Dietary red palm oil inclusion on growth performance, haematology, serum biochemistry, lipid profile, and oxidative stress markers of Wistar rats

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Abstract

Red palm oil (RPO), rich in carotenoids, tocopherols and tocotrienols, has been associated with antioxidant, lipid-modulating and haematopoietic effects. However, its effects on growth performance, haematology, serum biochemistry, lipid metabolism and oxidative status remain debated. This study evaluated graded dietary RPO inclusion in Wistar rats over 42 days. Forty-eight 60-day-old rats (initial body weight 149 ± 1.0 g; 50:50 males and females) were individually housed and randomly assigned to four treatments (n = 12): basal diet containing 0 (control), 200, 300, or 400 mg RPO kg diet^{-1}. RPO was manually incorporated into the diet and was not chemically characterised. Growth performance was monitored daily and weekly. At the end of the trial, blood samples from eight rats per treatment were analysed for haematological, serum biochemical, lipid and oxidative stress indices. Growth data were analysed using a 2×4 factorial arrangement (gender×diet), while treatment effects were assessed by analysis of variance and Student–Newman–Keuls mean separation ($p < 0.05$). Dietary RPO significantly influenced weight gain and daily weight gain in males compared to females ($p < 0.05$). Treatment × gender interactions were observed for daily weight gain, weight gain, and feed conversion ratio, whereas feed intake and feed efficiency were unaffected. Red blood cell counts were higher in the control and 200 and 400 mg kg^{-1} groups than in the 300 mg kg^{-1} group. White blood cell counts declined progressively across RPO-supplemented groups. Haemoglobin concentrations were higher at 200 and 400 mg kg^{-1} than in the control and 300 mg kg^{-1} groups. Mean corpuscular volume and mean corpuscular haemoglobin were higher at 300 and 400 mg kg^{-1}, while platelet counts were lower at 200 mg kg^{-1} than in the other groups. Serum urea increased with RPO inclusion, whereas creatinine was highest at 300 mg kg^{-1} ($p < 0.05$). Total cholesterol (55.62 mg dL^{-1}), triglycerides (83.70 mg dL^{-1}), and low-density lipoprotein cholesterol (13.00 mg dL^{-1}) decreased significantly following RPO inclusion ($p < 0.05$). High-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, and total cholesterol-to-HDL ratio were unaffected ($p > 0.05$). Catalase (9.78: $p < 0.04$), glutathione peroxidase (44.26: $p < 0.05$), superoxide dismutase (1.51: $p < 0.05$), glutathione (0.36: $p < 0.04$), and malondialdehyde (15.31: $p < 0.02$) were significantly influenced by dietary treatment, with the highest mean values varying across inclusion levels. Dietary RPO at 200–400 mg kg^{-1} produced non-linear, biomarker-specific and gender-dependent responses, with no inclusion level uniformly favourable. These findings indicate a modulatory rather than consistently protective effect under the conditions investigated. Larger, longer-term, gender-stratified studies using chemically characterised RPO and incorporating tissue- and pathway-level analyses are needed before optimal inclusion levels can be recommended.

Keywords: red palm oil, Wistar rats, haematology, lipid profile, oxidative stress.

Inclusão dietética de óleo de palma vermelho no desempenho de crescimento, hematologia, bioquímica sérica, perfil lipídico e marcadores de estresse

oxidativo de ratos Wistar

Resumo

O óleo de palma vermelho (OPV), rico em carotenoides, tocoferóis e tocotrienóis, tem sido associado a efeitos antioxidantes, moduladores do perfil lipídico e hematopoéticos. Contudo, seus efeitos sobre o desempenho de crescimento, a hematologia, a bioquímica sérica, o metabolismo lipídico e o status oxidativo permanecem controversos. Este estudo avaliou a inclusão gradual de OPV na dieta de ratos Wistar durante 42 dias. Quarenta e oito ratos com 60 dias de idade (peso corporal inicial de 149 ± 1,0 g; 50:50 machos e fêmeas) foram alojados individualmente e distribuídos aleatoriamente em quatro tratamentos (n = 12): dieta basal com 0 (controle), 200, 300 ou 400 mg de OPV kg de dieta^{-1}. O OPV foi incorporado manualmente à dieta e não foi caracterizado quimicamente. O desempenho foi monitorado diariamente e semanalmente. Ao final do ensaio, amostras de sangue de oito ratos por tratamento foram analisadas quanto aos índices hematológicos, bioquímicos séricos, lipídicos e de estresse oxidativo. Os dados de desempenho foram analisados em arranjo fatorial 2 × 4 (sexo × dieta), e os efeitos dos tratamentos foram analisados por análise de variância e pelo teste de Student–Newman–Keuls ($p <$ 0,05). O OPV influenciou o ganho de peso e o ganho de peso diário dos machos em relação às fêmeas ($p < 0,05$). Houve interação tratamento × sexo para ganho de peso diário, ganho de peso e conversão alimentar, enquanto o consumo e a eficiência alimentar não foram afetados. A contagem de eritrócitos foi maior nos grupos controle, 200 e 400 mg kg^{-1} do que no grupo 300 mg kg^{-1}. Os leucócitos diminuíram progressivamente nos grupos suplementados. A hemoglobina foi maior com 200 e 400 mg/kg do que no controle e com 300 mg kg^{-1}. O volume corpuscular médio e a hemoglobina corpuscular média foram maiores com 300 e 400 mg kg^{-1}, e as plaquetas foram menores com 200 mg kg^{-1}. A ureia sérica aumentou com a inclusão de OPV, e a creatinina foi maior com 300 mg kg^{-1} ($p <$ 0,05). O colesterol total (55,62 mg dL^{-1}), os triglicerídeos (83,70 mg dL^{-1}) e o LDL-colesterol (13,00 mg dL^{-1}) diminuíram com a inclusão de OPV ($p < 0,05$). O HDL-colesterol, o VLDL-colesterol e a razão colesterol total/HDL não foram afetados ($p > 0,05$). Catalase (9,78; $p < 0,04$), glutationa peroxidase (44,26; $p < 0,05$), superóxido dismutase (1,51; $p < 0,05$), glutationa (0,36; $p < 0,04$) e malondialdeído (15,31; $p < 0,02$) foram influenciados pelo tratamento, com as maiores médias variando entre os níveis de inclusão. O OPV entre 200 e 400 mg kg^{-1} produziu respostas não lineares, específicas de cada biomarcador e dependentes do sexo, sem nível de inclusão uniformemente favorável. Esses resultados indicam efeito modulador, e não consistentemente protetor, nas condições investigadas. Estudos maiores, mais longos, estratificados por sexo, com OPV quimicamente caracterizado e análises teciduais e de vias metabólicas são necessários antes da recomendação de níveis ótimos de inclusão.

Palavras-chave: óleo de palma vermelho, ratos Wistar, hematologia, perfil lipídico, estresse oxidativo.

1. Introduction

Red palm oil (RPO; *Elaeis guineensis* Jacq.) is a widely consumed vegetable oil extracted from the mesocarp of the oil palm fruit. Two distinct oils are derived from the fruit: RPO and palm kernel oil. Although they share the same botanical origin, they differ markedly in fatty acid composition and nutritional profile. Originating in West Africa, oil palm cultivation has expanded to other tropical regions, including Southeast Asia and South America. Indonesia is currently the world's leading producer and exporter of palm oil. Global palm oil production is forecast at approximately 80 million metric tonnes in 2025/26, out of 234.5 million metric tonnes of major vegetable oils, representing about 34% of total production (USDA, 2025). Because of its stability, affordability, and nutritional qualities, RPO has become an important substitute for animal fats and hydrogenated oils in the food industry.

The characteristic reddish colour of RPO is primarily attributable to its high carotenoid content, while its other phytonutrients include vitamin E compounds (tocopherols and tocotrienols), phytosterols, phospholipids, glycolipids, and coenzyme Q10 (Madoromae; Lertcanawanichakul, 2025). These bioactive compounds endow the RPO with strong oxidative stability and confer multiple health benefits. Carotenes, particularly α- and β-carotene, serve as provitamin A precursors, thereby helping to prevent vitamin A deficiency, protect vision, and support tissue integrity and growth.

Tocotrienols, members of the vitamin E family, have been reported to inhibit cholesterol biosynthesis by suppressing the activity and promoting the degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key regulatory enzyme in cholesterol synthesis (Ranasinghe et al., 2022). Additionally, tocotrienols act as potent antioxidants with roles in protecting against cancer, cardiovascular disorders, neurodegeneration, immune dysregulation, and oxidative stress (Meganathan; Fu, 2016). Madoromae & Lertcanawanichakul (2025)

reported that although palm oil contains a considerable proportion of saturated fatty acids, its naturally occurring bioactive compounds, including carotenoids, tocopherols, and tocotrienols, may contribute to its antioxidant and potential cardioprotective effects.

The authors concluded that palm oil consumed in realistic amounts as part of a balanced diet does not necessarily increase cardiovascular disease risk. Furthermore, these compounds exhibit antioxidant, cardioprotective, cholesterol-lowering, anti-inflammatory, and other health-promoting activities. They may protect tissues from oxidative damage and contribute to the beneficial biological effects associated with palm oil consumption (Loganathan et al., 2017).

Beyond its micronutrient content, the fatty acid composition of RPO has drawn considerable attention. Approximately 44% of its fatty acids are palmitic acid, accompanied by 5% stearic acid, 39% monounsaturated oleic acid, and 11% polyunsaturated linoleic acid (Madoromae; Lertcanawanichakul, 2025). Importantly, palmitic acid is often criticised for its hypercholesterolemic potential; in RPO it is primarily esterified in the sn-1 and sn-3 positions of triacylglycerols rather than the sn-2 position, a structural arrangement that mitigates its cholesterol-raising effect compared to animal fats (Syarifah-Noratiqah et al., 2020). Unlike many animal fats, RPO contains negligible amounts of lauric and myristic acids—saturated fatty acids most strongly associated with increases in serum cholesterol. Consequently, RPO has been suggested to reduce risks of arterial thrombosis and atherosclerosis, suppress platelet aggregation, lower blood pressure, and inhibit endogenous cholesterol synthesis, with primary or meta-analytic evidence (Mancini et al., 2015).

Despite these promising attributes, the role of RPO in human health remains controversial. Concerns about saturated fats in general, and palmitic acid in particular, have fuelled debates linking palm oil consumption to cardiovascular disease (Loganathan et al., 2017). Yet, both animal and human studies suggest that RPO is not inherently atherogenic and does not consistently elevate serum cholesterol levels (Utama et al., 2024). In fact, several investigations have demonstrated that RPO supplementation can increase serum retinol in populations at risk of vitamin A deficiency, improve lipid profiles relative to other dietary fats, and attenuate cholesterol-induced atherosclerosis in experimental animals (Loganathan et al., 2017).

From a culinary perspective, RPO contributes to the sensory qualities of food by enhancing palatability, flavour release, and satiety (Gesteiro et al., 2019). It also facilitates the absorption of fat-soluble vitamins and provides a dense source of energy with a relatively low thermogenic effect. Its wide use in processed foods is partly due to its trans-fat-free profile, which makes it a safer alternative to hydrogenated oils. In contrast, trans fats are strongly associated with adverse health outcomes, including cardiovascular diseases, diabetes, infertility, and neurodegenerative conditions such as Alzheimer's disease (Abd El-aal et al., 2019). Thus, while palm oil's saturated fat content remains contentious, its absence of harmful trans fats positions it more favourably in modern diets.

Red palm oil (RPO) is a natural edible oil characterized by a balanced ratio of saturated and unsaturated fatty acids and is recognized as a rich source of fat-soluble antioxidants, including vitamin E, tocopherols, tocotrienols, and carotenoids. These bioactive constituents confer multiple health benefits, notably antioxidant, anti-inflammatory, cardioprotective, and hepatoprotective effects, particularly under pathological conditions (Ajuwon et al., 2022). Haematological indices are essential biomarkers for evaluating the physiological and pathological status of both humans and animals.

They provide critical insights into the functional integrity of the blood and immune systems. Alterations in these parameters often serve as early indicators of systemic pathology (Ugbogu et al., 2019). In rodent models, acute liver injury is often precipitated by oxidative stress and inflammatory responses. The liver plays a central role in nutrient metabolism and detoxification, processing exogenous substances such as medications and xenobiotics (Rahmouni et al., 2017). Serum biochemical parameters, therefore, serve as important indicators of hepatic and renal status in response to dietary interventions.

Recent scientific attention has focused particularly on the antioxidant potential of RPO. Its carotenoids and vitamin E compounds act synergistically to enhance endogenous antioxidant defences. Studies in Wistar rats have shown that dietary RPO increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase, while reducing malondialdehyde (MDA), a biomarker of lipid peroxidation (Ajuwon et al., 2022). Moreover, RPO supplementation has been linked to the restoration of glutathione redox balance and activation of Nrf2-mediated antioxidant signalling, thereby protecting against oxidative damage (Ajuwon et al., 2022). In models of lipopolysaccharide-induced liver injury, RPO has also been shown to suppress NF-κB-mediated inflammatory pathways (Ajuwon et al., 2022). These findings highlight RPO's dual role as both a dietary fat and a functional food with therapeutic potential.

Nevertheless, despite the growing body of evidence, the literature on the physiological impact of RPO remains incomplete. Many studies have focused narrowly on specific biochemical or antioxidant outcomes, with limited integration of haematological parameters, lipid metabolism, and oxidative stress markers under a 42-day dietary exposure. Addressing these gaps is important for clarifying the health implications of RPO, especially in the context of global dietary transitions and increasing reliance on palm oil as a major fat source. The present study was therefore designed to investigate the effects of graded dietary inclusion levels of RPO on growth performance, haematological indices, serum biochemistry, lipid profiles, and oxidative stress in Wistar rats.

The hypothesis was that increasing dietary RPO inclusion would improve lipid profiles, enhance growth performance, antioxidant status, and influence haematological indices and serum biochemical parameters. The inclusion levels were selected to assess the physiological responses of Wistar rats to graded RPO supplementation over a 42-day feeding period.

2. Materials and Methods

2.1 The study location

The study was performed at the Animal Science Unit of the Teaching and Research Farm, Department of Agricultural Science, Alvan Ikoku Federal University of Education, Owerri, Imo State, Nigeria. The study area lies between 4°45'N and 7°15'N latitude and 6°50'E to 7°25'E longitude, with an altitude of 200 m above sea level (Imo State, Nigeria). The weather conditions of the area are characterized by a mean annual rainfall of 1850 mm with high relative humidity of over 75%, wet season of 6-8 months, average annual ambient temperature of 27 °C (80 °F) on a vegetation of humid rainforest (Onafeso, 2023).

2.2 Experimental animals and housing

The Wistar rats were housed in metabolic cages. The Wistar rats (n = 48), both genders (male/female - 50:50), aged 60 days and initial body weight of 149 ± 1.0 g bought from Covenant Farm, Gbolasire area, Iwo Road, Ibadan, Oyo State were used for the study. They were housed individually in 14 (length) x 14 (width) x 15 (height) cm metabolic cages, at a room temperature of 27 ± 2 °C. A light: dark cycle of 13 h: 11 h. All the animals were acclimated for one week in the animal house before the 6-week feeding trial with free access to feed and water. The experimental animals were maintained under standardised animal house conditions and observed daily throughout the feeding trials. The Wistar rats were randomly allocated to the experimental groups, with initial body weights considered to ensure comparability among the groups at the commencement of the study.

2.3 Animal feed and RPO preparation

The conventional rat diet prepared by Top Feed Mills, Sapele, Delta State, Nigeria, on a dry matter basis was used for the study. Red palm oil (RPO) was purchased from a local factory in Owerri, Imo State. The rats were fed a commercial basal diet containing soybean meal, grains and grain by-products, vegetable oil, limestone, dicalcium phosphate, salt, and vitamin–mineral premixes. The manufacturer's stated specifications were 20% crude protein, 5% crude fat, 5% crude fibre, 1% calcium and 0.45% available phosphorus. However, the ingredient proportions and nutrient composition of the diet were not independently verified, and the red palm oil (RPO) was not analysed to determine its chemical composition.

The experimental diets were prepared by incorporating graded levels of RPO (0, 200, 300, and 400 mg kg of diet^{-1}) into the basal diet. The RPO was manually mixed with the basal diet and left in a cool, dry place at room temperature overnight before the commencement of the feeding trial. Forty-eight Wistar rats were randomly assigned to four dietary treatments, with 12 animals per treatment, receiving 0, 200, 300, and 400 mg RPO kg of diet^{-1} for treatments 1 (control), 2, 3, and 4, respectively. The animals were fed the treatment diets for six weeks. For each animal, 15 g of feed was weighed and offered daily, and the residual feed was removed, weighed, and recorded the following morning.

2.4 Growth indices

The growth indices of Wistar rats (n = 12) were monitored and measured daily and weekly. Weight gain, daily weight gain (DWG), feed intake, daily feed intake (DFI), feed conversion ratio (FCR), and feed efficiency of individual Wistar rats were measured and recorded. FCR is calculated by dividing DFI by DWG. No mortality or

any signs of disease were reported in any feeding group during the feeding period.

2.5 Blood collection and serum separation

At the end of the six-week feeding trial, all the Wistar rats were fasted for 12-h overnight thereafter, blood samples (1 - 2 mL) were collected from eight (8) animals per treatment (n = 8) between 08: 00 - 09: 00 hours from by cardiac puncture, under chloroform anaesthesia (with an approved and appropriate anaesthetic protocol) into sterile tubes with ethylene diamine tetra-acetic acid (EDTA) for haematology and plain tubes for serum biochemistry, lipid profile and oxidative stress markers analysis. The contents of the plain tubes were centrifuged at 1,500 × g for 15 min at 4 °C to separate the serum. The separated sera were decanted into *Eppendorf* tubes and stored in a -20 °C freezer until analysed.

2.6 Haematological and serum biochemical analysis

Haematological indices such as red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and packed cell volume (PCV) were determined according to the described protocol. Red blood cell (RBC) count was determined using a haemocytometer after appropriate dilution of the blood sample. Haemoglobin concentration was measured using the cyanmethaemoglobin method, while packed cell volume (PCV) was determined using the microhaematocrit method.

Platelet count was determined using a haemocytometer, and differential leukocyte count was performed on blood smears stained with Giemsa stain by identifying and counting the different types of white blood cells under a light microscope. Total WBCs were determined using a Neubauer haemocytometer after dilution. Blood constants: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined using Equations 1, 2, and 3.

$$\text{MCV (fL)} = \frac{\text{Haematocrit (\%)} \times 10}{\text{RBC count (million/µL)}} \quad (1)$$

$$\text{MCH (pg/cell)} = \frac{\text{Haemoglobin (\%)} \times 10}{\text{RBC count (million/µL)}} \quad (2)$$

$$\text{MCHC (g/dL)} = \frac{\text{Haemoglobin (g/100 mL of blood)} \times 100}{\text{Packed cell volume (mL/100 mL of blood)}} \quad (3)$$

Where: MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RBC = red blood cell.

Total protein was determined using the Biuret method, while albumin was measured using the bromocresol green method. Globulin was calculated by subtracting albumin from total protein, and the albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value. Glucose, urea, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were determined using spectrophotometric methods with kits from Randox Laboratories Ltd., Crumlin, United Kingdom, according to the manufacturer's protocols.

2.7 Lipid profile

Total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were evaluated in eight (8) animals per treatment. The lipid profile analysis was carried out using a chemistry auto-analysing kit, with an appropriate enzymatic method and a digital colorimeter. The serum TC was determined by the enzymatic colorimetric method. The serum HDL was determined by the dextran sulphate-magnesium (II) precipitation method. The glycerol phosphate oxidase enzymatic method was used to determine the serum triglyceride. The very low-density lipoprotein (VLDL) was calculated by dividing the serum triglyceride by 5, while the serum LDL was calculated using the Friedewald formula (Meeusen et al., 2015).

2.8 Measurement of antioxidant enzymes

After allowing the blood to clot, the serum was separated by centrifugation, decanted, and stored at -20 °C until analysis of serum enzyme activity. Oxidative stress markers, including total antioxidant capacity, catalase, superoxide dismutase, glutathione, glutathione peroxidase, and lipid peroxidation (malondialdehyde), were measured according to the previously established protocols described by Jimoh (2019).

2.8.1 Catalase

A volume of 50 µL of the serum supernatant was mixed with 2 mL of phosphate buffer (pH 7.0) and 1 mL of 30 mM H_2O_2 in a cuvette. Catalase (CAT) activity was measured spectrophotometrically using a Spectroquant Pharo 300 (Merck, Germany) at 240 nm and was expressed as micromoles of H_2O_2 consumed per minute per mg of protein.

2.8.2 Superoxide dismutase

The final assay mixture (1 mL) contained 75 mM Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM pyrogallol. At the end, 50 µl of serum supernatant was added as the enzyme source, and the absorbance was measured spectrophotometrically at 420 nm. The activity was expressed as units per mg of protein.

2.8.3 Glutathione Peroxidase

The final reaction mixture (1 mL) contained 0.05 M phosphate buffer (pH 7.0) with 2 mM EDTA, 0.025 mM sodium azide, 0.15 mM reduced glutathione, 0.25 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 100 µL of 5% serum supernatant as the enzyme source. The reaction was initiated by adding 0.36 mM H_2O_2. Absorbance was measured spectrophotometrically at 340 nm, and the activity was expressed as nmol of NADPH oxidized per minute per mg of protein.

2.8.4 Glutathione

A volume of 1.0 mL of the 5% serum supernatant was combined with 0.1 mL of 25% trichloroacetic acid (TCA), and the mixture was centrifuged at 5000×g for 10 min to remove the precipitate. Then, 0.1 mL of the resulting supernatant was added to 2 mL of 0.6 mM Ellman's reagent prepared in 0.2 M sodium phosphate buffer (pH 8.0). Absorbance was measured at 412 nm, and results were expressed as nmol per mg of protein.

2.8.5 Lipid peroxidation

Lipid peroxidation was assessed using the thiobarbituric acid (TBA) method by measuring the formation of malondialdehyde (MDA), a key end-product of lipid peroxidation. To 1 mL of 10% serum supernatant, 2.5 mL of 20% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3500×g for 10 min. The resulting precipitate was dissolved in 2.5 mL of 0.05 M sulfuric acid, followed by the addition of 3 mL of TBA reagent. The samples were then incubated in a hot water bath for 30 min. After cooling, MDA was extracted with 4 mL of butanol, and the resulting pink color was measured spectrophotometrically at 530 nm. Results were expressed as nmol of MDA per mg of protein.

2.8.6 Total antioxidant capacity

Serum total antioxidant capacity (TAC) was determined using the method described by Jimoh (2019). Serum total antioxidant capacity (TAC) was assessed using a spectrophotometric assay to estimate the overall antioxidant activity of the sample.

2.9 Statistical analysis

For the experiment, data are presented as mean ± SEM (standard error of the mean) or pooled SEM, and $p < 0.05$ was considered statistically significant. For performance indices, all animals per group (n = 12) were included in the analysis using a 2 × 4 factorial design, with gender and dietary treatment as factors, including their main effects and interaction. Eight animals per group (n = 8) were used for the analysis of haematological variables, serum biochemical indices, lipid profiles, and oxidative stress biomarkers.

Each sample was analysed in triplicate. Performance data were analysed using a 2 × 4 factorial design, while haematological variables, serum biochemical indices, lipid profiles, and oxidative stress biomarkers were analysed using one-way analysis of variance (ANOVA) in GenStat, 18th Edition (VSN International, Hemel Hempstead, United Kingdom), with dietary inclusion levels as treatments. The normality of residuals and homogeneity of variances were assessed to verify the assumptions of ANOVA. Student–Newman–Keuls multiple-comparison test was used to determine differences among dietary inclusion levels.

3. Results

3.1 Growth Performance

Growth performance of Wistar rats fed varied levels of red palm oil (RPO) inclusion is expressed in (Figure 1). The results showed that the varied levels of RPO inclusion in the diet of Wistar rats increased ($p < 0.05$) weight gain and daily weight gain in male rats compared to female rats (Figure 1). However, daily feed intake (DFI), feed intake (FI), feed efficiency (FE), and feed conversion ratio (FCR) ($p > 0.05$) were not affected by gender and treatment. Additionally, there was a treatment by gender interaction effect ($p < 0.05$) on daily weight gain, weight gain, and feed conversion ratio. This revealed that daily weight gain, weight gain, and feed conversion ratio of males were improved across treatments.

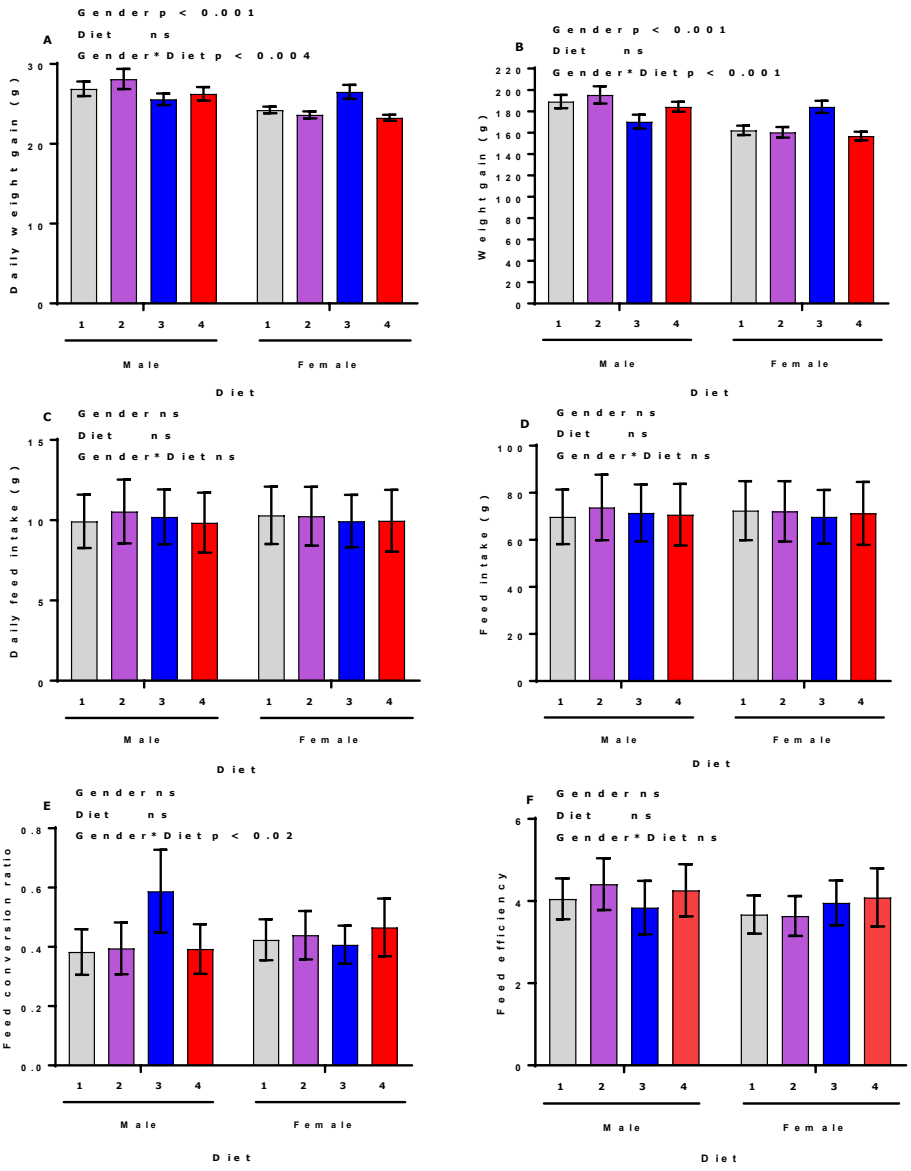

Figure 1. Effect of red palm oil inclusion on growth performance of Wistar rats. The varied levels of red palm oil inclusion were added at 0, 200, 300, and 400 mg kg^{-1} to animal feeds of both genders (males and females: n = 12/treatment). The effect of treatment is shown on (a) Daily weight gain (g), (b) Weight gain (g), (c) Daily feed intake (g), (d) Feed intake (g), (e) feed conversion ratio, and (f) feed efficiency. The data are mean ± SEM (SEM: standard error of the mean). Source: Authors, 2026.

3.2 Haematological variables

The effect of varied levels of RPO inclusion on the haematological variables of Wistar rats is shown in (Table 1). The results showed a significant difference ($p < 0.05$) of the control and the dietary treatment groups in red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelets, whereas packed cell volume, mean corpuscular haemoglobin concentration (MCHC), and all the differential counts were unaffected. The effect on the concentration of RBC (10^6 mm^3) was significantly higher ($p < 0.05$) in groups 2, 1, and 4 than in group 3.

Similarly, the WBC decreased significantly ($p < 0.05$) in a dose-related trend within the RPO-treated groups. The PCV (%) of the animals was not significantly different ($p > 0.05$) between the RPO-treated groups, although a numerical increase was observed in group 4 compared to the control group and groups 2 and 3, respectively. The

haemoglobin (Hb) level (g dL) was significantly higher in the 400 mg kg^{-1} and 200 mg kg^{-1} RPO-treated groups of 4 and 2 than those in groups 1 and 3 (Table 1).

The effect of RPO on MCV (μm^3) and MCH (pg) had the same trend. The animals fed diets 3 and 4 had significantly higher MCV and MCH ($p < 0.05$) compared to the control and diet 2, respectively. The platelets ($\times 10^3/\mu L$) of the group fed the control, diets 3 and 4 were significantly higher ($p < 0.05$) than those fed diet 2 (Table 1).

Table 1. Effect of red palm oil inclusion on haematological variables of Wistar rats.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Pooled SEM	P value
Red blood cell (10^6 mm^3)	6.19^{ab}	6.44^{a}	4.66^{b}	5.93^{ab}	0.40	0.05
White blood cell (10^3 mm^3)	2.13^{a}	2.15^{a}	1.96^{ab}	1.80^{b}	0.20	0.03
Packed cell volume (%)	44.33	45.33	44.00	49.33	1.70	0.19
Haemoglobin (Hb) (g dL)	14.78^{b}	15.11^{ab}	14.67^{b}	16.44^{a}	0.60	0.04
Mean corpuscular volume (fL)	72.00^{b}	70.40^{b}	95.80^{a}	83.90^{ab}	25.40	0.05
Mean corpuscular haemoglobin (pg)	23.88^{b}	23.46^{b}	31.48^{a}	27.72^{ab}	1.50	0.04
Mean corpuscular haemoglobin concentration (g dL)	33.33	33.32	33.33	33.34	0.00	0.19
Platelets (10^3 µL)	8033.33^{ab}	6800.00^{b}	8466.77^{a}	7833.33^{ab}	27.06	0.02
Neutrophils (%)	43.70	42.30	41.70	37.70	4.16	0.80
Lymphocytes (%)	56.30	57.70	58.30	62.30	4.17	0.80
Monocytes (%)	0.00	0.00	0.33	0.33	0.19	0.45

Note: a, ab, b: Means in the same row with different superscript letters are significantly different ($p < 0.05$). Diets (n = 8/treatment; both genders (male/female rats): 50:50 ratio). SEM: Standard Error of the Mean. Diet 1: Basal diet (control) 0 mg kg^{-1}; Diet 2: Diet 1 + Red palm oil at 200 mg kg^{-1}; Diet 3: Diet 1 + Red palm oil at 300 mg kg^{-1}; Diet 4: Diet 1 + Red palm oil at 400 mg kg^{-1}. Source: Authors, 2026.

3.3 The serum biochemical indices

The effect of varied levels of RPO inclusion on the serum biochemistry of Wistar rats is shown in Table 2. The results of the concentrations of urea, creatinine, and alkaline phosphatase showed significant differences ($p < 0.05$) between the control and the treated groups. The control recorded increased ($p < 0.05$) urea concentration (mg/dl) than those fed diets 2, 3, and 4 in a dose-related trend within the RPO-treated groups.

The creatinine concentration (mg dL^{-1}) of the animals in group 3 was significantly higher ($p < 0.05$) than those in group 2, control, and 4. The alkaline phosphatase (U L^{-1}) level was significantly higher ($p < 0.05$) in the control and group 3 compared to group 2 and 4. The effect on glucose, total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the AST/ALT ratio was not significantly influenced ($p > 0.05$) between the control and treatment groups (Table 2).

Table 2. Effect of red palm oil inclusion on serum biochemical indices of Wistar rats.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Pooled SEM	P value
Glucose (mg dL^{-1})	72.20	63.60	69.90	69.40	3.39	0.40
Urea (mg dL^{-1})	26.37^{a}	19.93^{b}	21.38^{ab}	22.03^{ab}	1.72	0.05
Creatinine (mg dL^{-1})	0.77^{ab}	0.81^{ab}	0.82^{a}	0.65^{b}	0.04	0.02
Total protein (mg dL^{-1})	6.23	5.87	5.98	6.19	0.36	0.73
Albumin (mg dL^{-1})	4.05	3.97	3.83	3.98	0.25	0.93
Globulin (mg dL^{-1})	2.18	1.90	2.11	2.21	0.38	0.94
Albumin/Globulin ratio	1.86	2.09	1.82	1.80	1.02	0.80
Alkaline phosphatase (U L^{-1})	34.45^{a}	30.39^{ab}	33.36^{a}	28.84^{b}	1.33	0.04
Aspartate aminotransferase (U L^{-1})	287.50	254.40	263.40	275.50	10.90	0.26
Alanine aminotransferase (U L^{-1})	126.03	125.60	128.80	125.01	1.91	0.51
AST/ALT ratio	2.282	2.028	2.043	2.204	0.12	0.20

Note: $^{a, b}$ mean that values in the same row with different superscript letters are significantly different ($p < 0.05$). Diets (n = 8/treatment; both genders (male/female rats): 50:50 ratio). SEM: Standard Error of the Mean. Diet 1: Basal diet (control) 0 mg kg^{-1}; Diet 2: Diet 1 + Red palm oil at 200 mg kg^{-1}; Diet 3: Diet 1 + Red palm oil at 300 mg kg^{-1}; Diet 4: Diet 1 + Red palm oil at 400 mg kg^{-1}. Source: Authors, 2026.

3.4 The serum lipid profile

The serum lipid profile of Wistar rats fed varied levels of RPO inclusion is shown in Table 3. The total cholesterol (TC) (mg dL^{-1}), triglycerides (TG) (mg dL^{-1}), and low-density lipoprotein (LDL) (mg dL^{-1}) levels of the animals fed varied levels of RPO inclusion differed significantly ($p < 0.05$) between the treated groups and the control group. The animals fed the control diet had a significantly increased concentration of TC, more than those in groups 4 and 2, but similar to group 3.

The TG (mg dL^{-1}) level of the control group significantly increased ($p < 0.05$) more than those in groups 3 and 2, but was similar to group 4. The groups 4, 2 and control were significantly increased LDL (mg dL^{-1}) levels ($p < 0.05$) more than group 3. High-density lipoprotein (HDL), very low-density lipoprotein (VLDL) (mg dL^{-1}), and the cholesterol/HDL ratio were unaffected ($p > 0.05$) by the varied levels of RPO inclusion.

Table 3. Effect of red palm oil inclusion on serum lipid profile of Wistar rats.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Pooled SEM	P value
Total Cholesterol (mg dL^{-1})	59.24^{a}	57.67^{ab}	55.62^{b}	58.54^{ab}	3.65	0.04
Triglycerides (mg dL^{-1})	89.40^{a}	86.20^{ab}	88.70^{ab}	83.70^{b}	5.70	0.05
High-density lipoprotein (mg dL^{-1})	25.76	23.61	24.88	23.45	2.31	0.44
Low-density lipoprotein (mg dL^{-1})	15.60^{ab}	16.82^{ab}	13.00^{b}	18.35^{a}	5.35	0.05
Very low-density lipoprotein (mg dL^{-1})	17.88	17.24	17.74	16.74	1.14	0.53
Cholesterol/HDL ratio	2.313	2.451	2.244	2.497	0.21	0.46

Note: ^{ab} means that values in the same row with different superscript letters are significantly different ($p < 0.05$). Diets (n = 8/treatment; both genders (male/female rats): 50:50 ratio). SEM: Standard Error of the Mean. Diet 1: Basal diet (control) 0 mg kg^{-1}; Diet 2: Diet 1 + Red palm oil at 200 mg kg^{-1}; Diet 3: Diet 1 + Red palm oil at 300 mg kg^{-1}; Diet 4: Diet 1 + Red palm oil at 400 mg kg^{-1}. Source: Authors, 2026.

3.5 The oxidative stress indicators

Table 4 presents the effects of red palm oil inclusion on oxidative stress markers in Wistar rats. Dietary red palm oil inclusion significantly affected all measured oxidative stress indicators—total antioxidant activity, catalase, glutathione peroxidase, superoxide dismutase, glutathione, and malondialdehyde ($p < 0.05$). Wistar rats fed diet 4 showed significantly higher ($p < 0.05$) total antioxidant activity than those fed diets 1 (control) and 2, but similar to those on diet 3.

Catalase activity was significantly higher ($p < 0.05$) in rats fed diet 2 than in those on diets 3 and 4, but similar to the control group. Glutathione peroxidase activity was significantly higher ($p < 0.05$) in rats fed diet 4 than in those fed diet 3, but similar to diets 1 (control) and 2. Superoxide dismutase levels were significantly higher ($p < 0.05$) in rats fed diet 4 than those in diets 1 and 3, but similar to diet 2.

Glutathione levels were significantly higher ($p < 0.05$) in rats fed diets 2 and 4 than those on diet 3, but similar to the control group (diet 1). Lipid peroxidation (MDA) levels were significantly higher ($p < 0.05$) in rats fed diet 2 than those in diet 4, but similar to diet 1 (control) and 3.

Table 4. Effect of red palm oil inclusion on oxidative stress indicators of Wistar rats.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Pooled SEM	P value
Total antioxidant activity (TAC) (mM)	3.67^{ab}	3.67^{ab}	3.20^{b}	3.71^{a}	0.29	0.05
Catalase (CAT) (mmol/ml)	6.47^{b}	9.78^{a}	8.77^{ab}	8.31^{ab}	1.06	0.04
Glutathione peroxidase (GSH-Px) (u L^{-1})	32.63^{b}	33.76^{b}	40.70^{ab}	44.26^{a}	9.92	0.05
Superoxidase dismutase (SOD)(u mL^{-1})	1.12^{ab}	0.86^{b}	1.18^{ab}	1.51^{a}	0.09	0.05
Glutathione (GSH) (mM)	0.22^{b}	0.35^{a}	0.28^{ab}	0.36^{a}	0.03	0.04
Lipid peroxidation (MDA) (μM)	12.36^{b}	15.31^{a}	12.35^{b}	13.74^{ab}	0.65	0.02

Note: ^{ab} means that values in the same row with different superscript letters are significantly different ($p < 0.05$). Diets (n = 8/treatment; both genders (male/female rats): 50:50 ratio). SEM: Standard Error of the Mean. Diet 1: Basal diet (control) 0 mg kg^{-1}; Diet 2: Diet 1 + Red palm oil at 200 mg kg^{-1}; Diet 3: Diet 1 + Red palm oil at 300 mg kg^{-1}; Diet 4: Diet 1 + Red palm oil at 400 mg kg^{-1}. Source: Authors, 2026.

4. Discussion

4.1 Growth indices

Growing Wistar rats fed different levels of RPO exhibited differences in daily weight gain and body weight between males and females. In this study, weight gain and daily weight gain in male rats suggest that RPO has growth-promoting properties, possibly related to an energy-efficient diet. Additionally, treatment-by-gender interactions were observed for daily weight gain, weight gain, and feed conversion ratio, with males showing greater improvements than females. These findings may indicate differences in growth responses between male and female Wistar rats. Greater growth responses in male rats may reflect gender-related differences in nutrient utilisation, energy metabolism and hormonal regulation.

The feed conversion ratio further suggested that RPO inclusion positively influenced feed utilisation and efficiency in males more than in females. The interaction between dietary treatment and gender suggests that growth responses to red RPO may depend on the physiological characteristics of the animals rather than on dietary inclusion alone. Furthermore, the interaction effect also indicates that treatment responses differed between genders, have to be cautiously interpreted of the overall dietary effect. However, the absence of significant effects on feed intake and overall feed efficiency limits the extent to which improved growth can be attributed to increased feed consumption or consistently enhanced feed utilisation. Experimental evidence from RPO-fed Wistar rats provides some support for its potential influence on growth.

Ayeleso et al. (2012) investigated dietary RPO supplementation at 1, 2 and 4 mL daily for seven weeks and reported increased body-weight gain at the 2- and 4-mL levels relative to the control. However, the study used male rats only and therefore could not establish whether the response differed by gender. In another experiment, Ayeleso et al. (2014) investigated RPO in streptozotocin-induced diabetic male Wistar rats, focusing on antioxidant status rather than gender-related growth responses. These studies provide relevant experimental context, but direct comparisons remain limited by differences in animal models and study objectives. Tibaes et al. (2022), using male and female Wistar rats fed low-fat or high-fat diets for nine weeks, similarly found greater weight gain in males, irrespective of diet.

Maric et al. (2022) also demonstrated gender-dependent differences in dietary responses in rodent models, reinforcing the importance of considering gender when evaluating nutritional interventions. These studies provide context for the observed interaction, although their dietary models differ from RPO supplementation. Consequently, the present findings suggest gender-dependent growth responses to RPO, but the underlying mechanisms cannot be established from growth performance data alone. Moreover, our results agree with findings of Albrahim et al.

(2022), who reported reduced body weight in rats receiving olive oil or palm oil alongside a high-cholesterol diet, although these groups did not differ from the control or high-cholesterol diet-only groups. The specific effects of varied RPO inclusion levels on feed intake, body weight and feed efficiency between genders were difficult to establish from the treatment-by-gender interactions.

Previous studies suggest that age, genetic background, and dietary composition may affect male and female rats differently. Recent experimental studies indicate that age, genetic background, and dietary composition can influence gender-specific responses to nutritional interventions in rats. Gual-Grau et al. (2023), using Wistar and Fischer 344 rats exposed to a cafeteria diet for 10 weeks, demonstrated that both rat strain and gender influenced metabolic and physiological responses to the diet. Similarly, Lékó et al. (2024) reported gender-dependent responses to high-fat feeding in male and female Wistar rats, demonstrating differences in body-weight regulation and food intake. These findings support the consideration of gender, strain, and dietary composition when interpreting growth responses in experimental rats. These factors may partly explain the treatment-by-gender effects observed in this study. Further research is needed to clarify the effects of RPO and other nutrients on physiological responses in male and female rats.

4.2 Haematological characteristics

In this study, the impact of graded red palm oil (RPO) inclusion on the hematological profile of Wistar rats was investigated to understand the nutraceutical implications of RPO consumption. The findings revealed significant effects of dietary RPO on several key hematological variables, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count.

The RBC count was significantly elevated in the 200 mg kg^{-1}, 0 mg kg, and 400 mg kg^{-1} RPO-fed groups compared to the 300 mg kg^{-1} group. The significantly lower RBC count at 300 mg kg-1 RPO, compared with the control, 200 mg kg^{-1}, and 400 mg kg^{-1} groups, indicates a non-linear response to supplementation. However, the isolated reduction cannot be confidently attributed to a specific biological mechanism based on the present findings, as gender, age, and animal-related variation can influence Wistar rat haematological parameters (Patel et al., 2024). This suggests that RPO may enhance red blood cell synthesis and, consequently, oxygen transport capacity. These findings are consistent with earlier studies linking the erythropoietic potential of RPO to its rich content of vitamin E, tocotrienols, and carotenoids, which collectively help mitigate oxidative stress in haematopoietic tissues (Khor et al., 2021).

Alwahaibi et al. (2019) investigated the effects of palm oil-derived tocotrienol-rich fraction (TRF) on erythrocyte membrane lipid composition in streptozotocin-induced diabetic rats. After four weeks of supplementation with 200 mg kg^{-1} TRF, the findings suggested that TRF may help preserve erythrocyte membrane lipid composition and protect against lipid peroxidation, although haemolysis and red blood cell lifespan were not directly measured. Interestingly, WBC counts declined in a dose-dependent manner with increasing RPO supplementation. While leukocytes are vital for host defense, excessive proliferation can be detrimental, leading to inflammation and organ stress. The observed reduction may reflect an anti-inflammatory and immunomodulatory influence of RPO, attributable to its antioxidative constituents.

This trend corroborates findings by Di Vincenzo et al. (2019) and Zainal et al. (2022), who reported that tocotrienols and carotenoids downregulate pro-inflammatory cytokines and reduce systemic oxidative stress. Although this decline could represent a beneficial suppression of subclinical inflammation, further research is warranted to determine if such reductions might impair immune competence. Packed cell volume (PCV) did not differ significantly among the treatment groups; however, numerical increases were observed in the 400 mg kg^{-1} RPO group. The haemoglobin concentration (g dL^{-1}) was significantly higher in the 400 mg/kg and 200 mg kg^{-1} RPO-treated groups than in the 0 mg kg^{-1} and 300 mg kg^{-1} RPO-treated groups. These observations align with previous findings from Emmanuel et al. (2021), who reported improved PCV, Hb, and RBC levels in rats fed RPO, suggesting enhanced erythropoiesis and iron utilization.

The phytochemical constituents of RPO may stimulate hematopoietic activity in the bone marrow, enhancing the synthesis of erythrocytes and hemoglobin. Platelet counts exhibited significant variation across treatment groups, with the 200 mg kg^{-1} RPO group showing a notably lower count compared to the control, 300 mg kg^{-1}, and 400 mg kg^{-1} groups. This may imply a regulatory role of RPO in thrombopoiesis, possibly through its influence on platelet production and aggregation mechanisms. Vitamin E-rich oils have previously been shown to affect platelet physiology, potentially altering clotting factor activity and reducing the risk of thrombotic events (Loganathan et al., 2017).

MCV and MCH followed a non-linear trend, with significantly higher values in the 300 mg kg^{-1} group compared to the 400 mg kg^{-1} group, but similar to those in the control and 200 mg/kg groups. This suggests that while moderate RPO inclusion may support normal erythrocyte morphology and hemoglobin content, excessive intake may disrupt iron metabolism or erythrocyte development. Similar findings reported that high-fat diets, including those containing palm oil, may alter erythrocyte fatty acid composition and membrane-related properties, potentially affecting red blood cell structure and function (Alwahaibi et al., 2019).

These findings are also consistent with Asiriuwa et al. (2026), who reported improved haematological indices in rats fed fresh palm oil, suggesting that the antioxidant-rich components of red palm oil. The increase in haemoglobin concentration observed in our study may reflect enhanced iron absorption from the gut, facilitated by the antioxidant properties of RPO, leading to greater iron bioavailability and improved oxygen transport; this is speculative. In summary, the results demonstrate that RPO exerts dose-dependent effects on hematological parameters in Wistar rats.

Moderate supplementation appears to support improved hematological function, while higher doses may influence immune regulation and thrombopoiesis. These outcomes highlight the potential of RPO as a functional dietary lipid with hematopoietic and immunomodulatory properties. However, the biphasic nature of some responses underscores the importance of dose optimization. Future investigations should explore the molecular mechanisms underpinning these effects and evaluate the long-term safety and efficacy of RPO supplementation in animal and human models.

4.3 Serum biochemistry

In the present study, dietary inclusion of RPO at various concentrations significantly influenced serum levels of urea, creatinine, and alkaline phosphatase (ALP), whereas glucose, total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the AST/ALT ratio were unaffected. Urea and creatinine are established biomarkers of renal function. Elevated serum urea often signifies renal impairment, especially when sustained over time. Here, a dose-dependent decrease in urea concentrations was observed across RPO-treated groups, with the lowest levels recorded at 200 mg kg^{-1} (19.93 mg dL^{-1}) compared to the control (26.37 mg dL^{-1}).

These values fall within the normal physiological range for Wistar rats (15-45 mg dL^{-1}), indicating preserved renal integrity (Patel et al., 2024). The trend aligns with a report by Emmanuel et al. (2021), who demonstrated that antioxidant-rich diets, including RPO, enhance renal function by mitigating oxidative stress in renal tissues. Thus, the observed decline in urea may reflect improved nitrogen metabolism, potentially mediated by RPO's tocopherol and tocotrienol content. Creatinine, a more specific index of glomerular filtration rate, followed a slightly different pattern. Although a mild elevation was observed in the 300 mg kg^{-1} group (0.82 mg dL^{-1}), it remained within the acceptable physiological range (0.30-0.87 mg dL^{-1}) and did not suggest renal dysfunction (Patel et al., 2024).

Interestingly, the creatinine levels decreased in the group receiving a higher RPO dose (0.65 mg dL^{-1} in group 4), supporting the hypothesis of improved renal clearance, possibly due to RPO's antioxidative defense mechanisms (Ugbogu et al., 2019). Alkaline phosphatase (ALP), a marker for hepatobiliary function, bone turnover, and intestinal activity, was significantly reduced in groups 2 (30.39 U L^{-1}) and 4 (28.84 U L^{-1}) compared to the control (34.45 U L^{-1}). ALP values of 28–34 U L^{-1} appear low, below published ranges (36.47–209 U L^{-1}) and need verification of assay procedures, units, and reference intervals (Patel et al., 2024).

ALP activity suggests enhanced hepatocellular membrane stability and reduced hepatic stress (Emmanuel et al., 2021). This finding corroborates studies by Meganathan & Fu (2016) in human studies and Ajuwon et al. (2022) in a lipopolysaccharide-induced hepatic injury model, who reported that RPO administration in rats led to a decrease in hepatocellular damage markers, including ALP, AST, and ALT, attributed to its high vitamin E content. The hepatoprotective action may be due to membrane stabilization, with decreased enzyme leakage from hepatocytes suggesting reduced cellular degeneration or necrosis. Consistent with Emmanuel et al. (2021), the present study found no significant changes in serum glucose, total protein, albumin, globulin, or the AST/ALT ratio across all treatment groups. These parameters are essential indicators of liver synthetic function and metabolic homeostasis.

Their stability further supports the absence of hepatotoxicity at the administered RPO doses. Moreover, Ayeleso et al. (2014) and Szulczewska-Remi et al. (2019) demonstrated that palm oil consumption did not impair glycemic regulation and was associated with increased hepatic vitamin E bioavailability, respectively, affirming RPO's potential metabolic benefits. Additionally, the unchanged levels of hepatic transaminases (AST and ALT), which

are sensitive markers of hepatocellular integrity, suggest that RPO inclusion did not induce liver injury. AST (254–288 U L^{-1}) and ALT (125–129 U L^{-1}) across treatments, including controls, exceeded rats' reference ranges (AST 34–109; ALT 13–56 U L^{-1}) (Patel et al., 2024).

This might suggest chloroform-induced hepatotoxicity from the chloroform anaesthesia (ATSDR, 2024). This observation is in line with the findings of Ajuwon et al. (2022), who reported no elevation in liver enzymes in rats fed fresh palm oil, contrasting with the hepatotoxic effects of oxidized palm oil. The protective action was attributed, at least in part, to the antioxidant properties of bioactive constituents of red palm oil. Taken together, these results suggest that dietary RPO, when administered at 200-400 mg kg^{-1}, may provide mild hepatoprotective and renoprotective effects without negatively impacting glycemic control or protein metabolism.

Nonetheless, the dose-dependent responses observed in some markers, such as creatinine, underscore the need for further investigations to determine the optimal inclusion rate for maximum physiological benefit. Future studies should explore the molecular mechanisms underlying these effects, including gene expression profiling of key metabolic and oxidative stress-related enzymes.

4.4 Lipid profile

Lipid profiling is a crucial diagnostic tool employed in the evaluation of dyslipidemia and associated cardiovascular risks. It involves the quantification of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels, serving as an indicator of metabolic and cardiovascular health status. Red palm oil (RPO) has historically attracted scrutiny due to its relatively high saturated fatty acid (SFA) content, particularly palmitic acid, which has been implicated in the elevation of plasma cholesterol and the development of cardiovascular disease (CVD).

Palmitic acid is predominantly esterified at the sn-1 and sn-3 positions of triacylglycerols in red palm oil (RPO), whereas its distribution in animal fats varies by source of sn-2 position. The potential atherogenic effects of palmitic acid depend partly on its dietary context and metabolic handling (Mancini et al., 2015). Consequently, dietary intake of RPO is often associated with the potential risk of hypercholesterolemia. However, the findings from this study indicate that RPO may, in fact, exert favorable or neutral effects on lipid metabolism.

Unlike trans fats, which are known to raise TC and LDL levels while reducing HDL, RPO appears to behave more like an unsaturated fat due to its nearly equal proportions of saturated and unsaturated fatty acids. Our results also corroborate studies reporting improvements in lipid profiles following RPO administration (Albrahim et al., 2022; Femi-Oloye et al., 2020). May & Nesaretnam (2014) similarly observed that palm oil influences blood lipid profiles in a manner comparable to monounsaturated fats, such as olive oil, emphasizing its neutral or beneficial role in cardiovascular health.

In the present study, the inclusion of RPO at varying levels significantly influenced serum lipid parameters in Wistar rats. The TC levels were significantly lower in the RPO-supplemented groups compared to the control, particularly in groups 2 and 4, but similar to group 3, which exhibited a comparable reduction, though not statistically distinct. These findings are consistent with the observations of Ayeleso et al. (2014), who reported hypocholesterolemic effects of RPO in experimental models, attributing this to its rich content of tocotrienols and carotenoids, which modulate cholesterol biosynthesis by downregulating HMG-CoA reductase.

Similarly, Dauqan et al. (2011) demonstrated that dietary RPO supplementation led to dose-dependent reductions in serum cholesterol concentrations. The observed effects may be attributed to the antioxidant components in RPO—β-carotene and vitamin E—which have been shown to enhance lipid profiles more effectively than oils like soybean oil. Serum triglyceride levels were similarly reduced in RPO-treated groups, notably in groups 2 and 3. These results suggest that RPO supplementation does not promote hypertriglyceridemia, but rather may ameliorate it. Ojieh et al. (2015) also reported a decline in plasma TG levels following dietary RPO inclusion, which was attributed to improved fatty acid oxidation mediated by tocotrienol-rich fractions.

However, the lack of an increase in TG levels in this study undermines that hypothesis and suggests a more nuanced interaction between RPO constituents and lipid metabolism. Low-density lipoprotein (LDL), regarded as the most atherogenic lipoprotein class, was significantly lower in group 3, indicating a potential optimal level of RPO inclusion for lipid regulation. This LDL-lowering effect is congruent with prior findings by Meganathan & Fu (2016), who observed significant reductions in LDL levels with RPO supplementation.

The mechanistic basis may involve enhanced hepatic LDL uptake or inhibition of cholesterol synthesis. Interestingly, while other groups exhibited higher LDL levels than group 3, all values remained within physiological ranges, supporting the safety profile of RPO consumption (Ayeleso et al., 2012; Mancini et al., 2015).

Furthermore, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and the cholesterol/HDL ratio remained statistically unchanged across treatment groups. These findings are in agreement with those of Unhapipatpong et al. (2021), who reported no significant changes in HDL and VLDL levels in rats administered fresh RPO.

The stability of these parameters further underscores the non-atherogenic nature of RPO when included at moderate dietary levels. The cholesterol/HDL ratio, a well-established predictor of cardiovascular risk, also remained unaffected, suggesting that RPO consumption does not elevate atherogenic risk markers. This is corroborated by Unhapipatpong et al. (2021), who observed the preservation or improvement of HDL levels following palm oil supplementation. Again, our study agrees with Syarifah-Noratiqah et al. (2020), who reported from a systematic review of animal studies that palm oil and palm olein generally exert neutral or beneficial effects on serum lipid profiles, with evidence of reductions in total cholesterol and LDL-cholesterol in several experimental models.

However, responses in triglycerides and HDL-cholesterol were inconsistent, suggesting that the lipid effects of palm oil may depend on dietary dose, duration of supplementation, and experimental conditions. In the present 42-day study, RPO was incorporated into the diet at 200, 300, and 400 mg kg^{-1}. By comparison, Ayeleso et al. (2012) administered 1, 2, or 4 mL of RPO per rat daily for seven weeks. These studies differed from the present experiment in dose expression, dietary inclusion level, and duration, limiting direct comparisons. Furthermore, a systematic review of animal intervention studies reported inconsistent responses in TG and HDL-C following palm oil or palm olein supplementation, with increases, decreases, or no significant changes depending on the study (Syarifah-Noratiqah et al., 2020).

These variations suggest that lipid responses may also depend on the amount and type of dietary fat and other experimental conditions. Therefore, the observed TG and HDL-C responses should be interpreted in the context of the specific dietary and experimental conditions rather than attributed solely to RPO supplementation. In conclusion, dietary RPO supplementation was associated with modest changes in lipid parameters, including reductions in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), although the responses were not consistent across all inclusion levels. HDL-C, very-low-density lipoprotein cholesterol (VLDL-C), and lipid ratios were not adversely affected.

These findings suggest that RPO may influence lipid metabolism under the conditions of the present study; however, the modest changes do not establish a consistent lipid-improving effect or demonstrate cardiovascular benefits. The bioactive components of RPO, particularly tocotrienols and carotenoids, may contribute to its potential lipid-modulating properties. Further research should investigate the molecular pathways involved, including the roles of PPARα and SREBP-1c, and evaluate the long-term effects of RPO supplementation on lipid metabolism and cardiovascular health.

4.5 Oxidative stress indicators

Total antioxidant capacity (TAC), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were all significantly influenced by dietary red palm oil (RPO) inclusion, but the responses were biomarker-specific rather than uniformly dose-dependent. RPO is rich in tocotrienols, tocopherols, carotenoids, and coenzyme Q10 (Ajuwon et al., 2022; Loganathan et al., 2017), and tocotrienols in particular have been reported to attenuate oxidative stress and inflammation in metabolic disease models (Pang; Chin, 2019).

These constituents may explain the modulatory effects observed, but their presence does not guarantee improved antioxidant status at every inclusion level. Earlier dietary studies in Wistar rats similarly found that RPO modified individual antioxidant components rather than the defence system as a whole (Ayeleso et al., 2012). The inclusion levels used here (200–400 mg kg^{-1}) are not directly comparable with earlier work, which administered RPO by volume or as g/kg diet (Ajuwon et al., 2022). TAC was higher at 400 than at 300 mg kg^{-1}, with the control and 200 mg/kg groups intermediate, so total capacity did not rise progressively with dose. Ajuwon et al. (2016) reported higher plasma antioxidant capacity following rooibos–RPO co-supplementation, whereas Ayeleso et al. (2012) found no change in plasma TAC in RPO-fed rats despite changes in individual enzymes. This is because TAC reflects the aggregate response of the assay used; it should not, in isolation, be read as evidence of reduced oxidative damage.

CAT activity was higher at 200 mg/kg than in the control, with the 300 and 400 mg kg^{-1} groups intermediate. This peak neither defines an optimal inclusion level nor demonstrates feedback inhibition at higher doses, although the

lower activity at 300 and 400 mg kg^{-1} may reflect an oxidative burden exceeding enzymatic capacity (Ajuwon et al., 2022). GPx and SOD activities increased in a dose-related trend within the RPO-treated groups. GPx was highest at 400 mg kg^{-1}, above the control and 200 mg/kg groups, whereas SOD at 400 mg kg^{-1} exceeded only the 200 mg kg^{-1} value. Earlier work likewise reported tissue-discordant responses: RPO raised erythrocyte, but not hepatic GPx, and SOD was unchanged in both tissues (Ayeleso et al., 2012).

SOD converts superoxide to hydrogen peroxide, which CAT and GPx subsequently remove, with GPx also reducing lipid hydroperoxides (Ajuwon et al., 2022). A plausible driver of enzyme induction is Nrf2 signalling, which Ajuwon et al. (2022) found to be upregulated by RPO in lipopolysaccharide-challenged Wistar rats alongside restored hepatic SOD, GPx and glutathione reductase. Nrf2 was not measured here, and that model involved an oxidative challenge, so this explanation remains speculative. GSH was significantly higher in the 200 and 400 mg kg^{-1} groups than in the 300 mg kg^{-1} group but remained lower than in the control. Ajuwon et al. (2022) reported that RPO upregulated glutamate cysteine ligase, which catalyses the rate-limiting step of glutathione synthesis, consistent with enhanced synthesis.

Variation in GSH may reflect compensatory responses to oxidative stress or differences in precursor availability. GSH alone does not define redox state, and neither oxidised glutathione (GSSG) nor the GSH: GSSG ratio was measured here. MDA, a marker of lipid peroxidation, was highest at 200 mg kg^{-1}, whereas the 400 mg kg^{-1} group did not differ from any other group. The increase at 200 mg kg^{-1} is unexpected, because RPO lowered MDA in Wistar rats exposed to oxidants (Ajuwon et al., 2022). Those studies, however, differed in oxidative challenge, tissue, and duration, which limits direct comparison.

The elevated GPx and SOD activities at 400 mg/kg should not be interpreted as improved antioxidant status; rather, they likely reflect an adaptive response to increased oxidative load, consistent with the compensatory rise in CAT activity attributed to hydrogen peroxide overproduction in challenged rats (Ajuwon et al., 2022). The present data cannot confirm such a mechanism, as no direct measure of oxidative load was taken. RPO produced non-linear, biomarker-specific changes in antioxidant indices without a consistent reduction in lipid peroxidation, and no inclusion level was uniformly favourable: 200 mg kg^{-1} gave the highest CAT activity but also the highest MDA. These findings support a modulatory effect of dietary RPO rather than consistent protection against oxidative damage.

This study had several limitations: small, mixed-gender groups (n = 8), a short intervention, no feed intake data, no tissue analyses, and an RPO that was not chemically characterised. Because its carotenoid and tocotrienol contents were unknown, the effects cannot be attributed to any individual compound or pathway. Future studies should use larger groups, a longer intervention, and a characterised oil, and should compare direct oral administration with dietary incorporation of RPO across a wider dose range. They should also include tissue-specific damage measures, the GSH: GSSG ratio and pathway-level analyses, and assess long-term effects.

5. Conclusions

Dietary red palm oil (RPO) elicited dose-dependent and sex-related responses in Wistar rats, indicating that its physiological effects were not uniformly beneficial across the evaluated parameters. Growth performance showed more pronounced responses in males, whereas feed intake and overall feed efficiency remained largely unchanged. Haematological, biochemical, lipid, and antioxidant profiles exhibited variable responses, suggesting a complex modulatory effect of RPO on physiological and metabolic processes.

Although changes in selected renal and hepatic biochemical markers were observed, the findings provided no clear evidence of adverse organ function under the experimental conditions. Similarly, improvements in lipid-related parameters were modest, and antioxidant responses varied according to the biomarker assessed. Overall, these findings suggest that the effects of dietary RPO depend on sex and the physiological parameter evaluated, precluding the identification of a universally favourable inclusion level. Further studies incorporating longer experimental periods, sex-stratified analyses, chemical characterisation of the oil, and tissue- and molecular-level investigations are warranted to clarify the underlying mechanisms and establish the safety and efficacy of RPO supplementation in animal nutrition.

6. Authors' Contributions

Chinwe Uchechi Nwachukwu: conceived and designed the study, methodology support, experimented, wrote the first draft of the manuscript. *Olatunji Abubakar Jimoh*: data analysis, proofreading, and reviewing the manuscript. *Peter Kelechi Ajiogu*: co-performed the experiment and edited the manuscript. Jovi Richard Otite: co-supervision,

co-performed the experiment.

7. Conflicts of Interest

The authors declare no conflicts of interest.

8. Ethics Approval

The institutional research and ethics committee of Alvan Ikoku Federal University of Education, Owerri, Imo State, Nigeria, approved the study and its experimental protocols (AIFUE/DAS/005), which complied with the standard guidelines for institutional animal scientific procedures. All experimental animal care procedures and welfare conformed to the Guide for the Care and Use of Laboratory Animals (8th edition, 2011), published by the U.S. National Institutes of Health (NIH). The authors confirm that the ethical policies of the journal were adhered to as noted on the journal authors' guideline page. The authors confirm that the standards for the protection of animals used for experimental/scientific purposes were followed.

9. Artificial Intelligence Statement: Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

Generative AI was used exclusively to assist with language editing and improve the clarity of the manuscript. The Portuguese-language version was reviewed using AI tools and also by a human translator. The authors reviewed and verified the final text and take full responsibility for the content of the manuscript. AI tools were not listed as authors and do not qualify for authorship.

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