EFFECT OF FRYING AN EGG YOLK WITH RED OIL ON SOME LIPID AND HISTOLOGY ON THE HEART OF A WISTAR RAT

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BY

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LSC2103751

FEBRUARY, 2025

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DECLARATION

I IKHAYERE EZEKIEL MIRACLE with the Matriculation number LSC2103751, declare that this project, entitled "Effect of frying an egg yolk with red oil on some lipid profile and histology of the Wistar rat heart", is my original work and has not been submitted elsewhere for a degree. All sources used have been duly acknowledged.

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All thanks, praise and glory to God, the father of all, and the lord of mercy for His unmeasurable love, strength and faithfulness with which he has seen me through the course of the work/study, to Him be thanks and honour forever, amen. This work is also dedicated to every member of the Iriah family for their immense support at every point in time during the course of my study.

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CERTIFICATION

This is to certify that this project was carried out by **IKHAYERE EZEKIEL MIRACLE,** with matriculation number **LSC2103751**, in the department of Biochemistry, University of Benin, Benin city, Edo sate, Nigeria.

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DR. O.C. UGBENI DATE

(Project Supervisor)

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PROF. E.C. ONYENEKE DATE

(Head of Department)

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(External Supervisor) DATE

DEDICATION

I dedicate this project to the Almighty God who is rich in mercy, grace, and love, for strength and help at every point of my need. Also, to my parent Mr and Mrs Iriah to their huge support morally and financially.

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ACKNOWLEDGEMENT

All thanks to the Almighty God. I want to genuinely appreciate my parent Mr. and Mrs. Iriah and my siblings Helen, Emmanual and Timothy for their moral and financial support.

To my supervisor, DR. O.C. UGBENI for making this work a success. And to the Head of Department Prof. E.C Onyeneke and to all my lecturers, I thank you all for your wonderful work, support and dedication to my academics. God bless you all.

I want to say a big thanks to the house of TACSFON and also to my fellow project partners for their great support.

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ABSTRACT

The impact of dietary fats on cardiovascular health is of growing concern, particularly in regions where red palm oil is a staple cooking fat. This study investigates the effect of frying an egg with red palm oil on egg cholesterol levels and its influence on the lipid profile and heart histology of Wistar rats. Sixteen female Wistar rats were divided into four dietary groups fried egg with red oil, raw cholesterol, positive control, and negative control. After 42 days, lipid profile analysis on some lipid (total cholesterol and triglyceride) revealed that the fried egg with red oil group had a total cholesterol level of (251.90 ± 2.64 mg/dL), which was significantly lower than the raw cholesterol group (307.63 ± 7.74 mg/dL). Triglyceride levels also varied, with the fried egg group showing (554.19 ± 9.16 mg/dL), compared to (577.09 ± 9.53 mg/dL) in the raw cholesterol group. Histological analysis of the heart tissues showed vascular stenosis and perivascular infiltration in the fried egg with red oil group, while the raw cholesterol group exhibited mild perivascular fibroblast mobilization. These results indicate that frying eggs in red palm oil alters lipid metabolism and affects cardiovascular health, potentially modifying cholesterol bioavailability. The study highlights the significance of cooking methods in influencing dietary cholesterol impact and underscores the need for further research on the long-term effects of palm oil consumption on heart health.

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CHAPTER ONE

INTRODUCTION

Despite the widespread consumption of eggs and palm oil in various diets, their combined effect on cholesterol metabolism and cardiovascular health remains unclear. Eggs are known to contain cholesterol, while red oil (palm oil) is rich in saturated and unsaturated fats. studies suggest that excessive dietary cholesterol may contribute to lipid abnormalities, leading to cardiovascular diseases.

Cardiovascular diseases (CVDs) are a predominant cause of global mortality, with dietary considerations significantly influencing lipid metabolism and cardiac function. Cholesterol intake, particularly from animal sources like eggs, has been extensively researched in relation to cardiovascular disease (CVD). Although eggs are acknowledged as a nutrient-dense food, offering high-quality protein, vitamins, and vital fatty acids, their cholesterol concentration continues to be a topic of scholarly contention (McNamara, 2015). Historically, dietary cholesterol has been linked to elevated plasma cholesterol levels, particularly low-density lipoprotein (LDL), which is a recognized risk factor for atherosclerosis and cardiovascular illnesses. Recent research indicates that the influence of dietary cholesterol on serum cholesterol levels is contingent upon genetic predisposition, dietary context, and other metabolic variables (Krittanawong *et al*., 2020). Nonetheless, the possible impacts of cooking techniques, especially frying, on cholesterol bioavailability and its ensuing metabolic consequences necessitate additional research.

The frying procedure, particularly with oils rich in saturated fats, can modify the chemical structure of food and influence its physiological effects. Red oil (palm oil) is a widely utilised cooking oil, especially in Africa and Asia, owing to its cost-effectiveness, stability, and elevated

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smoke point (Sundram *et al*., 2017). Palm oil is abundant in carotenoids and tocotrienols, which has antioxidant characteristics; yet, it is also elevated in saturated fatty acids, prompting over its impact on lipid metabolism and cardiovascular health. Research indicates that unrefined red palm oil may confer advantages owing to its antioxidant properties, but repeatedly heated or oxidised palm oil can exacerbate oxidative stress, endothelial dysfunction, and lipid irregularities (Chong *et al.,* 2010). The frying of eggs in palm oil may result in intricate metabolic interactions due to the interplay between dietary cholesterol and heat-induced lipid oxidation, potentially affecting lipid homeostasis and cardiovascular health.

The lipid profile, comprising total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG), is essential for evaluating cardiovascular health. LDL is commonly designated as "bad cholesterol" because it facilitates the transfer of cholesterol to tissues, where excessive accumulation may lead to arterial plaque development and consequent cardiovascular difficulties (Goldstein & Brown, 2015). In contrast, HDL, known as "good cholesterol," promotes reverse cholesterol transport by transporting surplus cholesterol from tissues to the liver for elimination, thus diminishing cardiovascular risk (Khera *et al*., 2011). Triglycerides, the principal storage form of fat in the body, function as an energy source but are associated with obesity, insulin resistance, and metabolic syndrome when present in excess (Toth, 2016). Given that dietary fat composition and processing methods affect lipid metabolism, it is essential to assess whether the combination of egg cholesterol and palm oil frying results in significant modifications to some lipid that may predispose individuals to cardiovascular disease. Alongside biochemical indicators of cardiovascular risk, dietary impacts on heart tissue histology elucidate structural and pathological alterations linked to diet-induced dyslipidaemia. The heart comprises three main layers: the epicardium, myocardium, and endocardium, with the

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myocardium containing the cardiomyocytes that facilitate contractile action (Raj *et al.*, 2020). Under normal physiological settings, cardiomyocytes have organised striations, undamaged intercalated discs, and robust vascular networks to facilitate efficient circulation. Chronic exposure to high-fat diets, oxidised lipids, and excessive dietary cholesterol can result in myocardial lipid buildup, oxidative damage, and fibrosis, ultimately causing progressive cardiac dysfunction (Dos Santos *et al.*, 2019). Prior research indicates that oxidised lipids facilitate the generation of reactive oxygen species (ROS), instigating inflammatory pathways and endothelial damage, potentially resulting in cardiac remodelling, ventricular hypertrophy, and heart failure (Ighodaro & Akinloye, 2018). Examining the histological alterations in Wistar rats consuming a diet of fried eggs in palm oil may yield significant insights into the potential mechanisms connecting diet-induced dyslipidemia and cardiovascular disease.

Wistar rats (Rattus norvegicus) are extensively utilised as an experimental model in biomedical and nutritional research owing to their consistent lipid metabolism, cardiovascular physiology, and genetic homogeneity, rendering them suitable for regulated dietary investigations (Zhou *et al.*, 2018). These rodents demonstrate metabolic reactions to dietary cholesterol and lipid alterations that closely resemble those seen in people, facilitating translational insights into human cardiovascular health. This study will assess if frying eggs in palm oil affects lipid metabolism and the structure of cardiac tissue.

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1.2 Statement of Research Problem

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Despite extensive research on dietary cholesterol and lipid metabolism, the combined effects of frying eggs in red oil (palm oil) on some lipid and heart histology remain poorly understood. While eggs are a rich source of high-quality protein and essential fatty acids, their cholesterol content has raised concerns about cardiovascular risk, particularly when consumed in fried form.

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Similarly, palm oil, widely used in cooking due to its stability and antioxidant properties, has been linked to oxidative stress and lipid abnormalities when repeatedly heated.

Previous studies have shown that egg cholesterol alone does not significantly raise blood cholesterol levels due to compensatory mechanisms in the body (McNamara, 2015). However, these studies fail to account for the impact of cooking methods, particularly frying, which may alter cholesterol bioavailability and contribute to oxidative modifications that influence lipid metabolism. Similarly, research on heated palm oil has indicated that repeated heating leads to lipid peroxidation and the generation of free radicals, which can disrupt normal lipid homeostasis (Chong *et al*., 2010). Despite these findings, no study has specifically evaluated the combined effect of egg cholesterol and frying in palm oil on lipid profile alterations and oxidative stress markers, leaving an important knowledge gap.

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Beyond lipid metabolism, the structural integrity of the heart is a key indicator of dietary impact on cardiovascular health. High-fat diets have been shown to induce cardiac fibrosis, inflammatory infiltration, and lipid accumulation in myocardial tissue, leading to progressive cardiac dysfunction (Dos Santos *et al*., 2019). Studies have also suggested that oxidized lipids play a major role in promoting endothelial damage, myocardial apoptosis, and oxidative stress (Ighodaro & Akinloye, 2018).

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Additionally, while changes in lipid profile are commonly used as biomarkers for cardiovascular risk, their direct relationship with histological alterations in the heart is not well established. Research has confirmed that elevated LDL and reduced HDL levels contribute to atherosclerosis and myocardial damage (Khera *et al*., 2011), but few studies have correlated these lipid profile changes with actual structural modifications in heart tissues. Without this correlation, it is

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difficult to determine whether changes in lipid metabolism due to frying in palm oil translate into observable cardiac damage.

This study seeks to fill these research gaps by investigating the effects of frying eggs in red oil on some lipid and histological changes in the heart of Wistar rats. The study will determine whether this dietary combination leads to significant alterations in lipid metabolism, induces oxidative stress, and contributes to cardiac remodeling.

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1.3 Justification of the study

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Cardiovascular diseases (CVDs) represent a worldwide health issue, with dietary cholesterol and lipid oxidation significantly contributing to their pathogenesis. Although considerable study has investigated the separate effects of egg cholesterol and palm oil intake on lipid metabolism, the synergistic effect of frying eggs in red oil on some lipid and cardiac histology is predominantly unexamined. Egg eating does not consistently affect cholesterol levels appreciably due to compensatory metabolic processes (McNamara, 2015). Nevertheless, these investigations neglect to account for the oxidative alterations induced by frying, which could affect cholesterol metabolism. Research on heated palm oil indicates that repeated heating generates lipid peroxidation products, hence elevating oxidative stress and cardiovascular risk (Chong *et al.*, 2010). Notwithstanding these findings, few studies have investigated the relationship between fried egg cholesterol and oxidised lipids, resulting in a significant research gap.

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Moreover, although alterations in some lipid in lipid profiles are commonly utilised as cardiovascular indicators, their direct correlation with cardiac histology is ambiguous. Prior research indicates that oxidised lipids play a role in cardiac fibrosis, lipid accumulation, and inflammation (Ighodaro & Akinloye, 2018); however, the impact of ingesting fried eggs in red oil on these consequences has not been investigated.

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1.4 Aim of the study

The purpose of this research is to examine how the some lipid and heart histology of Wistar rats are affected when an egg is fried in red oil. In order to improve knowledge of diet-induced cardiovascular risks, this study aims to assess the possible effects of dietary cholesterol and lipid changes on cardiovascular health.

This study aims to assess whether the interaction between these two dietary components alters lipid levels and induces structural changes in the heart, potentially increasing cardiovascular risk. Furthermore, understanding the impact of this dietary practice is essential for public health recommendations and nutritional guidelines. If significant negative effects are observed, the findings could support the development of dietary modifications to minimize cardiovascular risks associated with high-cholesterol and high-fat diets. On the other hand, if no detrimental effects are found, this study may contribute to refining misconceptions about dietary cholesterol intake. By using Wistar rats as a model, this research will provide foundational data that could be applicable to human dietary studies in the future.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Dietary Cholesterol and Lipid Metabolism

Cholesterol is a vital lipid that is crucial for numerous physiological processes, including the maintenance of cellular membrane integrity, hormone synthesis, and bile acid generation. Cholesterol exists in two principal forms: endogenous cholesterol, synthesized internally by the liver and intestines, and exogenous cholesterol, acquired from dietary sources (Feingold & Grunfeld, 2022). Primary dietary sources of cholesterol include animal-derived items such as eggs, dairy, meat, poultry, and seafood, with eggs being especially high in cholesterol, comprising roughly 186 mg per yolk (Drouin-Chartier *et al*., 2018). In contrast to animal products, plant-based diets lack cholesterol and include phytosterols, which have demonstrated the ability to compete with cholesterol absorption and aid in reducing serum cholesterol levels (Ostlund, 2018).

Cholesterol metabolism is a meticulously regulated process essential for sustaining equilibrium. It serves as a precursor for steroid hormones like oestrogen, testosterone, and cortisol, in addition to vitamin D and bile acids, which are crucial for lipid digestion and absorption (Rozner & Garti, 2016). Notwithstanding its significance, elevated cholesterol levels in circulation might result in detrimental health consequences, especially for cardiovascular diseases (CVDs). Cholesterol transport in the bloodstream is facilitated by lipoproteins, namely low-density lipoprotein (LDL) and high-density lipoprotein (HDL), which play crucial roles in lipid metabolism (Goldstein & Brown, 2015). Low-density lipoprotein (LDL), sometimes termed "bad cholesterol," facilitates the transfer of cholesterol to peripheral tissues. Excessive LDL accumulates cholesterol in artery walls, resulting in plaque formation and atherosclerosis, which markedly elevates the risk of

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heart disease and stroke (Ference *et al*., 2017). Conversely, HDL, referred to as "good cholesterol," promotes reverse cholesterol transport, extracting surplus cholesterol from tissues and delivering it to the liver for excretion, thus providing protective benefits against cardiovascular illnesses (Khera *et al*., 2011).

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Cholesterol absorption commences in the small intestine, where it is integrated into micelles, facilitating its reception by enterocytes (intestinal cells). Following absorption, cholesterol is encapsulated within chylomicrons, which facilitate its transport via the lymphatic system into the bloodstream (Hussain, 2014). Upon entering the bloodstream, LDL transports cholesterol to tissues, whereas HDL promotes its removal and excretion. A crucial element of lipid metabolism is triglycerides (TG), which function as an energy store. Elevated triglyceride levels, particularly when associated with increased LDL and decreased HDL, are correlated with a heightened risk of metabolic diseases and cardiovascular problems (Toth, 2016).

Krittanawong *et al*. (2020) established that egg consumption does not markedly increase blood cholesterol levels in healthy persons, as the liver modulates endogenous cholesterol synthesis in accordance with food intake. However, those identified as hyper-responders, possessing genetic predispositions to cholesterol sensitivity, may exhibit elevated LDL levels upon the consumption of cholesterol-laden diets.

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Although cholesterol is not necessarily the principal cause of cardiovascular disease, oxidised cholesterol, produced during frying, has been associated with inflammation, endothelial dysfunction, and atherosclerosis (Staprans *et al*., 2000). This raises concerns about frying techniques and the possible oxidative alterations of dietary cholesterol, especially in frequently utilised oils like palm oil (red oil). Research indicates that the interplay between cholesterol and oxidised lipids may exacerbate lipid peroxidation, oxidative stress, and vascular injury, rendering

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the effects of dietary cholesterol significantly contingent upon cooking techniques and overall dietary composition (Sundram *et al*., 2017).

2.2 Egg Cholesterol and Its Nutritional Composition

Eggs are regarded as one of the most nutrient-rich foods, supplying vital proteins, vitamins, and minerals. Nonetheless, the cholesterol content has been a topic of much discussion, especially about its impact on human health. A single large egg has roughly 186 mg of cholesterol, predominantly located in the yolk (McNamara, 2015). Historically, eggs were seen as a significant dietary factor in increasing blood cholesterol levels, resulting in limitations on their consumption. Recent research, however, contests this idea, indicating that dietary cholesterol does not substantially influence blood cholesterol levels in the majority of persons because of compensatory homeostatic processes (Drouin-Chartier *et al*., 2018).

Notwithstanding their cholesterol level, eggs offer various health advantages owing to their abundant protein and important minerals. They provide a superior source of high-quality protein, delivering all nine essential amino acids necessary for muscle repair, satiety, and immunological function (Shin *et al*., 2017). Egg yolks are abundant in choline, an essential ingredient for cerebral development, hepatic function, and neurotransmitter production (Zeisel & da Costa, 2009). Moreover, eggs are rich in antioxidants like lutein and zeaxanthin, which contribute to ocular health by diminishing the likelihood of macular degeneration (Abdel-Aal *et al*., 2013). The nutritious attributes of eggs render them a significant element of a balanced diet, notwithstanding apprehensions regarding cholesterol consumption.

The correlation between egg yolk consumption and lipid profile has been extensively researched. Historically, it was posited that dietary cholesterol directly elevated plasma cholesterol levels, especially low-density lipoprotein (LDL) cholesterol, which is linked to atherosclerosis and

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In summary, dietary cholesterol is integral to lipid metabolism, facilitating vital biological tasks and significantly influencing cardiovascular health control. The transportation of cholesterol through LDL and HDL is essential in influencing its impact, since elevated LDL levels augment cardiovascular disease risk, whereas HDL provides preventative advantages.

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cardiovascular illnesses (Goldstein & Brown, 2015). Contemporary studies indicate that the impact of dietary cholesterol on blood cholesterol levels differs among individuals. Most healthy individuals exhibit negligible elevations in blood cholesterol when the body modulates endogenous cholesterol production in response to increased dietary consumption (Feingold & Grunfeld, 2022). Moreover, eggs not only raise low-density lipoprotein (LDL) but also enhance high-density lipoprotein (HDL) cholesterol, frequently preserving a favourable LDL/HDL ratio (Krittanawong *et al*., 2020). Conversely, specific populations, including hyper-responders and those with metabolic problems, may demonstrate more significant elevations in LDL cholesterol with egg consumption (Drouin-Chartier *et al*., 2018).

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The cholesterol level of eggs is no longer a primary nutritional concern; however, the preparation process substantially affects their metabolic impact. Cholesterol is susceptible to oxidation when subjected to heat, air, and cooking oils, resulting in the production of oxidised cholesterol derivatives (oxysterols) (Staprans *et al*., 2000). Oxidised cholesterol molecules are more detrimental than native cholesterol since they can induce inflammation, endothelial dysfunction, and plaque accumulation in arteries (Ighodaro & Akinloye, 2018). Research indicates that frying eggs, especially in oils abundant in polyunsaturated fatty acids (PUFAs), enhances cholesterol oxidation, resulting in a greater production of oxysterols compared to boiling or poaching (Sundram *et al*., 2017). Dietary oxidised cholesterol is associated with heightened oxidative stress and an elevated risk of cardiovascular disease, underscoring the necessity of selecting cooking techniques that reduce cholesterol oxidation.

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Eggs are a very nutritious food that offers numerous health benefits, including contributions to muscle synthesis, cognitive function, and ocular health. The frying method significantly influences their effect on lipid metabolism and cardiovascular risk.

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2.3 Palm Oil (Red Oil): Composition and Health Effects

Palm oil, referred to as red oil in its unrefined form, is a prevalent edible oil derived from the fruit of the *Elaeis guineensis* palm tree. It is esteemed for its distinctive chemical composition, comprising a blend of saturated and unsaturated fatty acids, along with substantial quantities of bioactive substances like tocotrienols and carotenoids. Palmitic acid (C16:0) is the predominant saturated fatty acid in palm oil, constituting around 44% of its total fatty acid composition, but other saturated fatty acids, such as stearic acid (C18:0) and myristic acid (C14:0), are found in lesser amounts (Sundram *et al*., 2003). Besides its saturated fat concentration, palm oil comprises a significant proportion of monounsaturated and polyunsaturated fatty acids, with oleic acid (C18:1) accounting for 39–43% and linoleic acid (C18:2) constituting approximately 10% of the overall fatty acid profile (Sambanthamurthi *et al*., 2011). These fatty acids affect the oil's physical characteristics, rendering it semi-solid at ambient temperature and comparatively stable for culinary use.

In addition to its fatty content, palm oil contains tiny yet nutritionally significant components. Tocotrienols, a subset of vitamin E, demonstrate significant antioxidant capabilities associated with neuroprotection and anti-inflammatory actions (Sen *et al*., 2010). These antioxidants are thought to mitigate oxidative stress, a contributing component in neurological illnesses like Alzheimer's. Carotenoids, especially β-carotene, impart a deep red-orange hue to unrefined palm oil and act as precursors to vitamin A, vital for immunological function, eyesight, and cellular health (Sundram *et al*., 2003). These bioactive components augment the nutritional value of palm oil, rendering it a significant dietary source of fat-soluble vitamins.

A significant benefit of palm oil is its elevated oxidative stability, rendering it suitable for frying and food preparation. Saturated fatty acids enhance its resistance to oxidation, inhibiting the fast generation of free radicals and oxidative destruction (Edem, 2002). Additionally, the natural

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antioxidants included in palm oil, including tocotrienols and carotenoids, effectively impede lipid peroxidation, hence preserving the oil's quality even during high-temperature cooking (Sambanthamurthi *et al*., 2011). Nevertheless, when palm oil is repeatedly heated, it experiences chemical alterations that undermine its oxidative stability. Extended exposure to heat results in the generation of detrimental oxidative byproducts, including hydroperoxides, aldehydes, and polymeric compounds, which are associated with heightened oxidative stress and inflammation in the body (Choe & Min, 2007). The severity of these detrimental consequences is contingent upon elements including temperature, heating duration, and frequency of oil reuse.

The health implications of palm oil use have been extensively discussed, with studies indicating both possible advantages and hazards. Red palm oil provides a significant source of vital nutrients, including carotenoids and tocotrienols, which support immunological function, cardiovascular health, and neuroprotection (Sen *et al*., 2010). Certain studies indicate that tocotrienols in palm oil may contribute to the reduction of cholesterol levels and offer protection against neurodegenerative disorders (Yap *et al*., 2010). Furthermore, palm oil is devoid of trans fats, which are well acknowledged as detrimental to cardiovascular health. Concerns regarding palm oil consumption primarily arise from its elevated palmitic acid content, which has been linked to an increase in low-density lipoprotein cholesterol (LDL-C), often termed "bad" cholesterol, and a heightened risk of cardiovascular disease (Fattore & Fanelli, 2013). Some studies contend that the cholesterol-raising effect of palm oil is comparable to other dietary fats; however, excessive intake, especially from processed foods, may exacerbate cardiovascular risk factors (Sundram *et al*., 2003).

Research involving animals has shown that the recurrent intake of thermally degraded palm oil results in notable metabolic changes, such as elevated total cholesterol, triglycerides, and LDL-C

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Deleted[THE SPIRITMAN]: A significant issue related to palm oil intake is the impact of repeatedly heated palm oil on lipid metabolism. Research demonstrates that the reutilization of palm oil for frying results in the generation of oxidised lipids, trans fatty acids, and polymeric compounds, which may disturb normal lipid homeostasis (Choe & Min, 2007).

levels, alongside a decrease in high-density lipoprotein cholesterol (HDL-C), which serves as a safeguard against cardiovascular disease (Adam *et al*., 2007).

2.4 Frying Process and Lipid Oxidation

Frying is one of the most commonly used cooking methods, particularly for preparing foods rich in fat and protein. It involves the submersion of food in hot oil at high temperatures, typically between 150°C and 200°C, which induces complex chemical and physical changes in the food matrix and the cooking oil (Choe & Min, 2017). The frying process leads to changes in lipid composition, the formation of volatile compounds, oxidation of fatty acids, and structural modifications in food lipids, particularly cholesterol. These reactions impact the nutritional quality and safety of fried foods, with the extent of these alterations depending on factors such as the type of oil used, frying duration, temperature, and frequency of oil reuse (Debnath *et al*., 2012).

One of the primary concerns in frying is the oxidation of lipids, which leads to the formation of oxidized lipids, free radicals, and reactive oxygen species (ROS). When oils are exposed to high temperatures and oxygen, their unsaturated fatty acids undergo oxidation, producing lipid peroxides and aldehydes, which are harmful to health (Cheng *et al*., 2021). Lipid oxidation is influenced by the degree of unsaturation in the oil, with polyunsaturated fatty acids (PUFAs) being more susceptible to oxidation than saturated or monounsaturated fats. The presence of antioxidants, such as tocopherols and carotenoids in oils like red palm oil, can delay oxidation, but excessive heating and prolonged frying degrade these protective compounds, increasing the formation of harmful oxidation products (Zhang *et al*., 2020). Repeated heating of oils, a common practice in food preparation, accelerates the degradation process, leading to the

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accumulation of toxic compounds that contribute to oxidative stress, inflammation, and lipid metabolism disorders (Farhoosh & Johnny, 2018).

The frying process also has significant effects on cholesterol structure and absorption. Cholesterol, a major lipid found in animal-based foods such as eggs, meat, and dairy, is highly sensitive to oxidation when exposed to high temperatures (Pegg & Shahidi, 2017). During frying, cholesterol undergoes auto-oxidation, producing oxidized cholesterol derivatives known as oxysterols, which are linked to atherosclerosis, endothelial dysfunction, and increased cardiovascular risk (Staprans *et al*., 2000). Studies indicate that oxysterols promote pro-inflammatory responses, interfere with normal cholesterol metabolism, and contribute to plaque formation in arteries (Ighodaro & Akinloye, 2018). The extent of cholesterol oxidation is higher in fried foods than in raw or boiled counterparts, making frying a key contributor to the dietary intake of oxidized cholesterol compounds (Zhang *et al*., 2020). Additionally, the presence of oxidized cholesterol alters lipid digestion and absorption, influencing lipoprotein metabolism and increasing LDL cholesterol levels while reducing HDL cholesterol, thereby negatively impacting cardiovascular health (Debnath *et al*., 2012).

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The differences between fresh and repeatedly heated palm oil play a critical role in determining its health impact. Fresh palm oil contains bioactive compounds such as tocotrienols and carotenoids, which act as natural antioxidants that help protect against lipid peroxidation (Nagendran *et al*., 2010). However, when palm oil is repeatedly heated, its polyunsaturated fatty acids break down into hydroperoxides, aldehydes, and trans fatty acids, leading to the loss of its beneficial properties and an increase in toxic oxidation products (Adam *et al*., 2008). Studies have shown that consumption of repeatedly heated oils results in lipid abnormalities, increased levels of LDL cholesterol, and endothelial dysfunction (Owu *et al*., 2016). Furthermore, the

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oxidative stress induced by degraded oil components contributes to metabolic disorders such as insulin resistance and hypertension (Kamsiah *et al*., 2018). Research indicates that rats fed repeatedly heated palm oil showed higher levels of oxidative stress markers and cardiovascular dysfunction compared to those consuming fresh palm oil, emphasizing the health risks associated with prolonged oil reuse (Berger *et al*., 2019).

Frying significantly alters the chemical composition of lipids, leading to oxidation, the formation of free radicals, and changes in cholesterol structure, which affect nutritional quality and health outcomes. The formation of oxidized lipids and cholesterol derivatives during frying contributes to cardiovascular risk and metabolic disorders.

2.5 Histological Structure of the Heart

The heart is a highly specialized muscular organ responsible for pumping blood throughout the body to supply oxygen and nutrients to tissues while removing metabolic waste. Structurally, the heart consists of four chambers: the right atrium, right ventricle, left atrium, and left ventricle, each playing distinct roles in systemic and pulmonary circulation (Mohrman & Heller, 2018). The heart's walls are composed of three primary layers: the epicardium (outer layer), myocardium (middle muscular layer), and endocardium (inner lining) (Hall & Hall, 2020). The myocardium, which is predominantly made up of cardiomyocytes (cardiac muscle cells), connective tissue, and a network of capillaries, is the most functionally important layer, as it generates contractile force to propel blood into circulation. Cardiac tissue is highly vascularized, allowing for efficient oxygen exchange and metabolic support, critical for maintaining continuous heart function (Klabunde, 2017).

Histologically, the myocardium is characterized by branched, striated cardiomyocytes that are connected via intercalated discs, which contain desmosomes and gap junctions that facilitate

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2.1.5 Lipid Profile as a Marker of Cardiovascular Health

Frying is one of the most commonly used cooking method

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Oxidative stress plays a significant role in the pathogenesis of cardiovascular diseases (CVDs) by contributing to endothelial dysfunction, lipid peroxidation, and atherosclerosis. It is

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mechanical cohesion and electrical conductivity (Severs, 2000). These specialized junctions enable synchronous contraction of the heart muscle, ensuring efficient pumping action. Unlike skeletal muscle, cardiomyocytes exhibit a unique ability to sustain rhythmic contractions without fatigue, owing to their abundant mitochondria and reliance on oxidative metabolism (Lopaschuk *et al*., 2010). The presence of Purkinje fibers in the subendocardial region further enhances cardiac function by ensuring rapid electrical impulse transmission across the ventricles, coordinating contraction (Opthof, 2016).

Lipid metabolism plays a crucial role in maintaining cardiac energy homeostasis and structural integrity. The heart derives up to 70% of its energy from fatty acid oxidation, with the remainder coming from glucose oxidation and ketone metabolism (Stanley *et al*., 2005). Disruptions in lipid metabolism, such as excessive lipid accumulation or defective fatty acid oxidation, contribute to cardiac dysfunction, lipotoxicity, and myocardial stress, leading to conditions such as cardiomyopathy and heart failure (Fukushima *et al*., 2015). Oxidized lipids and free fatty acids, which are often elevated in metabolic disorders, are known to induce inflammatory responses, mitochondrial dysfunction, and endothelial damage, further exacerbating cardiovascular risk (Jia *et al*., 2019).

2.6 Diet-Induced Histological Changes in the Heart

The heart is highly dependent on a well-regulated balance of lipids for its structural integrity and optimal function. However, excessive dietary fat intake, particularly from high-fat diets, has been linked to profound histological changes in cardiac tissue. A high-fat diet (HFD) leads to increased lipid accumulation in myocardial cells, a condition known as cardiac lipotoxicity, which promotes metabolic stress and disrupts normal cardiac function (Fukushima *et al*., 2015). This excess lipid accumulation results in cardiomyocyte hypertrophy, mitochondrial dysfunction,

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and apoptosis, which ultimately contribute to myocardial fibrosis and contractile dysfunction (Jia *et al*., 2019). The infiltration of lipids into myocardial tissue also triggers oxidative stress and inflammation, further exacerbating structural damage to the heart (Bugger & Abel, 2014).

One of the key mechanisms linking high-fat diets to cardiac pathology is the role of oxidized lipids in inducing myocardial inflammation and fibrosis. Lipid oxidation generates reactive oxygen species (ROS), which activate inflammatory pathways and promote the infiltration of immune cells into the cardiac interstitium (Madamanchi *et al*., 2014). This inflammatory response leads to the activation of fibroblasts and excessive deposition of extracellular matrix proteins, resulting in fibrosis and cardiac stiffness (Lyon *et al*., 2015). Over time, this fibrotic remodeling compromises cardiac contractility and contributes to the development of heart failure with preserved ejection fraction (HFpEF) (Zhao *et al*., 2020). Furthermore, oxidized lipids interfere with normal endothelial function by reducing nitric oxide (NO) bioavailability, increasing vascular resistance and predisposing the heart to ischemic injury (Libby, 2021).

Lipid accumulation in the heart is a hallmark feature of metabolic syndrome and obesity-related cardiac dysfunction. Under normal physiological conditions, the heart derives most of its energy from fatty acid oxidation, with a minor contribution from glucose metabolism (Stanley *et al*., 2005). However, excessive lipid intake leads to an imbalance in lipid uptake and oxidation, resulting in the accumulation of triglycerides, ceramides, and other toxic lipid intermediates within cardiomyocytes (Fukushima *et al*., 2015). These lipid species interfere with mitochondrial function and induce lipotoxic stress, which impairs ATP production and promotes apoptotic cell death (Jia *et al*., 2019). Cardiac remodeling in response to chronic lipid overload is characterized by increased myocardial stiffness, left ventricular hypertrophy, and impaired diastolic function, all of which are common features in obesity-related cardiomyopathy (Lyon *et al*., 2015).

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High-fat diets contribute to significant histological changes in the heart, primarily through lipid accumulation, oxidative stress, and inflammatory fibrosis. The interplay between oxidized lipids, myocardial inflammation, and fibrotic remodeling underlies the pathogenesis of diet-induced cardiac dysfunction.

2.7 Wistar Rats as a Model for Nutritional Research

Wistar rats have been widely utilized nutritional research due to their well-characterized genetics, metabolic similarities to humans, and physiological responses to dietary interventions. Their controlled breeding and availability make them ideal models for studying lipid metabolism, dietary cholesterol impact, and other aspect of the health (Sengupta, 2013).

One of the main justifications for using Wistar rats in lipid metabolism studies is their metabolic resemblance to human lipid processing mechanisms. Rats, like humans, rely on hepatic regulation of cholesterol metabolism, lipid absorption, and lipoprotein distribution (Wang *et al*., 2016). The liver in both species plays a pivotal role in cholesterol synthesis and clearance, making Wistar rats an excellent model for studying the effects of dietary fats and cholesterol on lipid homeostasis. Additionally, Wistar rats exhibit diet-induced lipid imbalances, such as hypercholesterolemia and hypertriglyceridemia, which closely mirror human cardiovascular risk factors (Liu *et al*., 2018). This makes them a valuable tool in testing lipid-lowering drugs, dietary modifications, and their impact on atherosclerosis and other metabolic disorders.

Another important reason for using Wistar rats in nutritional studies is their ability to develop diet-induced metabolic diseases, including obesity, dyslipidemia, and cardiovascular dysfunction. Studies have shown that when fed a high-fat or high-cholesterol diet, Wistar rats develop significant alterations in lipid profiles, including elevated LDL cholesterol, reduced HDL cholesterol, and increased triglyceride levels, conditions that mimic human dyslipidemia (Shen *et*

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al., 2019). These diet-induced changes in lipid metabolism make Wistar rats particularly useful in evaluating the effects of dietary fats, antioxidants, and lipid peroxidation on cardiovascular health.

In addition to their role in lipid metabolism studies, Wistar rats have been extensively used to investigate the relationship between dietary cholesterol and heart histology. Several studies have demonstrated that a cholesterol-rich diet leads to histopathological changes in the heart, including increased lipid infiltration, myocardial fibrosis, and inflammatory responses (García-Ruiz *et al*., 2016). Histological examinations of the heart tissue in Wistar rats following high-cholesterol feeding have revealed cardiac hypertrophy, increased deposition of extracellular matrix proteins, and fibrosis, which are hallmarks of atherosclerosis and cardiovascular dysfunction (Huang *et al*., 2018). These findings have reinforced the importance of dietary cholesterol regulation in preventing cardiovascular diseases and have provided key insights into the molecular mechanisms underlying lipid-induced cardiac damage.

Their metabolic similarity to humans, susceptibility to diet-induced lipid disorders, and ability to develop atherosclerosis make them an ideal experimental model for studying the mechanisms of testing dietary interventions.

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Apparatus and Equipment

EDTA bottles

Test tube

Water bath (model:isotem)

Spectrophotometer (model:Evolution 201)

Cuvette

pH meter (model:Orion star A321)

Nose mask

Hand gloves

Universal bottle

Micropipettes

Syringe 5ml

Aluminium foil

Cotton wool

Mortar and pestle

Deleted[THE SPIRITMAN]: Plain

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Universal bottle

Beaker

Conical flask

Cuvettes

Centrifuge

Wash bottle

Test tubes

Micro pipette

Syringes

Mortar and pestle

Cotton wool

Aluminium foil

Spectrophotometer (model: Evolution 201)

Cages

Hand gloves

Paper tape

Polythene bag

Test tube rack

Scissor

Weighing balance

Water bath (model: isotem)

3.1.2 REAGENTS

Distilled water

Saline

Chloroform

Cholesterol Kits

Triglyceride Kits

3.1.3 ANIMAL

A total of 16 female Wistar rat were purchased from the animal house, Department of Biochemistry, Faculty of Life Science, University of Benin, Edo State. On average each rat weighed 160 ± 0.5g before sacrificing.

3.1.4 Experimental Design

A total of seventeen female Wistar rats were acquired and grouped into four categories. They were kept in individual cages at the Animal House of the Biochemistry Department at the University of Benin. During the entire experiment, the rats had constant access to both water and food. The animals were not allowed to acclimate and were given small amounts of the formulated diet.

3.1.5 Feed Formulation

A significant quantity of corn, soybean premix, and soybean oil was purchased from the market. Furthermore, one and a half crates of eggs and raw cholesterol were also obtained. And the animals were fed on the formulated pelleted feed and growers mash on a daily basis.

Group/ Formulated diet	Group 1 Egg+Red oil diet	Group 2 Cholesterol	Group 3 Positive control	Group 4 Negative control
Corn	3.2kg	3.2kg	3.2kg	3.2kg
Soyabeans	750g	750g	750g	750g
Egg yolk	250g	--	--	--
Corn oil	400g	400g	400g	400g
Vitamins	400g	400g	400g	400g
Synthetic cholesterol	--	2g	--	--

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3.1.6 Animal Sacrifice

The rats were placed on overnight fast for 12 hours before sacrifice. At the end of the feeding period of 42 days, the animal (rats) were anesthetized in a chloroform chamber on the 43rd day. Organs were excised, placed in sterile sample bags and stored in a refrigerator for further biochemical assays such as cholesterol and triglycerides analysis. Blood was also collected from the veins of the Wistar rats and 2ml was placed in each of the EDTA bottles for biochemical analysis. The homogenate was prepared by homogenizing 1g of heart in 5ml of saline water. The homogenate was centrifuge at 3000rpm for 10min.

3.2 Method

3.2.1 TRIGLYCERIDES

Principle

Lipase hydrolyzes the triglycerides to fatty acids and glycerol which in the presence of glycerolkinase and ATP, is phosphorylated to glycerol-3-phosphate and converted to glycerol-3-phosphate oxidase into Dihydroxyacetone phosphate and H_2O_2. The formed hydrogen peroxide reacts, in the presence of peroxidase, with 4-Chlorophenol and 4-Aminoantipyrin, giving a red colored compound, whose Absorbance, measured photometrically at 546nm, is proportional to the concentration of triglycerides.

Enzymatic colorimetric test:

Triglycerides + $3H_2O$ → Glycerol + 3RCOOH

Glycerol + ATP → Glycerol-3-phosphate +ADP

Glycerol-3-phosphate + O_2 → Dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminoantipyrine + p-chlorophenol → 4-(p-benzoquinone-monoiminol)-phenazone + $2H_2O$ + HCL

Procedure

Plasma volume of 10µL was pipetted into 10µL standard distilled water and 1000µL of Reagent 1(pipes buffer pH 7.8, 50mmol/l, p-Chlorophenol 2mmol/l, Lipoprotein Lipase 150000 U/I, Glycerolkinase 800 U/I, Glycerol-3-P-oxidase 400 U/I, Peroxidase 440 U/I, 4-Aminoantipyrine 0.7mmol/l, ATP 0.3mmol/l, Mg2+ 40mmol/l, Na-cholate 0.20mmol/l, Potassium-Hexacyanoferrate(II) 1µmol/l. These were mixed, incubated for 5 minutes and absorbance was read at 546nm within 60 seconds.

The working procedure is as follow:

Wavelength: 546nm

Temperature: 37°C

Measurement: Against distilled water

Pipetted as follows:

Reagent 1: 1000µL

Sample, Std/Cal/H_2O: 10µL

Calculation

Triglycerides conc. = $\frac{\Delta A \text{ sample}}{\Delta A \text{ stand}} \times$ Stand conc.

It was then mixed and incubated for 5 minutes and absorbance was read within 60 minutes.

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3.2.2 CHOLESTEROL

Principle

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Cholesterol is present in serum as cholesterol esters and free cholesterol. The cholesterol esters present in the serum are hydrolyzed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.

Procedure

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10µL of DDH_2O was added to blank with 1000µL of cholosterol reagent R1. 10µL of sample was added to 1000µL of cholesterol reagent R1. The content of each test tube was mixed and allowed to incubate for 5 minutes at 37°C, then the absorbance was read of the sample against the reagent blank at 500nm.

Calculation

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$$\text{Cholesterol conc. in sample} = \frac{\Delta\text{abs Sample}}{\Delta\text{abs standard}} \times \text{conc of standard}$$

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3.2.3 HISTOPATHOLOGICAL EXAMINATION

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Procedure

At the end of the experiment, the heart was collected and preserved in 10% neutral buffered formalin. Then it was later subjected to histological processing and dehydrated in a series of ethanol solutions for 1-2 hours. Then clear the dehydrated specimen using xylene or toluene for a period of 1-2 hours. Soak the cleared specimen in molten paraffin wax. Position the infiltrated specimen within a paraffin block, ensuring correct orientation of the heart.

Cut the paraffin block into sections that are 5-7µm thick using a microtome. Place the sections onto glass slides that have been treated with a suitable adhesive (e.g., albumen or poly-L-lysine) and apply Hematoxylin and eosin (H&E) stain to the sections to visualize the morphology of cardiac tissue. Inspect the stained sections under a light microscope to assess the morphology of the cardiac tissue, then capture photomicrographs of representative regions for documentation and further examination.

3.2.4 Statistical Analysis

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The data was presented as mean ± SEM, Analysis of Variance (ANOVA) was used to test for significant differences between the mean.

SPSS version 25 was used as the statistical tool for analyzing the data.

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CHAPTER FOUR

4.1 RESULTS

4.1.1 Measurement of the Body Weight

The average weight of female wistar rats in grammes measured before feed administrations and average weight in grammes of female wistar after feed administration is shown in the table below:

Table 4.1.1: Weight of Female Wistar

Average weight(g)	Group 1 Red oil diet	Group 2 Cholesterol	Group 3 Negative	Group 4 Positive
Weight before diet administration	42.58	39.34	45.42	42.08
Weight after diet administration (42 days)	143.54	128	126.4	132.65
Percentage increase %	245.21%	225.37%	175%	216.53%

4.1.2 LIPID PARAMETERS

Table 4.1.2: Triglycerides and Cholesterol levels in the heart of Wistar rats.

Group	Triglyceride(mg/dL)	Cholesterol(mg/dL)
Fried egg with red oil	554.19±9.16	251.90±2.64
Raw Cholesterol	577.09±9.53	307.63±7.74
Positive	569.46±5.50	259.53±4.03
Negative	558.77±7.93	277.09±9.16

KEY: TG: Triglyceride, TC: Total Cholesterol

INTERPRETATION OF RESULT

Base on the result, it shows that

TRIGLYCERIDES

Raw cholesterol fried Egg yolk with palm oil shows significant difference of 22.9mg/dL

Positive control and fried Egg yolk with palm oil, shows a significant difference of 15.27mg/dL

Negative control and fried Egg yolk with palm oil, shows a significant difference of 4.58mg/dL

TOTAL CHOLESTEROL

Raw cholesterol and fried Egg yolk with palm oil, shows a significant difference of 55.73mg/dL

Positive control and fried Egg yolk with palm oil, shows a significant difference of 7.63mg/dL

Negative control fried Egg yolk with palm oil, shows a significant difference of 25.19mg/dL

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4.1.3 Histological Result of the rats from different groups.

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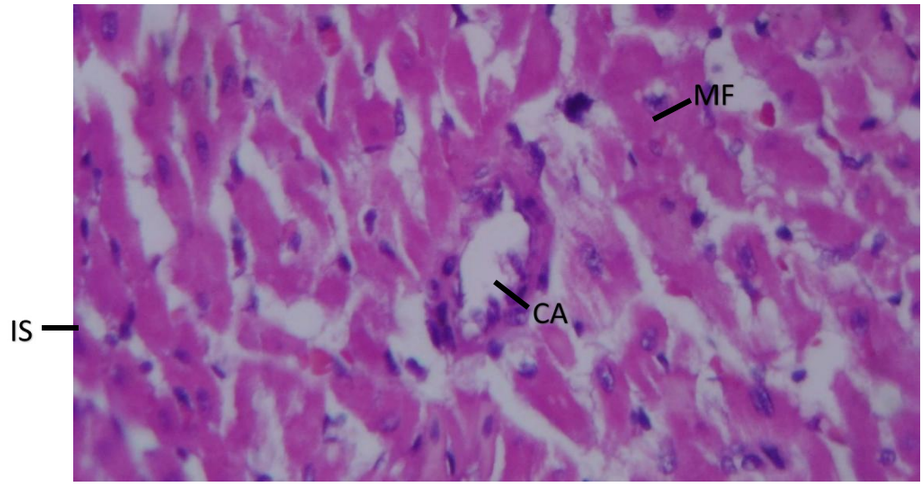

Plate 1. Rat heart normal control, show normal bundles of myocardiac fibres (MF), interstitial space (IS) and coronary artery (CA): H&E 400 X

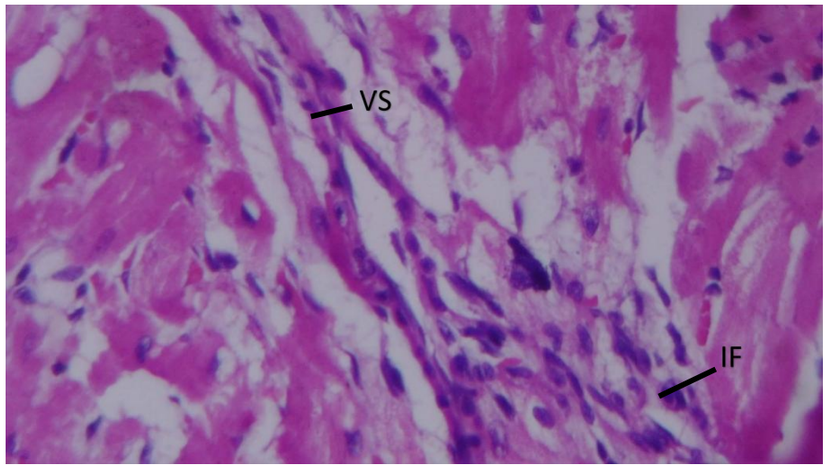

Plate 3, Rat heart given fried egg + red oil show: vascular stenosis (VS) and

Perivascular infiltrates of fibroblasts (IF): H&E 400 X

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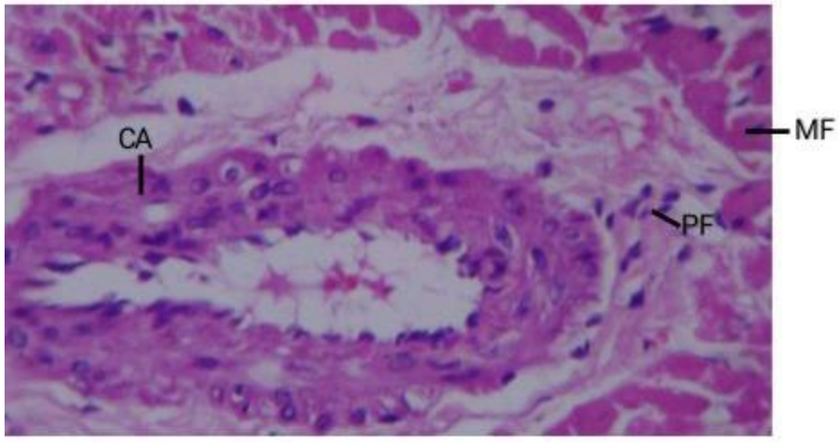

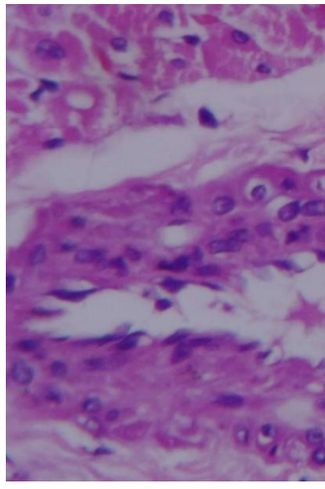

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Plate 5. Rat heart given cholesterol only show: normal bundles of cardiomyocytes (MF), coronary artery (CA) and mild perivascular mobilization of fibroblasts (PF): H&E 400 X

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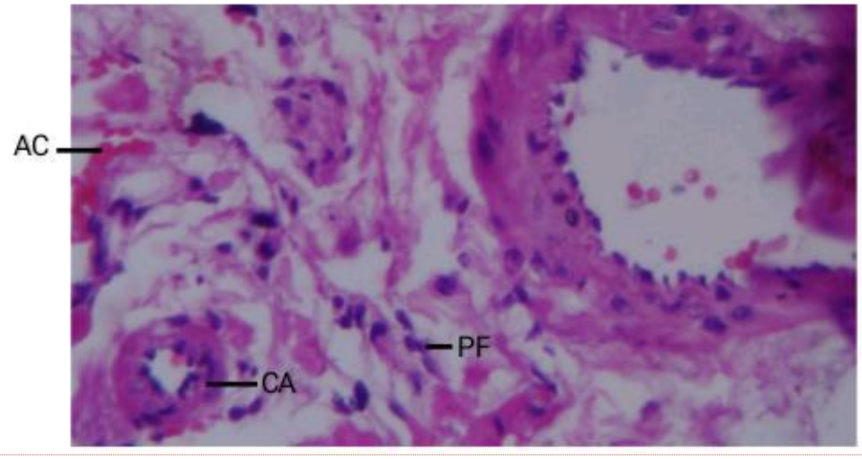

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Plate 6. Rat heart given Soya oil show: active interstitial congestion (AC), normal vascular architecture (CA) and perivascular mobilization of fibroblasts (PF): H&E 400 X

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CHAPTER FIVE

DISCUSSION AND CONCLUSION

The results of this study emphasize the effect of dietary lipids on cardiac structure and vascular dynamics. Egg yolks are heavy in cholesterol and fat, and excessive consumption can cause hyperlipidemia, which is an abnormally high level of any lipid (fat, triglycerides, cholesterol, phospholipid) or lipoprotein in the blood. The method of preparing eggs may affect the lipid's bioavailability and metabolic impact. This study examines the effect of frying egg yolk with red oil and raw cholesterol on certain lipids of wistar rats and compares the results to positive and negative groups.

TRIGLYCERIDES LEVEL

According to the results of the study, rats given fried egg with red oil (554.19±9.16mg/dL) had TG levels that were 22.9mg/dL lower than those given raw cholesterol (577.09±9.53mg/dL). This implies that frying egg with red oil could lessen the egg yolk's hyperlipidemic effects. In a similar vein, the fried egg yolk with red oil group's TG levels were 15.27mg/dL lower than those of the positive control group (569.46±5.50mg/dL) and 4.58mg/dL lower than those of the negative control group (558.77 ± 7.93 mg/dL). These results are consistent with earlier studies showing that consuming too many egg yolks fried with red oil can cause hyperlipidaemia.

TOTAL CHOLESTEROL LEVEL

The rats given fried egg with red oil (251.90±2.64mg/dL) had TC levels that were 55.73mg/dL lower than those given raw cholesterol group (307.63 ± 7.74 mg/dL). Similarly, the fried egg with red oil group's TC level were 25.19mg/dL lower than those of the positive control group (259.53±4.03) and 7.63mg/dL lower than those of the negative control group (277.09 ± 9.16 mg/dL).These results shows that lipid profiles may be impacted by the way eggs are prepared, also eating egg yolks fried with red oil can raise TC levels.

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5.1 CONCLUSION

According to this study, frying an egg with red palm oil influences lipid metabolism and cardiovascular health in Wistar rats. The results suggest that frying in red oil lowers total cholesterol (251.90 ± 2.64 mg/dL) and triglyceride levels (554.19 ± 9.16 mg/dL) compared to raw cholesterol (307.63 ± 7.74 mg/dL, 577.09 ± 9.53 mg/dL, respectively). However, histological analysis revealed vascular alterations, including vascular stenosis and perivascular fibroblast infiltration in the fried egg group. These results imply that the method of egg preparation significantly affects cholesterol bioavailability and its impact on lipid metabolism. Despite the potential cholesterol-lowering effect of frying, excessive consumption of fried eggs in red oil may still contribute to cardiovascular risks.

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Descriptives

VAR00001

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APPENDIX

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Triglycerides

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Descriptive

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	546.5567	2.64426	1.52667	539.9880	553.1254	545.03	549.61
2.00	3	554.1900	15.86559	9.16000	514.7777	593.6023	545.03	572.51

3.00	3	583.1967	6.99607	4.03918	565.8175	600.5759	577.09	590.83
4.00	3	577.0900	16.51342	9.53403	536.0684	618.1116	558.77	590.83
5.00	3	569.4567	9.53403	5.50447	545.7728	593.1405	558.77	577.09
6.00	3	558.7700	13.74000	7.93279	524.6379	592.9021	545.03	572.51
Total	18	564.8767	16.62513	3.91858	556.6092	573.1441	545.03	590.83

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2978.649	5	595.730	4.156	.020
Within Groups	1720.065	12	143.339		
Total	4698.714	17			

Multiple Comparisons

Dependent Variable: VAR00001

						95% Confidence Interval	
	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-7.63333	9.77544	.966	-40.4683	25.2016
		3.00	-36.64000*	9.77544	.026	-69.4749	-3.8051
		4.00	-30.53333	9.77544	.074	-63.3683	2.3016

		5.00	-22.90000	9.77544	.250	-55.7349	9.9349
		6.00	-12.21333	9.77544	.805	-45.0483	20.6216
	2.00	1.00	7.63333	9.77544	.966	-25.2016	40.4683
		3.00	-29.00667	9.77544	.095	-61.8416	3.8283
		4.00	-22.90000	9.77544	.250	-55.7349	9.9349
		5.00	-15.26667	9.77544	.635	-48.1016	17.5683
		6.00	-4.58000	9.77544	.996	-37.4149	28.2549
	3.00	1.00	36.64000*	9.77544	.026	3.8051	69.4749
		2.00	29.00667	9.77544	.095	-3.8283	61.8416
		4.00	6.10667	9.77544	.987	-26.7283	38.9416
		5.00	13.74000	9.77544	.724	-19.0949	46.5749
		6.00	24.42667	9.77544	.199	-8.4083	57.2616
	4.00	1.00	30.53333	9.77544	.074	-2.3016	63.3683
		2.00	22.90000	9.77544	.250	-9.9349	55.7349
		3.00	-6.10667	9.77544	.987	-38.9416	26.7283
		5.00	7.63333	9.77544	.966	-25.2016	40.4683
		6.00	18.32000	9.77544	.460	-14.5149	51.1549
	5.00	1.00	22.90000	9.77544	.250	-9.9349	55.7349
		2.00	15.26667	9.77544	.635	-17.5683	48.1016
		3.00	-13.74000	9.77544	.724	-46.5749	19.0949

		4.00	-7.63333	9.77544	.966	-40.4683	25.2016
		6.00	10.68667	9.77544	.875	-22.1483	43.5216
	6.00	1.00	12.21333	9.77544	.805	-20.6216	45.0483
		2.00	4.58000	9.77544	.996	-28.2549	37.4149
		3.00	-24.42667	9.77544	.199	-57.2616	8.4083
		4.00	-18.32000	9.77544	.460	-51.1549	14.5149
		5.00	-10.68667	9.77544	.875	-43.5216	22.1483
LSD	1.00	2.00	-7.63333	9.77544	.450	-28.9322	13.6655
		3.00	-36.64000*	9.77544	.003	-57.9388	-15.3412
		4.00	-30.53333*	9.77544	.009	-51.8322	-9.2345
		5.00	-22.90000*	9.77544	.037	-44.1988	-1.6012
		6.00	-12.21333	9.77544	.235	-33.5122	9.0855
	2.00	1.00	7.63333	9.77544	.450	-13.6655	28.9322
		3.00	-29.00667*	9.77544	.012	-50.3055	-7.7078
		4.00	-22.90000*	9.77544	.037	-44.1988	-1.6012
		5.00	-15.26667	9.77544	.144	-36.5655	6.0322
		6.00	-4.58000	9.77544	.648	-25.8788	16.7188
	3.00	1.00	36.64000*	9.77544	.003	15.3412	57.9388
		2.00	29.00667*	9.77544	.012	7.7078	50.3055
		4.00	6.10667	9.77544	.544	-15.1922	27.4055

		5.00	13.74000	9.77544	.185	-7.5588	35.0388
		6.00	24.42667*	9.77544	.028	3.1278	45.7255
	4.00	1.00	30.53333*	9.77544	.009	9.2345	51.8322
		2.00	22.90000*	9.77544	.037	1.6012	44.1988
		3.00	-6.10667	9.77544	.544	-27.4055	15.1922
		5.00	7.63333	9.77544	.450	-13.6655	28.9322
		6.00	18.32000	9.77544	.085	-2.9788	39.6188
	5.00	1.00	22.90000*	9.77544	.037	1.6012	44.1988
		2.00	15.26667	9.77544	.144	-6.0322	36.5655
		3.00	-13.74000	9.77544	.185	-35.0388	7.5588
		4.00	-7.63333	9.77544	.450	-28.9322	13.6655
		6.00	10.68667	9.77544	.296	-10.6122	31.9855
	6.00	1.00	12.21333	9.77544	.235	-9.0855	33.5122
		2.00	4.58000	9.77544	.648	-16.7188	25.8788
		3.00	-24.42667*	9.77544	.028	-45.7255	-3.1278
		4.00	-18.32000	9.77544	.085	-39.6188	2.9788
		5.00	-10.68667	9.77544	.296	-31.9855	10.6122

*. The mean difference is significant at the 0.05 level.

VAR00001

	VAR00002	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	3	546.5567	
	2.00	3	554.1900	554.1900
	6.00	3	558.7700	558.7700
	5.00	3	569.4567	569.4567
	4.00	3	577.0900	577.0900
	3.00	3		583.1967
	Sig.		.074	.095

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Cholesterol

Descriptive

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	258.0067	1.32213	.76333	254.7223	261.2910	256.48	258.77
2.00	3	251.9000	4.58000	2.64426	240.5226	263.2774	247.32	256.48
3.00	3	259.5333	5.28853	3.05333	246.3959	272.6708	256.48	265.64
4.00	3	307.6300	13.42187	7.74912	274.2882	340.9718	297.70	322.90
5.00	3	259.5333	6.99607	4.03918	242.1541	276.9125	251.90	265.64
6.00	3	277.0900	9.16000	5.28853	254.3353	299.8447	267.93	286.25
Total	18	268.9489	20.54837	4.84330	258.7304	279.1674	247.32	322.90

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ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6450.625	5	1290.125	21.284	.000

Within Groups	727.380	12	60.615		
Total	7178.005	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	6.10667	6.35689	.922	-15.2456	27.4589
		3.00	-1.52667	6.35689	1.000	-22.8789	19.8256
		4.00	-49.62333*	6.35689	.000	-70.9756	-28.2711
		5.00	-1.52667	6.35689	1.000	-22.8789	19.8256
		6.00	-19.08333	6.35689	.090	-40.4356	2.2689
	2.00	1.00	-6.10667	6.35689	.922	-27.4589	15.2456
		3.00	-7.63333	6.35689	.828	-28.9856	13.7189
		4.00	-55.73000*	6.35689	.000	-77.0823	-34.3777
		5.00	-7.63333	6.35689	.828	-28.9856	13.7189

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	6.00	-25.19000*	6.35689	.018	-46.5423	-3.8377

3.00	1.00	1.52667	6.35689	1.000	-19.8256	22.8789
	2.00	7.63333	6.35689	.828	-13.7189	28.9856
	4.00	-48.09667*	6.35689	.000	-69.4489	-26.7444
	5.00	.00000	6.35689	1.000	-21.3523	21.3523
	6.00	-17.55667	6.35689	.133	-38.9089	3.7956
4.00	1.00	49.62333*	6.35689	.000	28.2711	70.9756
	2.00	55.73000*	6.35689	.000	34.3777	77.0823
	3.00	48.09667*	6.35689	.000	26.7444	69.4489
	5.00	48.09667*	6.35689	.000	26.7444	69.4489
	6.00	30.54000*	6.35689	.004	9.1877	51.8923
5.00	1.00	1.52667	6.35689	1.000	-19.8256	22.8789
	2.00	7.63333	6.35689	.828	-13.7189	28.9856
	3.00	.00000	6.35689	1.000	-21.3523	21.3523
	4.00	-48.09667*	6.35689	.000	-69.4489	-26.7444
	6.00	-17.55667	6.35689	.133	-38.9089	3.7956
6.00	1.00	19.08333	6.35689	.090	-2.2689	40.4356
	2.00	25.19000*	6.35689	.018	3.8377	46.5423
	3.00	17.55667	6.35689	.133	-3.7956	38.9089
	4.00	-30.54000*	6.35689	.004	-51.8923	-9.1877
	5.00	17.55667	6.35689	.133	-3.7956	38.9089

LSD	1.00	2.00	6.10667	6.35689	.356	-7.7438	19.9571
		3.00	-1.52667	6.35689	.814	-15.3771	12.3238
		4.00	-49.62333*	6.35689	.000	-63.4738	-35.7729
		5.00	-1.52667	6.35689	.814	-15.3771	12.3238
		6.00	-19.08333*	6.35689	.011	-32.9338	-5.2329
	2.00	1.00	-6.10667	6.35689	.356	-19.9571	7.7438
		3.00	-7.63333	6.35689	.253	-21.4838	6.2171
		4.00	-55.73000*	6.35689	.000	-69.5805	-41.8795
		5.00	-7.63333	6.35689	.253	-21.4838	6.2171
		6.00	-25.19000*	6.35689	.002	-39.0405	-11.3395
	3.00	1.00	1.52667	6.35689	.814	-12.3238	15.3771
		2.00	7.63333	6.35689	.253	-6.2171	21.4838
		4.00	-48.09667*	6.35689	.000	-61.9471	-34.2462
		5.00	.00000	6.35689	1.000	-13.8505	13.8505
		6.00	-17.55667*	6.35689	.017	-31.4071	-3.7062
	4.00	1.00	49.62333*	6.35689	.000	35.7729	63.4738
		2.00	55.73000*	6.35689	.000	41.8795	69.5805
		3.00	48.09667*	6.35689	.000	34.2462	61.9471
		5.00	48.09667*	6.35689	.000	34.2462	61.9471
		6.00	30.54000*	6.35689	.000	16.6895	44.3905

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LSD	1.00	2.00	6.10667	6.35689	.356	-7.7438	19.9571
		3.00	-1.52667	6.35689	.814	-15.3771	12.3238
		4.00	-49.62333*	6.35689	.000	-63.4738	-35.7729
		5.00	-1.52667	6.35689	.814	-15.3771	12.3238
		6.00	-19.08333*	6.35689	.011	-32.9338	-5.2329
	2.00	1.00	-6.10667	6.35689	.356	-19.9571	7.7438
		3.00	-7.63333	6.35689	.253	-21.4838	6.2171
		4.00	-55.73000*	6.35689	.000	-69.5805	-41.8795
		5.00	-7.63333	6.35689	.253	-21.4838	6.2171
		6.00	-25.19000*	6.35689	.002	-39.0405	-11.3395
	3.00	1.00	1.52667	6.35689	.814	-12.3238	15.3771
		2.00	7.63333	6.35689	.253	-6.2171	21.4838
		4.00	-48.09667*	6.35689	.000	-61.9471	-34.2462
		5.00	.00000	6.35689	1.000	-13.8505	13.8505
		6.00	-17.55667*	6.35689	.017	-31.4071	-3.7062
	4.00	1.00	49.62333*	6.35689	.000	35.7729	63.4738
		2.00	55.73000*	6.35689	.000	41.8795	69.5805
		3.00	48.09667*	6.35689	.000	34.2462	61.9471
		5.00	48.09667*	6.35689	.000	34.2462	61.9471
		6.00	30.54000*	6.35689	.000	16.6895	44.3905

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5.00	1.00	1.52667	6.35689	.814	-12.3238	15.3771
	2.00	7.63333	6.35689	.253	-6.2171	21.4838
	3.00	.00000	6.35689	1.000	-13.8505	13.8505
	4.00	-48.09667*	6.35689	.000	-61.9471	-34.2462
	6.00	-17.55667*	6.35689	.017	-31.4071	-3.7062
6.00	1.00	19.08333*	6.35689	.011	5.2329	32.9338
	2.00	25.19000*	6.35689	.002	11.3395	39.0405
	3.00	17.55667*	6.35689	.017	3.7062	31.4071
	4.00	-30.54000*	6.35689	.000	-44.3905	-16.6895
	5.00	17.55667*	6.35689	.017	3.7062	31.4071

*. The mean difference is significant at the 0.05 level.

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VAR00001

			Subset for alpha = 0.05		
	VAR00002	N	1	2	3
Tukey HSD[a]	2.00	3	251.9000		
	1.00	3	258.0067	258.0067	
	5.00	3	259.5333	259.5333	
	3.00	3	259.5333	259.5333	

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	6.00	3		277.0900	
	4.00	3			307.6300
	Sig.		.828	.090	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

VAR00001

			Subset for alpha = 0.05		
	VAR00002	N	1	2	3
Tukey HSD[a]	2.00	3	251.9000		
	1.00	3	258.0067	258.0067	
	5.00	3	259.5333	259.5333	
	3.00	3	259.5333	259.5333	
	6.00	3		277.0900	
	4.00	3			307.6300
	Sig.		.828	.090	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

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