.3	11.1	1.1
6.	60.5	48.5	92.0	68.4	97.5	76.5	85.5	61.5	47.5	74.5	71.2	17.21	1.7
7.	60.0	62.0	94.5	58.4	113	88.0	96.5	67.0	61.5	75.5	77.6	9.2	1.9
8.	68.0	54.5	97.5	61.4	109	86.5	93.5	57.5	77.0	77.0	78.2	18.3	1.8
9.	60.5	42.5	94.0	50.9	106	82.0	84.0	23.5	78.5	83.0	70.5	25.4	2.5
10.	86.0	60.0	100	73.4	118	99.0	101	11.0	63.0	111	82.2	21.8	3.2
11.	63.5	74.0	117	69.4	120	95.0	116	12.7	70.5	113	85.1	33.9	3.4
12.	62.6	72.0	118	70.9	119	98.0	115	14.5	68.0	111	84.9	33.5	3.4
13.	64.0	75.0	120	71.4	120	99.0	117	17.5	67.0	112	86.3	33.3	3.3
14.	65.0	76.0	122	72.6	122	101	119	19.5	68.0	113	87.8	33.4	3.3
Mean Change in Weight	52.0	51.0	87.3	56.4	89.7	69.4	79.0	29.5	55.4	80.7			
S.D.	21.1	23.0	30.0	18.8	35.7	33.3	36.4	19.4	20.2	29.8			
S.E.	1.5	1.6	2.1	1.3	2.6	2.4	2.6	1.4	1.4	2.3			

APPENDIX B3: Weekly Change in Body Weight of Rats, following Consumption of Control Diet (Group C)

S/No	C1	C2	C3	C4	C5	C6	C7	C8	C9	Man Change in Weight	S.D	S.E
1.	20.5	54.5	19.5	39.0	20.5	24.0	24.0	13.0	18.0	25.9	12.9	1.4
2.	37.5	71.0	33.0	51.0	35.0	41.0	36.5	29.0	30.5	40.5	13.2	1.5
3.	75.5	81.0	51.0	60.6	51.0	71.5	52.1	50.5	46.5	60.0	12.8	1.4
4.	35.5	86.0	60.0	60.0	55.5	89.5	57.0	45.0	43.5	59.1	18.3	2.0
5.	50.0	105	70.0	65.0	65.0	93.5	70.0	47.5	63.5	69.9	18.6	2.1
6.	69.5	14.4	91.0	83.0	81.7	120	78.5	67.5	65.0	88.9	26.5	2.9
7.	57.5	165	108	106	96.5	73.5	93.0	73.5	82.0	95.0	31.0	3.4
8.	74.5	177	111	108	104	87.0	108	95.5	90.5	106.1	29.1	3.2
9.	96.5	194	118	118	115	85.5	122	108	96.5	117.1	31.4	3.4
10.	57.5	189	105	112	102	69.0	119	102	92.5	105.3	37.1	4.1
11.	68.5	191	105	125	106	77.0	125	104	95.5	110.8	35.5	3.9
12.	76.5	193	114	134	113	81.0	128	111	101	116.8	34.3	3.8
13.	81.5	195	118	138	118	85.5	140	116	106	122.0	33.9	3.8
14.	84.0	200	118	140	120	88.0	144	119	110	122.8	34.9	3.9
Mean Change in Weight	63.2	146.1	87.3	95.7	84.5	77.6	92.7	77.3	74.4			
S.D.	21.3	54.4	34.1	35.0	33.2	23.0	39.7	35.3	29.9			
S.E.	1.52	3.9	2.4	2.5	2.4	1.6	2.8	2.5	2.1			

APPENDIX B4: Showing the rate of food consumption (gm) in the Thermixidized Palm Oil Diet Group (A), Fresh Palm Oil Diet Group (B) and the Control Diet Group (C).

S/No.	A	B	C
1.	82	93	116
2.	94	89	131
3.	85	135	143
4.	85	94	146
5.	89	116	144
6.	88	91	126
7.	82	100	127
8.	87	104	126
9.	104	114	135
10.	97	85	138
11.	94	99	125
12.	99	107	145
13.	114	114	129
14.	102	115	134
15.	98	109	128
16.	102	118	130
Total	1502	1683	2123
Mean	93.88	105.19	132.69
S.D.	9.08	13.29	8.58

APPENDIX B5: Showing the effect on the Small Intestinal Vilous Length, Crypt Height and Stroma. In Thermoxidized Palm Oil Diet Group (A), Fresh Palm Oil Diet Group (B) and the Control Diet Group (C).

	GROUP C (um)			GROUP B (um)			GROUP A (um)		
S/N	Villi	Crypt	Stroma	Villi	Crypt	Stroma	Villi	Crypt	Stroma
1.	450	90	60	480	180	120	360	150	150
2.	480	120	60	480	150	90	300	120	190
3.	480	90	75	540	180	90	300	120	120
4.	480	120	30	540	180	180	360	150	60
5.	510	120	30	310	150	60	390	150	60
6.	480	120	120	390	120	120	360	120	60
7.	480	120	60	450	150	30	390	120	120
8.	480	120	60	450	120	60	390	120	60
9.	420	90	180	300	120	120	360	120	90
10.	450	120	150	510	120	105	390	150	120
11.	540	120	90	390	150	120	390	120	60
12.	450	90	60	480	180	180	390	150	60
13.	540	120	60	480	180	120	450	180	60
14.	480	120	90	420	120	90	450	150	90
15.	510	120	90	390	150	60	450	150	60
16.	570	150	60	390	120	75	390	120	60
Total	7800	1830	1275	7080	2370	1620	6120	2190	1320
Mean	487.5	114.38	79.69	442.5	148.13	101.25	382.5	136.88	82.5
S.D.	38.73	16.32	40.52	65.27	25.62	41.17	44.50	18.87	30.0

APPENDIX B6: The Effect of the Thermoxidized Palm Oil Diet, Group A, Fresh Palm Oil Diet, Group B and Control Diet Group C on the Surface Amplication due to the Villi Estimated using Transverse Sections of the Jejunum.

S/No.	GROUP A	GROUP B	GROUP C
1.	3.06	3.40	6.37
2.	3.19	4.08	5.10
3.	3.19	3.82	6.79
4.	3.06	3.82	5.10
5.	3.31	3.31	5.41
6.	3.82	4.14	5.10
7.	4.14	3.82	5.10
8.	4.14	4.78	5.10
9.	3.82	3.18	5.95
10.	3.31	5.41	4.78
11.	4.14	3.31	5.73
12.	3.31	3.40	6.37
13.	3.18	4.46	5.73
14.	3.82	3.31	5.10
15.	3.82	4.14	5.41
16.	4.14	3.40	4.84
Total	57.45	61.78	87.98
Mean	3.59	3.86	5.50
S.D.	0.42	0.62	0.60
C.V.	0.12	0.16	0.11

APPENDIX B7. Mean Change of Weight of Rats following consumption of Thermoxidized Palm Oil Diet Group (A), Fresh Palm Oil Diet Group (B) and Control Diet Group (C)

No. of Weeks	A	B	C
1.	12.36	15.1	25.9
2.	28.0	28.3	40.5
3.	39.8	48.9	60.0
4.	36.3	50.0	59.1
5.	48.2	53.3	69.9
6.	59.4	71.2	88.9
7.	59.8	77.6	95.0
8.	54.6	78.2	106.1
9.	62.1	70.5	117.1
10.	64.8	82.2	105.3
11.	65.1	85.1	110.8
12.	66.3	84.9	116.8
13.	70.3	86.3	122.0
14.	70.5	87.8	122.8
Mean	52.7	65.7	88.6
S. D.	17.6	23.1	32.0
S. E.	1.3	2.3	3.2

APPENDIX B8. Mean Change in Weight per Rat Throughout the feeding period, in the Thermoxidized Palm Oil Diet Group (A) Fresh Palm Oil Diet Group (B) and the Control Diet Group (C)

Rat No.	Mean Change in A	Mean Change B	Mean Change in C
1.	59.0	52.0	63.2
2.	55.3	51.0	146.1
3.	59.7	87.3	87.3
4.	76.3	56.4	95.7
5.	70.6	89.7	84.5
6.	45.3	69.4	77.6
7.	55.7	79.0	92.7
8.	45.0	29.5	77.3
9.	23.6	55.4	74.4
10.	36.1	80.7	86.8
Mean	52.7	65.0	88.6
S. D.	15.7	19.3	22.3
S. E.	1.6	1.9	2.6