UNIVERSITY OF CAPE COAST

EFFECT OF ROASTED PARA RUBBER (*HEVEA BRASILIENSIS*) SEED *KERNEL* MEAL *INCORPORATED FEED* ON GROWTH PERFORMANCE, BLOOD COMPOSITION, LIVER AND KIDNEY TISSUE HISTOLOGY OF INSTITUTE OF CANCER RESEARCH (ICR) ALBINO MICE

QUITA TOKPA

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BY

QUITA TOKPA

Thesis Submitted to the Department of Animal Science, School of Agriculture, University of Cape Coast, in Partial Fulfillment of the Requirement for Master of Philosophy Degree in Animal Science

NOVEMBER 2023

DECLARATION

By signing this document, I certify that this thesis is the result of my original study and that it has not previously been submitted in whole or in part for another degree at this University or elsewhere.

Candidate's Signature ………………………………… Date …………………...

Name: Quita Tokpa

Supervisors' Declaration

We state that the thesis preparation and presentation were overseen in accordance with the standards for thesis supervision established by the University of Cape Coast.

Principal Supervisors' Signature ………………………... Date …………

Name: Prof. Samuel O. Apori

Co - Supervisor' Signature ………………………... Date ………………

Name: Dr. Seth Y. Afedo

ABSTRACT

This study examined the impact of incorporating roasted para rubber seed kernel meal (RoPRSKM) into the feed of ICR albino mice, focusing on growth performance, haematology, blood biochemistry and liver and kidney tissue histology. In this study, four experimental diets, T1, which was the control made of Koudjis feed (21% CP and 2825 kcalsME/kgDm) and T2, T3 and T4 in which ME of RoPRSKM replaced 5%, 10 and 15%, respectively of T1 metabolizable energy content were used. Using a completely randomized design, 40 male albino mice were assigned to four dietary treatments. The proximate analysis of the RoPRSKM indicated 24.8% crude protein, 9.2% crude fibre, 46.6% crude fat, 0.26 mg/kgDM hydrogen cyanide (HCN), and estimated metabolisable energy of 4405.5 kcal ME/kgDM. Inclusion of RoPRSKM in the meal showed no significant effect on the weight gain and water intake of the albino mice. Feed intake significantly reduced in mice feed on treatments T3 and T4. At the end of the experiment, Mice were chosen randomly to collect blood for analysis of haematology and blood biochemistry. Using terminal standard operating procedure, blood samples were obtained from the orbit. To determine the packed cell volume, microhematocrit centrifugation method, hemoglobin (Hb) was employed to measured spectrophotometer, red blood cell (RBC), white blood cell and manually performed using Merck Veterinary Manual.While mean corpuscular volume (MCV),mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated Blood parameters (red blood cells, white blood cells, etc.) showed no significant detrimental effects in mice fed with RoPRSKM diets as compared to the control group. In terms of blood biochemistry, the diets did not exhibit any adverse effects across all the measured parameters, except for total protein, Alkaline Phosphatase, and Globulin. These three parameters displayed statistically significant differences among all the treatment groups. After the blood samples collection, the mice were immediately euthanized humanely by cervical dislocation. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. The liver and kidney samples were harvested and fixed in 10% formalin solution for a week. After that, Tissue samples were washed 24 hours under slow-speed running water, dehydrated in graded concentrations of ethanol and immersed in ethanol - xylene, xylene – paraffin1, paraffin wax I, paraffin wax II, paraffin wax III, and finally embedded in paraffin wax. 5 µm thick slices were cut from each paraffin block sample onto a glass slide, and stained with haematoxylin-eosin (HE) routinely. Tissue structure was then observed under the Hund H600 microscope, and photomicrographs were taken as images for presentation. However, the histology examination revealed no observable alterations or changes in the liver and kidney tissues of the mice when fed with RoPRSKM. The finding indicated that incorporating RoPRSKM into the diet of ICR albino mice did not have significant adverse effects on their growth, haematology, blood biochemistry, or liver and kidney tissue structure. However, some variations were observed in certain parameters, but were not significant as compared with the acceptable ranges.

KEYWORDS

Roasted

Haematology

Blood Biochemistry

Histology

Hydrogen Cyanide

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DEDICATION

To my cherished parents, beloved brothers, sisters and children this thesis is devoted.

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CHAPTER ONE

Introduction

The high cost of animal feed accounts for farmers' low involvement in poultry and livestock farming in Liberia, Ghana and other West African countries, thereby leading to high importation of frozen meat in these counties. This high cost is attributed to humans’ and animals' reliance on the same feed resources or ingredients, such as maize, soybean, ground nut etc. Consequently, this study is geared towards experimenting with para rubber seed meal as an alternative feed for albino mice to establish its influence on their growth performance, haematology, blood biochemistry and liver and kidney tissue histology. Para rubber seed kernel meal is used in this study because of its availability in abundance and less usability in Liberia. Albino-mice were used because they are scientifically proven to have digestive system and physiological processes similar to many domesticated animals.

Background to Study

The availability of conventional quality feed resources or ingredients for animals is getting increasingly challenging as croplands are used to grow crops for human consumption. This has led to a need for alternative or non-conventional good-quality feed to support the growth of the animal production industry. In West Africa, poultry and livestock farmers face various issues relating to inadequate feed supplies. According to Duruma *et* al. (2006), the issue persists due to the ongoing competition between humans and livestock for food ingredients and the raw materials they both rely on.

In addition, due to the increasing price of commodities, the feed price for domesticated animals has increased significantly. Studies have shown that feed accounts for about sixty (60 - 70) per cent of poultry and livestock production costs (Akinmutimi, 2007), (Adeyemi *et* al., 2008) in intensive production in West Africa. This rising cost is a huge burden that many small-scale farmers are facing.

Various factors contribute to the high feed cost, including but not limited to the non-availability of cheap feed ingredients, transportation expenses, and the use of some energy protein feed ingredients by humans and domesticated animals (Agbede, 2019; Igbasan, 2019). Therefore, the industry/farmers must find alternative feed sources or ingredients that can be more cost-effective. Cost-effectiveness leads to utilizing various non-conventional feed sources, such as produce residue and agricultural waste (Agbede, 2019; Igbasan, 2019).

According to Agbede (2019) & Igbasan (2019), replacing some of the expensive feed ingredients found in conventional feed with more sustainable alternative feed resources such as para rubber seed kernel can be done without compromising quality. Para rubber is extensively cultivated as a raw material for rubber. About 75% of its seeds are wasted, with only 25% being utilized as seedlings (Indonesian Directorate General of Plantation, 2010; Oluodo *et al.*, 2018; Lourith *et al.*, 2014; Reshad et al., 2015). Rubber seed yields have been estimated at around 136 to 2000 kg/hectare (Zhu *et al.*, 2014). According to Ikwuagwu *et al.*, (2000), & Widyaiani *et al.*, (2017), fresh rubber seeds comprise 42-48% shell and 52-60% kernel. Conversely, the kernel comprises 40-50% oil and 17-20% protein. Numerous studies are currently investigating the potential use of para rubber seeds as an unconventional feed resource for livestock feed, offering an alternative option within the livestock feed industry. Rubber seeds, as a feed component, are known to contain more readily digestible nutrients compared to certain traditional seed meals and hold promise as additional protein supplements in livestock diets (Mmereole, 2008; Babatunde *et al*., 1990). Previous studies showed that para rubber seed kernel meal protein contains high amounts of glutamic acid, aspartic acid and leucine content, which could replace most plant proteins in animal feed (Deng *et al*., 2017; Suprayudi *et al*., 2015). The feed source contains beneficial plant compounds that enhance livestock quality and reduce production costs. (Agbede, 2019; Igbasan, 2019). Its seed kernel shows great promise as an additional protein or energy source. However, according to Ukpebor *et al*. (2007) and Eka *et al.* (2010), cyanogenic glucoside's toxic component can prevent it from being utilized as a feedstuff. Cyanogenic glucoside occurs when the plant tissues are damaged, releasing hydrocyanic acid (Okafor *et al.*, 2006; Ahaotu *et al.,* 2010). Para rubber seed kernel meal can be used in livestock feed if the hydrocyanic acid is removed, similar to groundnut cake, fish meal, and soybean.

The para rubber industry is a vital component of the global economy and significantly contributes to the development and maintenance of infrastructure. Rubber is used to produce shoes, belts, tires, and plastics. However, para rubber seeds are of less importance to farmers. According to Noordin et al. (2012), in Nigeria, 20% of para rubber seeds were utilized for seedling production, while the remaining 80% were consumed by rodents or went to waste. Rubber tree is a commonly cultivated plantation produce in Africa. It is a long-lasting, persistent foreign exchange source for the region's economy. Para rubber tree starts to bear fruit from five to six years of age, depending on the care. When each fruit matures and splits open, it typically releases approximately three to four seeds, which drop to the ground. Each tree yields around 800 seeds annually, totaling approximately five kilograms (Bressani et al., 1983). Due to the large availability of unused rubber seeds in the Sub-Sahara African region, more studies are needed to establish its usefulness in serving as an alternative animal feed ingredient.

In 2016, Nath and colleagues reported that the seeds from para rubber (Hevea brasiliensis) contained 85.7% DM (dry matter) of nutrients, 26.1% crude protein, 43.0% crude fibre, 13.8% NFE, 1.8% ash, and 11.0% ether extract. The discovery of a metabolisable energy content of 2101.1 kilocalories per kilogram of dry matter in para rubber seeds led to considerations about their suitability as a feed source for livestock. Akinsanmi et al. (2020) conducted a study to evaluate how broilers' growth performance and carcass characteristics were affected after fed feeds that included para rubber seed kernel meal. In this study, two distinct processing methods for the rubber seed meal, roasting and hot water soaking, were employed, and all diets had equi-protein levels. The results indicated that the use of hot water-soaked para rubber seed meal did not negatively impact broilers' daily weight gain or feed intake. Rather, it increased their dressing and eviscerated weights compared to those fed roasted para rubber seeds kernel meal. Furthermore, the research demonstrated that para rubber seed kernel meal can be an effective alternative to soybean meal in broiler diets. Considering the affordability, availability, and low competition of para rubber seed kernel meal as an alternative livestock feed, further research in this area is warranted.

The availability of para rubber seeds in most West African countries, including Ghana, Nigeria, and Liberia, is increasing, and such a study is needed. In 2021, Liberia produced 90,800 tons of para rubber latex from 113,883 hectares of plantation. Production of Para rubber seed kernels in Liberia is estimated at 313,178.25 tons, but most of it goes to waste. Some earlier studies showed that treated Para rubber seed kernel fed at stated levels have been utilized without deleterious effects on test animals' growth performance, feed intake, gain in weight, feed conversion ratio, haematology, biochemistry and body organ weights (Farr, 2015). In this regard, this study will additionally determine the effect of roasted para rubber seed kernel (PRSK) incorporated diets on the histology of liver and kidney tissues apart from the listed parameters. The study aims to, therefore, investigate the nutrient composition of roasted rubber seed kernel meal, on the growth performance, the liver and kidney tissues histology, and blood composition of albino mice raised on para rubber seed meal-incorporated diets.

Statement of the Problem

Protein feed resources from animals are more beneficial for human consumption. Intensive livestock production relies heavily on easily digestible and highly nutritious feed ingredients. The availability of these high-quality feed resources at affordable prices is vital to the industry's expansion and sustainability. Protein, energy, vitamins, minerals, and water are essential nutrients livestock require for optimal health and production performance.

However, in developing countries such as Ghana, Liberia, and others, the availability of high-quality feed resources like cereals or grains as energy and protein sources like fish meal, oil seed cakes, meat meal, or industry-produced amino acid in large quantities at affordable prices all year round is a mirage (not feasible). Livestock producers in these countries should use alternative non-conventional feed sources for optimal production, as conventional feed resources may not be affordable or available all year.

The feed cost can be reduced by finding alternative ingredients or sources to replace all conventional ones. This can be achieved by utilizing agro-industrial by-products or crop residues. Agbede (2019) & Igbasan (2019) suggested that cheaper non-conventional feedstuffs should partially or wholly replace expensive conventional feed ingredients. In Agbede's view, one of the alternative feed resource is the kernel of para rubber seeds. This replacement would lead to sustainable and profitable animal production.

Conducting this study would motivate poultry and livestock farmers to include alternative and non-conventional feed resources or ingredients in their feed formulation. Insufficient literature is available concerning the use of milled, roasted para rubber seed kernel as a partial source of metabolisable energy in diets for poultry and livestock. This research stands to fill the gap by assessing the nutritional composition of para rubber seed kernel meal; investigate its impact on growth performance, hematological parameters, blood biochemistry, and the histological characteristics of liver and kidney tissues in albino mice that have been fed a diet containing para rubber seed kernel meal.

Purpose of the Study

The study assessed the impact of incorporating para rubber seed kernel meal into the diets of albino mice at varying inclusion levels. It examined how the addition of para rubber seed kernel meal affects the growth performance of the mice, their haematology and blood biochemistry, as well as the histology of their liver and kidney tissues.

Objectives of the Study

The study sought to:

1. evaluate the nutrient composition of para rubber seeds kernel;
2. assess the growth performance of albino mice fed para rubber seeds kernel meal;
3. examine the haematological, and blood biochemical indices of albino mice fed on para rubber seeds kernel meal; and
4. investigate liver and kidney tissue histology of albino mice-fed diet containing para rubber seed kernel meal.

Research Questions

The researcher shall use the following questions in collecting data for the study:

1. What is the nutrient composition of para rubber seeds kernel?
2. How does para rubber seeds kernel meal incorporated feed influence the growth performance of albino mice?
3. How does para rubber seed kernel meal impact the blood composition (haematology and blood biochemistry) of the albino mice fed para rubber seed kernel meal incorporated diets?

4. How does para rubber seed kernel meal affect albino mice liver and kidney tissue morphology and architecture?

Hypotheses

The following hypotheses guide this study:

H_0: Para rubber seeds kernel meal nutrient or proximate composition indicates that it can be used as a feed ingredient in the albino mice diet.

H_1: Para rubber seed kernel nutrient proximate composition indicates that it cannot be used as a feed ingredient for albino mice.

H_0: Para rubber seed kernel meal incorporated feed influences or affects haematology, and blood biochemistry of the albino mice.

H_1: Para rubber seed kernel meal incorporated feed does not affect or influence the haematology, and blood biochemistry of albino mice.

H_0: Para rubber seed kernel meal incorporated feed affects liver and kidney, tissue morphology and architecture of albino mice.

H_1: Para rubber seed kernel meal incorporated feed does not affect liver and kidney tissue morphology and architecture of albino mice.

Significance of the Study

The study informs livestock farmers about replacing costly conventional feed resources/ingredients with para rubber seed kernel meal. Additionally, the study adds value to waste and by-products (rubber seeds). Finally, this study increases livestock farmers' knowledge to stockpile rubber seeds for use as an alternative feed resource during its season, improving animal farmers' income and livelihood. In a broader sense, this research is expected to contribute to cost reduction in the production of poultry and livestock products, ultimately promoting sustainability within the industry.

Delimitation of the Study

The study was conducted at the University of Cape Coast, Cape Coast, School of Agriculture Teaching and Research farm, Cape Coast, Ghana. The variables captured in this study are the nutrient composition of para rubber seeds kernel meal; growth performance of albino mice fed para rubber seeds kernel meal, haematology and blood biochemistry of albino mice fed para rubber seeds kernel meal; and liver and kidney tissue histology of albino mice fed para rubber seeds kernel meal incorporated diets.

Limitation of the Study

The study did not cover other anti-nutritive factors or substances in the para rubber seed kernel that affected the nutritive value of the para rubber seed kernel being used as a feed ingredient apart from hydrogen cyanide. Due to resource constraints, albino mice were used as experimental animals, and the study could not be replicated with broiler chickens, which the agricultural by-product is intended to be used for. The parameters measured were restricted to growth performance, blood composition, liver, and kidney tissue histology.

Organization of the Study

This study is structured into five main sections. the first chapter, contains an overview of the study, the problem statement, the study's purpose, objectives, research questions, hypothesis, as well as its scope and limitations. The second chapter is dedicated to a review of relevant literature. Chapter three focuses on the methodology employed in conducting the experimental study, covering aspects such as research design, study area, population, sampling method, materials, data collection process, and data analysis. In chapter four, the results are analyzed and discussed. Finally, chapter five offers a summary, conclusions, and recommendations.

CHAPTER TWO

LITERATURE REVIEW

This study investigates the influence of para rubber seed kernel incorporated feed on the growth performance, blood composition, liver and kidney tissues histology of albino mice. Chapter two of this study reviews related literature on the influence of para rubber seed meal incorporated feed influence on poultry and livestock. It also contains the reviews and related literature on the parameters or indicators captured in this research when hydrogen cyanide-containing feed ingredients have been fed to animals.

Methods of Processing Rubber Seeds Meal

Various techniques have been employed to minimize the cyanogenic glycosides (hydrogen cyanide) from para rubber seeds kernel, which include boiling, sun-drying, roasting, soaking, heating, and decomposition of entire seeds. The use of these procedures helps to decrease the hydrogen cyanide content from the kernels. Additionally, to decrease hydrocyanic acid in para rubber seeds can be achieved through detoxification, which can be done either by soaking them in water or roasting them at a temperature of 80 degrees Celsius for fifteen minutes or in a two and a half per cent ash solution for 12 h0urs to help remove toxic substances and boost tastiness (Farr, 2015; Fuller, 1988).

Farr et al. (2019) reported that the para rubber seed kernel processing method centers around partial sun drying of the para rubber seed kernel for two days at a temperature of 30 degrees. Farr et al. (2019) employed soaking, boiling, roasting, and fermenting processes on rubber seeds to lower the cyanide levels in the seed kernels.

Soaking Method

Para rubber seed kernels were water-soaked for three days, then the water was removed and sundried for three days at a temperature ranging from 30c - 35c. The para rubber seeds kernel-to-water ratio of 1:3 was used while soaking the seeds in water (Farr *et al.*, 2019).

Boiling Method

Although para rubber seed kernel meal's protein content is promising, it also contains a cyanogen compound, which can be easily removed or reduced by boiling or storing it for a long time. The boiling and storing could increase nutrient accessibility and utilization of para rubber seed kernel as feed (Oluodo et al., 2018). The half-dried para rubber seeds must be boiled in water at 100 degrees Celsius for half an hour. During boiling the seeds, a water ratio of one to three (1:3) was used. The boiled para rubber seeds were sundried for three days at a temperature ranging from 30ºC to 35ºC (Farr et al., 2019).

Roasting Method

The roasting process was done by dry heating the para rubber seeds kernel for half an hour in a pot at 80 degrees Celsius. The seed kernels are regularly stirred to prevent burning, and the roasted seeds are grounded with a hammer mill.

A study by Aguihe et al. (2017) reported that roasting, soaking, boiling, or fermenting processing methods decrease the cyanide concentration in the para rubber seeds kernel. However, boiling or fermenting tended to reduce the cyanide concentration in para rubber seed kernel meals and made the cyanide content harmless to consuming animals. Other studies showed that a raw para-rubber seed kernel contained seven hundred seventy (770 ml/pp) milligrams per litre of Hydrogen Cyanide, which is harmful for livestock consumption. The researchers noted that the HCN content could be decreased to 38.20 milligrams per liter by sun-drying the para rubber seed kernel (PRSK) for six days, followed by incubating it in hot air at 70°C for 24 hours (Siriwathanannukul & Tantikapong, 2002).

Nutrient Composition of Para Rubber Seed

According to Nath *et* al. (2016), there are various amino acids in para rubber seeds kernel, such as lysine, leucine, isoleucine, valine, threonine, and methionine. The kernel also contains oil, crude protein, ash, and linoleic acid. In addition, a study conducted by Akinsanmi *et al.* (2017) & Eka et al. (2010) revealed that the metabolisable energy of the seed meal was approximately 1828.65 kilocalories per kilogram of dry matter, and it contained various nutrients, such as crude protein (22.16- 40.36 per cent), crude fibre (2.65 - 3.62%); ash (2.20%- 6.57%), and ether extract (12.41 to 55.67%).

Furthermore, para rubber seed kernel meal contained a moisture content of 3.99%, crude protein content of 23.87%, ash content of 4.3 g / 100 g, and crude fat content of 68.5 g / 100 g (Oluodo *et al.*, 2018). Its amino acid composition was low in Cysteine and high in glutamic acid. Because of its numerous components, para rubber seeds are believed to be an ideal feed resource for livestock. Other research studies by Pha-obnga *et al*. (2016) reported on para rubber seed kernel composition indicated its toxic compound as hydrocyanic acid at 80.6 mg/kgDM and aflatoxin as 316.75 ugl/kgDM.

Table1: Chemical composition and toxic compounds of rubber seed kernel

ITEMS	Value (%dry matter)
Chemical composition	
Moisture	2.43
Ash	2.84
Crude protein	19.82
Ether extract	47.67
Neutral detergent fiber	20.87
Acid detergent fiber	17.07
Acid detergent lignin	1.55
Toxic Content	
Hydrocyanic acid (mg/kg)	80.26
Aflatoxin (µg/kg)	316.75

Pha-obnga, et al. (2016)

Their studies also reported the fatty acid profiles of the para rubber seed kernel, as shown in Table 2.

Table 2: Fatty Acid Profiles of Rubber Seed Kernel

Items	Concentration (%)
Saturated fatty acids	
Butyric acid (C4:0)	1.55
Myristic acid (C14:0)	0.14
Palmitic acid (C16:0)	10.35
Stearic acid (C18:0)	6.73
Arachidic acid (C20:0)	0.36
Tricosanoic acid (C23:0)	0.03
Licnoceric acid (C24.0)	0.06
Total	19.23
Unsaturated fatty acid	
Palmitoleic acid (C16:1)	0.18
Oleic acid (C18:1)	25.12
Linoleic acid (C18:2)	37.62
Linolenic acid (C18:3)	17.64
Total	80.77

Pha-obnga, *et al*. (2016)

Eka *et al*. (2010), reported that the crude protein content of rubber seed kernel varied between 22% and 41% in unprocessed rubber seed and processed rubber seed meal. Para rubber seed kernels also contain harmful substances, as other feed ingredients sources of broilers' meals. It is worth noting that para rubber seed kernel meal's nutrient composition has hydrogen cyanide of 60.95 mg/100g (Farr, 2015).

Although para rubber seed meal is commonly described as having high cyanide content (Akinsanmi *et al*., 2018), its anti-nutritional factor contents are generally considered heat labile. Compared to other protein sources for animal feed, such as groundnut cake, soybean meal, and cotton seed cake of metabolizable energy such as 2750, 2460, and 2069 kcal ME kcal/kg DM. Para rubber seeds kernel meal has metabolizable energy levels (1828.65-2675.61kcal/kg) within the range of energy that can be utilized for producing poultry and livestock feed (Olomu, 2011). The high protein (21.90%) and energy contents of para rubber seeds kernel meal indicate that it can be utilized as a plant protein and energy source (Oyewusi *et al*., 2007) and can be included in poultry/livestock feed formulation and compounding.

The cyanide content in para rubber seed kernels can be reduced to make the kernel safe for animal consumption. Nkafamiya, *et al*. (2015), argued that HCN is also found in maize (0.5 mg/kg), baobab (130±1.00 mg/kg), tiger nut (210±1 mg/kg), 130±1.00 mg/kg and cashew nut (370±1 mg/kg) which are all well processed for animal industry consumption. Other studies have been carried out to analyse the cyanide content in specific food crops like maize, white beans, and soybeans, as well as in nuts like tiger nuts and groundnuts, using distilled water, acetone, n-hexane, and 70% ethanol. Data was analyzed qualitatively and quantitatively. Results revealed that soy beans, maize, white beans, ground nuts, and tiger nuts contain hydrogen cyanide (HCN). The quantitative measurements indicated by these researchers were: maize (n-hexane HCN level of 363.33±2.89 mg/kg) with n-hexane = 250.00±2.53), white beans (317.67±2.52), soya beans (282.33±7.02 mg/kg), and tiger nut (210.67±3.79 mg/kg). However, the use of food processing methods to eliminate harmful substances from food crops is considered a potential solution to the consumption of such crops as compared to para rubber seeds meal (Adejoh , *et a*l. 2020).

Para rubber seed kernel meal, which has not yet undergone commercial processing to make it suitable for inclusion in poultry and livestock diets, possesses a higher amount of digestible nutrients in comparison to certain conventional seed meals. As a result, it holds promise as a protein supplement in the feeds for both livestock and poultry (Oyewusi et al., 2007).

Udo, *et* al (2016) assessed the nutritional value of para rubber seed kernel meals and considered various factors such as mineral composition, anti-nutritional properties, and proximate composition. The para rubber seed kernels were processed, dried, and analysed. The crude protein content ranged from 21.08 to 24.60%.

On the other hand, the refined para rubber seeds' kernel crude fibre content was between 4.47% and 5.88%. Farr's 2015 study examined the metabolizable energy value and chemical composition of para rubber seed kernel meal. Based on processing methods, the seeds were sun-dried for around 24 hours before being split into different lots. They were then subjected to various methods, including boiling, sun-drying, soaking, and roasting. The researchers' findings revealed that the various techniques used for processing para rubber seeds resulted in varying results, as presented in Tables 3, 4 and 5.

Table 3: Proximate analysis of rubber seed kernel meal

	RRSM	SRSM	SDRSM	BRSM	RoRSM
Dry matter (%)	74.0^{d}	88.0^{c}	89.5^{b}	88.5^{c}	91.0^{a}
Moisture (%)	26.0^{a}	12.0^{b}	10.5^{c}	11.5^{b}	9.0^{d}
Crude protein (%)	16.1^{c}	18.0^{b}	18.3^{b}	18.4^{ab}	18.9^{a}
Crude fibre (%)	13.03^{a}	7.94^{c}	7.58^{c}	8.25^{c}	9.45^{b}
Ether extract (%)	16.5^{b}	20.0^{a}	13.5^{c}	17.5^{b}	17.0^{b}
Ash (%)	2.0^{a}	1.75^{b}	1.75^{b}	1.25^{c}	2.50^{a}
NDF (%)	16.0^{d}	36.5^{a}	21.0^{cd}	31.0^{ab}	25.5^{bc}
WS-DM	58.7^{c}	60.2^{b}	59.8^{b}	61.9^{a}	60.5^{b}
WS-N	75.8^{a}	73.2^{c}	75.0^{ab}	74.1^{b}	74.9^{b}
Calcium (%)	0.31$^{a\ ss}$	0.26^{a}	0.30^{a}	0.29^{a}	0.25^{a}
Phosphorus (%)	0.25^{c}	0.28^{c}	0.42^{a}	0.42^{a}	0.39^{b}
Potassium (%)	1.03^{d}	1.21^{c}	1.59^{a}	1.58^{a}	1.50^{b}
Magnesium (%)	0.28^{a}	0.29^{a}	0.27^{a}	0.25^{a}	0.26^{a}
ME (MJ/kg)[2]	11.79^{c}	12.97^{a}	11.76^{c}	12.56^{b}	12.87^{a}

Source:Farr (2015)[1]

RRSM means Raw Rubber seed mealSRSM means soak rubber seed meal, SDRSM means sun-dried Rubber Seed Meal, Biol Rubber Seed Meal and Roasted Rubber Seed Meal.

[1] Farr M.H (2015)

Table 4:) The amino acid profile of different processing methods of para rubber seeds meal (g/100g protein).

	RRSM	SRSM	SDRSM	BRSM
Lysine	2.45^{c}	3.25^{a}	3.04^{b}	3.05^{b}
Methionine	1.13^{a}	0.97^{b}	1.11^{a}	1.11^{a}
Cystine	1.77^{a}	1.63^{b}	1.70^{a}	1.73^{a}
Histidine	1.86^{a}	1.86^{a}	1.70^{a}	1.72^{a}
Phenylalanine	3.52^{b}	3.23^{c}	3.53^{a}	3.53^{a}
Threonine	2.45^{c}	2.69^{b}	2.85^{a}	2.84^{a}
Leucine	5.66^{a}	5.06^{c}	5.47^{a}	5.47^{a}
Isoleucine	2.89^{a}	2.77^{ab}	2.72^{ab}	2.69^{b}
Valine	6.44^{a}	6.05^{b}	6.00^{b}	6.06^{b}
Alanine	4.41^{a}	3.81^{c}	4.14^{b}	4.41^{a}
Aspartic acid	9.94^{a}	9.01^{b}	9.34^{a}	9.35^{a}
Arginine	9.71^{a}	8.05^{c}	9.37^{a}	9.55^{a}
Serine	4.14^{a}	3.69^{c}	3.97^{b}	3.96^{b}
Glutamic acid	14.48^{a}	12.87^{c}	14.03^{a}	13.75^{b}
Glycine	3.95^{a}	3.65^{b}	3.66^{a}	3.65^{b}
Proline	5.00^{a}	3.569^{d}	4.62^{bc}	4.82^{ab}

Source: Farr (2015)[2]

RRSM means Raw Rubber Seed Meal. SRSM means Soak Rubber Seed Meal; while SDRSM means Sun Dried Rubber Seed Meal. BRSM means Boil Rubber Seed Meal, and RoRSM means Roasted Rubber Seed Meal.

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Table 5: The anti-nutritional factors in para rubber seed meal.

	RRSM	SRSM	SDRSM	BRSM	RoRSM
HCN (mg/100g DM)	60.95[a]	10.90[c]	7.10[d]	4.6[e]	14.30[b]
Tannin (g/kg DM)	1.5[a]	0.5[b]	0.7[b]	0.1[c]	0.4[b]
Saponin (%)	0.88[a]	0.32[c]	0.45[b]	0.23[d]	0.33[c]
Phytate (%)	0.62[a]	0.24[c]	0.34[b]	0.08[e]	0.19[d]
Oxalate (%)	0.21[a]	0.11[c]	0.18[b]	0.07[d]	0.09[d]
Trypsin inhibitor (TIU/mg)	15.75[a]	0.06[c]	0.26[b]	0.00[d]	0.00[d]

Source: Farr (2015) [3]

RRSM means Raw Rubber Seed Meal. SRSM means Soak Rubber Seed Meal, while SDRSM means Sun Dried Rubber Seed Meal. BRSM means Boil Rubber Seed Meal, and RoRSM means Roasted Rubber Seed Meal.

Additionally, Farr et al. (2019) conducted a study to test four (4) processing methods of para rubber seed kernel. They reported that para rubber seed kernel meal contains 74.0 % - 91.0% dry matter, 9.0% - 26.0% moisture, crude protein 16.1% - 18.9%, and crude fibre 7.58% - 13.03%, oil fat content 13.5% - 20.0% and ash content of 1.25% - 2.50%.

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Udo et al. (2016) conducted a study, where two processing methods were used: boiled and toasted. They stated that para rubber seed kernel meal contains dry matter of 96.91% for raw, 85. 40% for boiled and 93.63% for toasted, crude protein 23. 31% for raw, 24.60% for boiled, 21.8% for toasted, crude fibre 5.88% for raw, 4.47% for boiled, 4.95% for toasted, ether extract 38.47% for raw, 23.13% for boiled, 32.57% for toasted and Ash content 3.77% for raw, 4.68% for boiled, 4.57% for toasted of para rubber seed kernel meal. They also reported that the metabolisable energy value of para rubber seed kernel ranges from 2.88 MJ/kg (raw), 2.32 MJ/kg (boiled) and 2.58 MJ/kg (toasted).

A study conducted by Mmereole in 2008, examined the substitution of groundnut cake with rubber seed meal in broiler diets and assessed its impact on hematological and serological indicators. In the course of the study, a proximate analysis was performed, revealing that rubber seed meal has the following composition: 3.10% ash, 34.10% crude protein, 10.12% crude fat, and 4.40% crude fiber.

In addition, Onwurah et al. (2010) conducted a study to test the nutritional value of rubber seed meal. The study found that rubber seed meal has 3.9 moisture content, 2.6% ash, 22.3% protein, 42.5% fat and 29.0% NFE. Additionally, Sharma et al. (2014) conducted proximate composition on detoxified rubber seed meal. They reported that rubber seed meal contains 16% moisture content, 0.24% ash, 25.4% crude protein, 39.1% crude fat, 75.00% crude fibre and 33.2% NFE.

Feed Intake by Albino Mice

Animals consume food to meet their energy needs, which include maintenance, growth, physical activity, heat generation, and other physiological processes. Water intake compensates for losses from evaporation and excretion in urine and faeces. Food and water intake exhibit significant daily fluctuations closely tied to the circadian rhythm of wake-sleep patterns. Various biochemical and physiological factors are associated with food and water consumption, and these daily variations can influence how animals respond to experimental stimuli. The presence of food and water significantly influences the traits and attributes of experimental animals.

According to the University of North Carolina (2023) report, the average feed intake per mice is around 5g\day. However, it will differ by more than two (2) fold depending on feed palatability, fat and sugar content of the feed or strain of albino mice. The fat content of the diet should be around a maximum of 8% or less. Daily food consumption for housed animals varies particularly, where they do not have access to exercise and are fed a uniform chow diet. In these circumstances, the daily food consumption, when adjusted for every 100 grams of body weight, is approximately 20 grams when the animals are five weeks old. The food intake declines during the early stages of life and levels off for adult mice at around 6-8 grams. Likewise, the daily water intake also decreases at a range of 9-12 millilitres per 100 grams of body weight. In addition, according to the National Research Council (NRC, 1995), in 1995, mice required 160 kcal/kg BW. To fulfill this requirement, a suitable diet should provide 3.9 kcal of metabolizable energy (ME) per gram. Growing mice required 263 kcal of ME per kilogram of 0.7 increase in body weight. During pregnancy, quest for energy rises significantly to 358.5 kcal of ME per kilogram of body weight increase to 0.75. Additionally, the diet's fat content should remain below 10-11%. According to Verma, et *al*. (2023). emphasize that the quantity and efficiency of amino acid absorption from the diet are essential considerations when determining the necessary protein intake. A diet containing 18% crude protein can sustain growth rates exceeding 1 gram daily.

Use of Para Rubber Seed Kernel Meal as Animal Feed Resource

A study by Onyimonyi et al. (2012) found that feeding para rubber seeds kernel meals to albino rats had no significant impact on their performance. However, another finding by Esonu et al. (2001) found that feeding para rubber seed kernels meal to albino rats decreased their growth rate.

Boateng et al. (2021) carried out research to assess how dried cashew apple meal influences the growth and internal organs of an albino rat. The results showed that incorporating dried cashew apple meal (DCAM) into albino rat diets at levels ranging from 10% to 15% did not result in any negative impacts on growth performance indicators and the final dressed weight. This result means that dried cashew apple meal can be used in livestock feed formulation at the inclusion rate of 15% without harming growth performance and carcass characteristics.

Deng et al. (2017) carrier out a study, to assess how substituting fish meals with para rubber seed meal affected nutrient utilization, cholesterol metabolism, and the growth of tilapia at the inclusion rate of zero (0%) control, one hundred fifty (150 g/kg), three hundred (300 g/kg), four hundred fifty (450 g/kg), and six hundred (600 g/ kg) of para rubber seed meal substituting fish meal in the diet. The findings showed that para rubber seed kernel meal's inclusion of one hundred fifty (150 g/kg) grams per kilogram diet (150%) did not affect the daily growth coefficient, protein and feef competence ratios, or weight gain. In addition, its inclusion inhibited digestive enzyme activity, cholesterol metabolism, and antioxidant capacity. They attributed their observation to fiber and cyanide, or the lack of lysine and methionine (Deng et al., 2017).

Fawole et al. (2017) analysed the various factors that affect the immunobiological, antioxidant, and histological changes in Labeo rhita fingerlings fed with para rubber seed kernel protein isolates at the inclusion rate of twenty (25%), fifty (50%), seventy-five (75%), and hundred (100%) per cent to replace soybean meal in the diet. The research findings indicated that the growth parameters of Labeo rohita fingerlings, including final body weight and feed efficiency ratio, showed no significant variations among the different groups in the analysis. The study noted that the fish effectively utilised the para rubber protein isolate, and the inclusion levels of this isolate did not lead to oxidative-induced stress. Deng, et al. (2015) examined how para rubber seed kernel meals used to substitute soy-bean affected the fish at the zero (0%) control, ten (10%), twenty (20%), thirty (30%), and forty (40%) per cent, respectively. The study revealed that consuming 30 percent para rubber seed kernel meal replacing soybean meal in a tilapia diet did not affect the fish's growth, immunity, or antioxidant capacity (Deng et al., 2015). However, para rubber seed meal substituting soy-bean meal in tilapia above thirty (30%) percent led to various adverse effects, including an inhibited growth rate, reduced liver function, and disease resistance. These deleterious effects were attributed to cyanide content in the meal (Deng et al., 2015).

Deng et al. (2015) study also examine how juvenile tilapia feed substitution with para rubber seed kernel meal would affect it. This replacement was done at various levels, including 0, 65, 130, 195, and 260 g per kilogram. Addition of 195 grams of para rubber seed kernel meal to replace plant proteins had no effect on the fish. Although using the experimental feed at the 195 g/kg level in the meal of the fish may lead to reduced feed digestibility and antioxidant capacity, it could also help maintain the growth rate of the fish. This is because the nutrients (fibre) present in para rubber seed meal can counteract the cyanide's adverse (Deng et al., 2015).

Akinsanmi, et al. (2020) examined how standard para rubber seed kernel meal-based diet affects broiler's growth routine and carcass features. Two strategies were applied to prepare the meal for the study. The study used a hot water-soaked sample and a roasted rubber seed meal. The researchers noted that standard para rubber seed kernel meal-based diet had no significant effect on broiler's growth routine and carcass features, and could therefore be used to replace soybean in chicken diet.

Khatun et al. (2015) examined where rubber seed meal substituted soy-bean to feed broilers and found that it had no toxic or detrimental effect on broiler production, and RSM can replace ten percent (10%) to twenty percent (20%) of soybean meal in feed supply for broiler chickens.

Nath et al. (2016) examined how incorporating Rubber Seed Meal and Saccharomyces yeast into broiler diets, with a yeast inclusion rate of 1% and para rubber seed kernel meal at 10% affect the broiler. The broilers were housed in an open-sided facility under consistent monitoring for 35 days. Findings showed that yeast and rubber seed meal inclusion in poultry diets led to enhanced growth performance in broiler chickens. It was suggested that the enhanced growth performance might be due to efficient nutrient digestion occurring in the ileum.

ljaiya et al. (2011) substituted a portion of broiler feed with para rubber seed cake, replacing soybean meal in the diet after oil extraction from the rubber seeds kernel. The inclusion rate was 0% (control), 25%, 50%, 75%, and 100% rubber seeds meal swapping certain quantity of the soy-bean meal in the diet. It was noted that a diet with a 50% inclusion level of rubber seed meal positively affects broiler chickens' weight gain and nutrient digestibility. In addition, the researchers reported that adding 75% rubber-seed feed to broiler chickens' meal could significantly improve feed comsumption due to increased fibre content in rubber seed meal, increasing the total fibre content and thereby diluting other nutrients. The birds need to consume increased amounts of meal satisfaction of their liveliness requirements to maintain rapid development and growth, as observed by Ijaiya et al. in 2011. However, increase in the kernel meal in the chicken feed could lead to reduced nutrient intake due to its fibre content, which might result in the birds being unable to meet their production and metabolic needs.

Furthermore, another study assessed how para-rubber meal affects the growth-performance, feed intake of poultry birds and its carcass quality. Four diets were formulated with varying proportions of rubber seed meal and blood meal: 0% rubber seed meal and 0% blood meal, 10% rubber seed meal and 8% blood meal, 20% rubber seed meal and 6% blood meal, and 30% rubber seed meal and 4% blood meal. This trial was carried out using a traditional management system. The findings indicated that combining 4% blood meal and 30% rubber seed meal did not harm the birds' performance.

However, above the 30% rubber seed meal is attributed to depression in broiler chicken because of the dietary fibre present in rubber seed meal (Ahaotu, 2018). it was noted that an increased dietary fibre content leads to a reduction in weight gain among broiler chickens.

The effect of Para rubber seeds meal as feed ingredient in diets on haematology and blood biochemistry of mice and other animals.

Using alternative feed ingredients in livestock feed without a thorough understanding of their nutrient content, anti-nutrient properties, and impact on animal health can be harmful to the animals' well-being. Animals' health risks from alternative feedstuffs can be mitigated by screening for impact of nutritional requirements. This involves assessing physiological conditions and using blood parameters to evaluate health and prognosis when using different protein feed ingredients, as these parameters change with physiological shifts (Obasoyo et al., 2005; Amel et al., 2006; Etim et al., 2013). The various components of blood (creatine, urea nitrogen, serum protein, blood count, haemoglobin, red blood cells, white blood cells, neutrophils, eosinophils, bilirubin, etc.) can be used as indicators to determine an animal health condition. They also help evaluate the quality of protein and amino acid levels in feeds fed to an animal (Animashaun, et al., 2006; Ojebiyi et al. 2007). Additionally, Bahman (2011) observed that blood analysis offers a rapid method for assessing the nutritional health and clinical conditions of animals in feeding trials. The typical hematological parameters for albino rats or mice with normal blood values are as follows: hematocrit/packed cell volume (36-54%), Red Blood Cell count (11-19.2 g/dL), mean cell volume (48-70 fL), mean corpuscular hemoglobin concentration (40 g/dL), white blood cell count (6-18 x 1000), segmented neutrophils (10-30%), lymphocytes (65-85%), monocytes (0-5%), eosinophils (0-6%), basophils (0-1%), and platelets (500-1300 x 1000) (Wikivet, 2012). In addition, Mcbean (2016-2017) also reported the normal mice haematology reference range: WBC 1.06 – 56.08, RBC 3.57 – 15.2, HCT 16.7 -69.8, MCHC 27 -37.6, MCV 39 -90.8, PLT 59-2633, LYM 0.12-23.46, HB 6.1-21.7, and MPV 5.2-13.1. These elements of the blood can be seen at a specific level. When the blood is average, these elements have specific bodily functions.

Red blood cells are essential for transporting oxygen from the lungs to body tissues, where it acts as a vital source of energy (Ruben, 2018). Ruben (2018) further indicated that an above-average red blood cell count signals dehydration and abnormal physiological processes that stimulate its excessive production in the marrow of the bone. Conversely, low average its count suggests anaemia is present, which can result from factors like blood loss, ongoing bleeding, bone marrow disorders, or increased breakdown of red blood cells in certain immune-related conditions due to malnutrition or exposure to toxins (Ruben, 2018).

White blood cell counts reflect the body's immune response to infections and diseases, with abnormal levels suggesting potential issues like infections, blood cancer, or immune system disorders (National Institute of Health, 2022). Platelets, responsible for blood clotting, can exhibit higher or lower levels than normal, indicating the possibility of clotting or bleeding disorders (NIH, 2022).

Haemoglobin is an iron-rich protein in red blood cells, serving as a vehicle for oxygen transport throughout the body (NIH, 2022). Hemoglobin contributes to the growth and function of organs and tissues, and it also helps in transporting carbon dioxide from the body to the lungs for removal (Kamfrath, 2022). When the levels of this iron-rich protein fall below the normal range, it can indicate conditions such as anaemia, sickle cell disease, or thalassemia (NIH, 2022).

Hematocrit or packed cell volume tests determine the proportion of blood volume that is occupied by red blood cells. Elevated levels may indicate dehydration, while lower levels suggest the possibility of anaemia (NIH, 2022). Moreover, hematocrit/packed cell volume can indicate reductions in blood toxicity, with abnormal levels suggesting the presence of toxic substances that may adversely affect blood formation (Oyawoye & Ogunkunle, 1998).

Mean Corpuscular Volume (MCV) assesses the average size of blood cells. If MCV levels are lower than usual, it can indicate conditions like anaemia or thalassemia (NIH, 2022). On the other hand, Mean Corpuscular Hemoglobin Concentration (MCHC) measures the amount of haemoglobin within a single red blood cell relative to its volume, serving as an indicator of red blood cell function and overall health, particularly for detecting signs of anaemia and other blood disorders (Kamfrath, 2022). Elevated MCHC levels suggest an excess of haemoglobin within the red blood cells, while low levels point to the possibility of anaemia (Ruben, 2018).

Lymphocyte Cell Count, a component of white blood cells in the immune system, consists of two primary lymphocyte types: B cells, responsible for producing antibodies to combat invading bacteria, viruses, and toxins, and T cells, which eliminate body cells that have been hijacked by viruses or have become cancerous. Elevated lymphocyte cell counts may signal the presence of infection, viral diseases, or specific cancers like lymphosarcoma. Conversely, lower lymphocyte cell counts can indicate viral infections affecting bone marrow and leading to anaemia (Ruben, 2018).

Monocytes are white blood cells that combat infections, and elevated levels suggest the presence of an infection (Ruben, 2018). Eosinophils are white blood cells primarily involved in fighting parasites, and high eosinophil counts indicate a parasite-induced illness. However, it is important to note that low eosinophil counts are impossible in standard blood samples as they can reach zero (Ruben, 2018). Basophils, although relatively uncommon among white blood cells, may be observed in specific parasitic infections, especially heartworms. High basophil levels can signal potential parasitism, but it is essential to mention that low basophil levels are impossible in standard blood samples as they can also reach zero (Ruben, 2018).

The standard blood chemistry reference comprises the various components of the body, such as the total protein (g/dL) 5.6 – 7.6, glucose (mg/dL) 50 – 135, albumin g/dL) 3.8 – 4.8, blood urea nitrogen (BUN) (mg/dL) 15 – 21, creatinine (mg/dL) 0.2 – 0.8, sodium (mEq/L) 143 – 156, chloride(mEq/L) 100 – 110, potassium (mEq/L) 5.4 - 7.0, phosphorus (mg/dL) 3.11 – 11, calcium (mg/dL) 5.3 – 13, Alanine aminotransferase, ALT (U/L) 17.5 – 30.2, Aspartate aminotransferase, AST (U/L) 45.7 – 80.8, Alkaline phosphorus, (U/L) 56.8 – 128, Cholesterol (mg/dL) 40 – 130; total bilirubin (mg/dL) 0.2 – 0.55, and Amylase (SU/dL) 128 – 313 (Queensbury & Carpenter, 2012). The liver is a multifunctional organ that plays a vital role in metabolism, detoxification, digestion, and other processes essential for an animal's overall health and survival (Baquerre, et al., 2021).

Sodium is a substance cells need to work and helps uphold the right balance of fluids in the body. It helps an animal's nerves and muscles to work. When sodium is higher than usual, it decreases live and daily body weight gains, water and feed intake, reducing the digestibility of animal nutrients (Fang et al., 2018).

Potassium’s significant role in an animal body is to help maintain a standard range of fluid inside the animal cells, and it also helps activate various cell and nerve functions. When potassium is low in an animal, it indicates it is suffering from hypokalemia. Hypokalemia is characterized by a low blood potassium concentration. The primary causes include severe or prolonged vomiting and chronic failure of the kidney. When the calcium level is lower than usual, the animal suffers hypocalcemia. A pair of parathyroid glands controls calcium concentration. AST (Aspartate transaminase), ALT (alanine transaminase), and ALP (alkaline phosphatase) are important markers for liver damage. These enzymes are found mainly in the liver, red blood cells, heart, pancreas, kidneys, and the biliary ducts of the liver. Measuring the levels of AST and ALT in the blood helps determine if there has been damage to specific tissues, especially the heart and liver (Kasarala & Tillmann, 2016). When body tissues are damaged, they release more AST and ALT into the bloodstream, leading to elevated serum enzyme levels. Therefore, the concentration of AST and ALT in the blood directly reflects the extent of tissue damage. In cases of acute viral hepatitis, a high AST to ALT ratio (>1.5) may suggest a more severe condition (Botros & Sikaris, 2013; Washington & Hoosier, 2012). The ALP test is used to detect blockages due to disorders in the bone, damage of the liver, or bile ducts. When liver cells are damaged, they release more ALP into the bloodstream. Elevated ALP levels in the plasma can also indicate significant bile duct obstruction, intrahepatic cholestasis, or infiltrative liver diseases.

Total protein measures the overall amount of proteins produced by the liver and found in the blood. High total protein levels can be influenced by dehydration or a metabolic disturbance. While low total protein levels are the result of chronic malnutrition, impaired absorption of proteins, or protein depletion stemming from kidney or liver issues (Washington & Hoosier 2012).

Albumin is a blood protein with crucial functions: it maintains blood vessel osmotic pressure, transports hormones, fatty acids, and drugs, regulates blood pH, and prevents fluid leakage. Low levels signal malnutrition, chronic inflammation, hepatitis, or severe liver damage, while high levels suggest dehydration or reduced blood volume (Washington & Hoosier, 2012).

Esonu et al. (2001) found that feeding para rubber seed kernel meals to albino rats decreased their red blood cell count, haemoglobin concentration, and hematocrit value. However, another study Onyimonyi et al. (2012), found that feeding albino rats with para rubber seed kernels meal has no significant effect on these blood parameters.

Fawole et al. (2017) conducted a feeding trial to test the haemato-biochemical, antioxidant, and histological characteristics of Labeorohita fingerlings fed with para rubber seed protein isolates. They replaced soybean meal with these para rubber seed protein isolates at an inclusion level of RPI 25, RPI 50, RPI 75, and RPI 100. The results of the study indicated that the various parameters of haematological analysis were similar to the control group. However, red blood cells significantly differed from the control group. Additionally, immuno-biochemical activities, including lysozyme activity, globulin levels, respiratory burst, and total immunoglobulin, were similar across the different groups. The researchers observed that fish effectively utilized rubber seed protein isolates without inducing oxidative stress. Therefore, rubber seed protein isolates could be considered a viable alternative to soybean protein isolates in fish diets, with no adverse effects. This makes protein isolation from rubber seed meal a promising option (Fawole et al., 2017).

Udo, et al. (2018) examined the various biochemical, growth, and haematological components of WAD (West African dwarf) goats that were fed different treated rubber seed kernel meal at the inclusion rate of 0, 10, 20 and 30% of para rubber seed kernel meal. They reported that as the rate of boiled rubber seed kernels meal inclusion level increases from 10% to 30% of the diet or ration in the feed, the level of feed consumption decreases. The researchers noted that the various parameters of the blood samples were not significantly different between treatments except for white blood cell and cholesterol levels. They also stated that 20% of the boiled rubber seed meal diet was generally better for West African dwarf goats' performance (Udo et al., 2018). In addition, they also stated that the abnormal value revealed in the study might not be connected with traces; it may stem from other source/s of infection and not anti-nutritional factors from boiled rubber seed meal.

Some feed resources or ingredients, including but not limited to para rubber seed feed, contain HCN (hydrogen cyanide). HCN is a liquid/gas by an almond-like odour. It can disrupt the body's ability to utilize oxygen and may cause harm to the other parts of the body {brain, heart, blood vessels, lungs, and liver} (National Institute for Occupational Safety and Health, NIOSH, 2023).

However, when the hydrogen cyanide-containing feed resources are subjected to various processing methods like ensiling, sun-drying, fermentation, boiling, roasting, and soaking, it helps to reduce or remove the hydrogen cyanide content and makes it safe for animal consumption. The extract from cassava peels contains linamarin and lostraulin, which are cyanogenic glycosides (Smith, 1988). These two compounds are derivatives of hydrogen cyanide. Because of the harmful effects of hydrogen cyanide on livestock (Mcdonald et al., 1995), the use of fresh, unprocessed cassava peels as feed has been restricted (Smith, 1988). Cassava peels can be detoxified through various methods, such as ensiling (Okeke et al., 1985), sun-drying (Ahamefule et al., 2003), and fermentation (Ijaiya, 2001).

Babatuyi, et al. (2020) analysed different characteristics (haematology, cyanide, and histopathology) of albion-rats that were fed feed made of fufu using two processing methods which were SWI (spontaneous-soaked method) and a grated preparation of fufu-based diets. The fermented product was identified and purified from the various microorganisms involved in the fermentation process. Some of these include (Bacteria only, Bacteria and Yeast, Yeast only, Bacteria and Mould, Mould and Yeast, and Mould only. They oven-dried and milled the fermented components. The rats were fed with fifty (50%) per cent of their commercial vital feed and fifty per cent of fifty (50%) of the balance samples of Fufu using the ration 1:1. The study results indicated that albino rats on MAP, and BMP meal showed high levels of neutrophil, with lower lymphocyte, packed cell volume in the blood, the highest white blood cell concentration was found in rats on BMP meal. It was because of low feed intake. The high level of neutrophils was prompted by mould (high metabolic level of the fungi) in sample of the fufu (Babatuyi, et al., 2020).

The rats consuming the MAP meal had the lowest white blood cell counts. This could be attributed to the elevated metabolic activity of fungi (mould) in 'Fufu' samples BMP and MAP. The additional increase in neutrophil levels may be a response to the presence of these fungi. The study suggests that red blood cells might counteract the antigens (foreign substances) by producing antibodies, indicating an infection. The decreased lymphocyte count could indicate lymphocytopenia, an immune system disorder often caused by insufficient B-lymphocyte secretion responsible for producing antibodies. This insufficiency may result from various factors, including metabolites from microbial activity in BMP and MAP samples. The rats fed 'Fufu' samples containing a combination of moulds, possibly Penicillium notatum and Aspergillus niger, could be at risk of food poisoning due to mycotoxins (Babatuyi et al., 2020).

Concerning hydrogen cyanide, the researchers reported no cyanide content was detected in the rats that consumed the fufu-based diets. This absence of cyanide can be attributed to the efficient processing methods that facilitated the breakdown of cyanogenic glycoside in the cassava tuber by increasing its surface area (Babatuyi et al., 2020).

Bamikole, et al. (2022) evaluated the effects of a formulated herbal mixture made from Sorghum bicolor L, Curcuma longa L, Bridelia ferruginea B. and honey (STBH) on weight, biochemical parameters, haematological profile, and histological effect on healthy Wistar rats. The herbal mixture was orally administered to rats at a dose of 0.65mg/g, 1.3mg/g and 2.6mg/g of rat weight for twenty-eight (28) days. They noted that the use of Sorghum bicolour L. Curcuma longa, Bridelia ferruginea, and honey (STBH) significantly improved the packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) blood parameter of rats.

A study by Raji, et al. (2020) examines the effect of sun-dried cassava peel meal on haematological and blood serum indices of broiler starter chicks at the inclusion rate of 0, 10, 20, 30 and 40% sun-dried cassava peel meal (SDCPM), as replacing wheat bran in the diets. The study revealed that the supplementation of sun-dried cassava peel meal did not significantly differ among the various parameters examined; however, the globulin and albumin ratios were significantly dissimilar. Albumin utilisation and synthesis were increased among treatments. They suggested that the increase might be attributed to the higher level of crude protein provided by the test ingredient at elevated inclusion levels in the diet, as albumin synthesis has been observed to correlate with the amount of available protein in the diet. Therefore, Sun-Dried Cassava Peel Meal (SDCPM) performed well at all inclusion levels. This good performance means cassava peal can replace wheat bran in chickens' diets without affecting their health.

In a different study, the effects of cassava composite meals on the growth performance, hematological indices, and serum biochemistry of broiler chickens were examined. Initially, cassava roots were cleaned, peeled, chopped into small pieces, oven-dried, and ground into a meal. Similarly, the petiole, tender stems, and leaves were cut and oven-dried to produce cassava foliage meal. Cassava root meal was prepared by drying and milling the cassava roots, while cassava composite meal was a blend of root meal and foliage meal in a 10:1 ratio. Diets were formulated with varying inclusion levels of cassava root meal and foliage meal (0%, 5%, 10%, 15%, and 20%). The study found that hematological indices stayed within normal ranges, but there were significant differences in all serum biochemistry parameters. The researchers noted that the considerable reduction in serum total protein levels in birds fed cassava-based diets might be due to insufficient dietary protein (Adedokun et al., 2017).

In contrast, Akapo et al. (2014) researched to investigate the impacts of feeding broiler chickens with cassava tuber meal at inclusion levels of 100 grams per kilogram (100 g/kg) and 200 grams per kilogram (200 g/kg), along with a maize-based controlled diet. They then fed them the two different diets: a control diet with no cassava root meal and a modified diet with both raw and cooked cassava root meal. The researchers found that the modified diet had a significant effect on the growth performance of the chicks. The result indicated that feeding peeled or unpeeled cassava root threatens broiler chicks' growth and health (Akapo et al., 2014). Providing broilers with a diet containing 10% peeled cassava root meal led to a decrease in their final body weight. This weight reduction in cassava-fed birds compared to the control group was attributed to the higher levels of dietary crude fibre and hydrogen cyanide, along with the reduced crude protein content, as cassava root meal inclusion levels increased (Akapo et al., 2014).

A different study by Oyedele et al. (2021) found that including dried Distilled Cassava with Soluble in broiler chicken diets at levels of 0%, 4%, 8%, and 12% dry matter had no significant difference when measuring parameters for the broilers fed with these experimental diets. The researchers suggested that adding distilled cassava with soluble at a rate of 12% inclusion in chicken meal did not affect the haematological and serum biochemical characteristics.

Adebiyi et al. (2008) conducted a biological study using sorghum bicolour starch hydrolysed with α-amylase from Rhizopus sp as a feed for albino rats. They prepared this feed by mixing 12 g of sorghum starch with 100 mL of water, 0.4 g of CaCO and 0.4 g of NaCl, maintaining a pH of 6.0. The mixture was then heated at 72°C for two hours in water, and at 60°C, 4 mL of partially purified enzyme from Rhizopus sp was added. Afterwards, the mixture was sun-dried for two to three days, and 65.5 hydrolysed sorghum starch was incorporated into the diet. The research results demonstrated improvements in blood formation and increased body weight in the animals, possibly due to essential nutrients like minerals and vitamins that support blood formation and maintenance. The researchers concluded that using sorghum bicolour starch hydrolysed with α-amylase from Rhizopus sp as a feed for albino rats is a favourable alternative.

A study was conducted to investigate how the inclusion of rubber seed meal (RSM) at various levels, along with different processing methods, in broiler chicken diets could impact the overall health of the birds. These methods were soaking in hot water and roasting the raw para rubber seed kernels was subjected. The equi-protein was incorporated into the diet at a rate of 15, 25 and 35%, replacing soybean. The result revealed that the para rubber seed kernel meal or cake had no significant adverse effects on the health status of poultry birds when included in broilers' diet from 15% to 35% of the seed meal (Akinsanmi et al., 2020).

Effect of Para Rubber Seed Meal as Feed Ingredient on Liver and Kidney Histology of Albino Mice/Rat and Other Animals

Histological analysis is the preferred method for examining tissues, serving both research and diagnostic purposes. It allows for qualitative and quantitative assessments, enabling the evaluation of inflammation, healing progress, and the existence and supply of degraded feeds in the neighboring tissue (Ragamouni et al., 2013). Research conducted by Esonu et al. (2001) found that including rubber seed meal in the diet of albino rats led to impaired liver and kidney function, evident from elevated levels of alanine aminotransferase (ALT) and blood urea nitrogen (BUN). However, another study by Onyimonyi et al. (2012) found no significant effect on these parameters. In a study carried out by Fawole et al. (2017), a feeding trial was conducted to examine the hematological, biochemical, antioxidant, and histological traits of Labeorohita fingerlings. The study involved substituting soybean meal with para rubber seed protein isolates at varying inclusion levels, RPI 25, RPI 50, RPI 75, and RPI 100. They found that the liver did not show signs of changes.

Adeyemo. et al. (2014) also studied the effect of cassava peel on poultry birds' (broilers) internal organs, replacing maise in the diet. The researchers discovered that the various organs of the birds, such as the liver, kidney, and heart, had increasing signs of coagulative necrosis and degeneration of the hepatocytes and vacuolation. The cardiac and hepatocellular tissues had also shrunk due to cassava peel's increased feed intake.

In addition, Oyedele. et al. (2021) found that the use of Dry Distilled Cassava with Soluble in broiler chickens' diets based on haematological, biochemical, and histological characteristics at the inclusion levels of 4, 8, and 12% results in liver, kidney, small intestine, and large intestine changes. They indicated that the changes might have been attributed to including Dry Distilled Cassava with Soluble in the diet, which might have caused changes in the liver, kidney, small intestine, and large intestine due to increased crude fibre levels in the diets.

Manyelo et al. (2019) studied sorghum bicolour as replacement for Zea mays on performance and gut histo-morphology of broiler at 0, 25%, 50, 75%, and 100% substituting maize graded levels in the diets. The result indicated that the tissue structure did not change histologically when the animals were fed either 75% or 100% substituted diets. They concluded that the various types of Macia sorghum could be utilised in broiler diets without adverse effects (Manyelo et al, 2019).

CHAPTER THREE

MATERIALS AND METHODS

The materials and methods used to conduct the research reported in this chapter. Experimental site, experimental diets, research design, data collection procedures and data analyses are the ingredients of this chapter.

Experimental Design

The research design employed was a Completely Randomized Design, which included four different dietary treatments, each with two replicates.

Table 6: Dietary Treatments

Replicates	T1	T2	T3	T4
R1	T1R1	T2R1	T3R1	T4R1
R2	T1R2	T2R2	T3R2	T4R2

Three months old, fifty male Institute of Cancer Research (ICR) albino mice were acquired from the Animal Unit of the Center for Plant Medicine Research (CPMR), Akuapem Mampong, Eastern Region of Ghana for this experiment. Forty (40) mice were randomly assigned to four dietary treatment groups (D1, D2, D3 and D4) with two replicates each. Ten (10) mice per treatment and five (5) per replicate were randomly selected using a completely randomized experiment design. The albino mice in each treatment were fed one of the four diets. Animals were the same breed (ICR), age (12 weeks), same-sex (male), and similar weight. The four diets were D1 (control: a commercially prepared chick starter diet containing 21.5% crude protein and 2825 kcalME/kgDM energy). In diets 2 (D2), 3 (D3) and 4 (D4) 5%, 10% and 15% of the ME (141.25 kcal ME, 288.5 kcal ME and 423.75 kcal ME) in the control diet, respectively was replaced with ME from full-fat Para rubber seed kernel meal (PRSKM) ME (other conditions held constant.) so the four diets were iso caloric. The same four experimental diets were fed to the albino mice assigned respectively through the 28 (twenty-) day feeding period. The mice were raised under 12 hours of light and 12 hours of darkness.

Area of the Study

This research was conducted at the University of Cape Coast, School of Agriculture Teaching and Research Farm in Cape Coast, Central Region of Ghana.

Experimental Site

The study was conducted at the Animal House of the Animal Science Department, School of Agriculture, University of Cape Coast, Cape Coast, located at the Teaching and Research Farm of the School of Agriculture. The Teaching and Research Farm is in the southwestern part of Ghana. It has an average temperature ranging from 24^{c} to 34 c and a relative humidity of 50% to 85%, as (Yangtul, 2013) reported. The Teaching and Research Farm area experiences a bimodal rainfall pattern that ranges from a minimum of 800mm to a maximum of 1500mm per annum and lies within latitude 05 05NL (north) and longitude 1x15’w (west) (Hagan and Apori 2013).

Feed Ingredients and Experimental Diets

Para rubber seed kernel was the main feed ingredient under study or investigation. Para rubber seeds were obtained from the Crop Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) in Kumasi. The para rubber seeds were part of the germplasm material of the Crops Research Institute. The seeds were collected from the Enyinase outstation around Elembele in the Western Region of Ghana. The para rubber seeds were collected in September, 2022 and stored in polyethylene bags under ambient conditions at the Crop Research Institute in Kumasi. The seeds were then delivered to the Animal Science Department, School of Agriculture, University of Cape Coast, Cape Coast, in mid-June 2023. The dried para rubber seeds was then decorticated or dehulled to obtain the seeds.

The seeds obtained were roasted for 30 minutes at a temperature of 80 degree Celsius. This method were used because there is insufficient literature on the use of milled roasted para rubber seed kernel as a partial provider of metabolizable energy in the diet for livestock. The seeds were milled with a hammer mill with one millimetre (1mm) sieve. The milled full-fat para seed kernel was stored in a cold room and used for the study.

The experimental diets were four equi or iso-calorie diets. The control diet was commercial chick starter crumble obtained from Kuodjis Limited, which had a metabolizable energy of 2825 Kilocalories per kilogram dry matter of the diet (2825 kcals ME/ kg DM). The control diet was formulated and compounded from corn, soybean, wheat by-products, minerals premix and soy oil. In experimental dietary treatments, 5%, 10%, and 15% represently treatments 2, 3, and 4 respectively, was used as replacements for metabolizable energy in the control diet. The process in which the replacement were done are as follows:

Metabolizable Energy of Koudjis Starter = 2825kcalME/kgDM

1g of Koudjis Starter =2.825 kcalME/kgDM

5% metabolizable energy in Koudjis Diet =141.25 kcalME/kgDM (2.825÷100×5)

10% metabolizable energy in Koudjis Diet =282.5 kcalME/kgDM (2.825÷100×10)

15% metabolizable energy in Koudjis Diet = 423.75 kcalME/kgDM (2.825÷100×15)

Grams (DM) of Koudjis feed to give 141.25 kcalME/kgDM

5% = 141.25 kcalME/kgDM ÷2.825 =50g

10% = 282.5 kcalME/kgDM ÷2.825 = 100g

15% = 423.75 kcalME/kgDM ÷2.825 = 150g

Metabolisable Energy of para rubber seed kernel meal = 4,405 kcalME/kgDM

1g of the para rubber seed kernel meal = 4.405 kcalME/kgDM

The grams of para rubber seed kernel meal that were used as a replacement for the ME in the Koudlis starter are as follows: grams of para rubber seed kernel meal were calculated to replace 141.25 kcalME/kgDM, 282.5 kcalME/kgDM and 423.75 kcalME/kgDM each of the value was divided by 4.405 kcalME/kgDM. Examples 141.25÷4.405, 282.5÷4.405 and 423.75÷4.405.

The calculated metabolizable energy of the full-fat para rubber seed kernel was 4405.5 kcalME/kgDM.

Experimental Animals

This study was conducted using three months old forty male ICR albino mice purchased from the Animal Unit of the Centre for Plant Medicine Research (CPMR) at Akuapem Mampong in the Eastern Region of Ghana. The mice were fed with commercially pelleted feed by Agri Care Company limited in Kumasi.

On arrival at Cape Coast, they were provided with Koudjis feed for seven days to get adjusted. After the seven-day adjustment, they were weighed and assigned to the experimental diets. Feed and water intake data were collected. Feed and water were supplied adlib in the morning at about 8:00 a.m.

Feed and water left over from previous days.

Feed supplied to the mice was collected and weighed during the twenty-eight-day study period. Feed was weighed daily in the morning into two feeding troughs. The graduated water bottle was emptied and refilled after reading leftover water from the previous day's supply. Shredded dried wood shaving was used as bedding for the mice, and it was changed daily at about 7:30 a.m. Five (5) mice were grouped and housed per standard cages model NC3RS manufactured to house six (6) mice. The mice were inspected daily for any signs of disease or behavioural abnormality. The standard mice housing cages were put in a bigger cage divided into four compartments. Two mice cages, each housing five animals forming the replicates of a dietary treatment, were placed in each compartment of the bigger cage.

Growth Performance of Albino Mice

Feed and Water Intake

Each morning, the designated amount of feed was measured and placed into a feeding trough within each housing cage for the mice in every replicate. The remaining feed that was not consumed was weighed the following morning before providing fresh feed. The difference between feed weighed in and leftover feed after twenty-four hours (the next morning) later was the feed intake per day per replicate. Similarly, the difference between the volumes of water put in the graduated automatic plastic drinker in the mice housing cage and the volume of water left the next morning after twenty-four hours were taken as the quantity of water drunk by the five mice in the replicate.

Live Weight Grain of Mice

In each replicate, the weight of the five mice was measured individually at the start of the experiment on day fourteen and the end of the study on day twenty-eight. An electronic scale (LA214Max220g d=0.1mg Serv.24 1A) was used for the weight measurements. The weight gain per mice was calculated by finding the difference between the weights recorded at the end and the beginning of the study.

Feed Conversion Ratio

The feed conversion ratio of mice on the four experimental diets was calculated using the formula:

$$FCR = \frac{\text{Feed taken during the experimental period}}{\text{Weight gained during the experimental period}}$$

Water to Feed Ratio

The water to feed ratio was calculated using the formula:

$$W{:}F = \frac{\text{Average water intake per mice per replicate cage}}{\text{Average feed intake per mice per replicate cage}}$$

Feed Analysis

Samples of the four experimental diets, diet one (1) or control and after formulation and compounding of the three full-fat para rubber seed kernel incorporated diets 2, 3, and 4 plus full-fat para rubber seed kernel (untreated) and treated full-fat para rubber seed kernel were taken for proximate analysis. In total, six (6) feed samples were analyzed. The proximate analysis was conducted at the Nutrition Laboratory of the Animal Science Department within the School of Agriculture. The procedures recommended by AOAC (2008) were followed during the analysis.

The the researcher put the moisture content of triplicate (30g) samples of raw and roasted para rubber seed meals in clean oven-dried crucibles and weighed. They were kept in a thermostatically controlled oven at 105^0C for 48 hours. After that the moisture content was then calculated as the percentage water loss by the sample. The ash contents of the samples were obtained after heating triplicate (30 g) samples in a furnace at 500oC for 24 h.

The crude fibre content of triplicate (3g) samples was weighed and placed in a boiling flask, 100ml of the 1.25% sulphuric acid solution was added and boiled for 30 minutes. After the boiling, filtration was done in a numbered sintered glass crucible. The residue was transferred back into the boiling flask and

100ml of the 1.25% Sodium hydroxide, solution was added and boiled for 30 minutes. the residue was washed with boiling water and methanol and placed in a crucible dried in an oven at 105 degrees overnight and weighed. The crucible was placed in a furnace at 500 degrees for about 4 hours. The crude fibre was calculated as

$$\% \text{ Crude fibre} = \frac{\text{weight loss thro ashing}}{\text{Sample weight}} \times 100$$

AOAC (2008)

The fat/oil content of triplicate (30g) samples was weighed into a 50 ×10mm soxhlet extraction thimble and transferred to a 50ml capacity soxhlet extractor AOAC (2008). 150ml Petroleum spirit was added and connected to the soxhlet extractor and extraction was done for 6 hours using a heating mantle as a source of heating AOAC (2008). After heating, the flask was removed and placed in an oven at 60^{o}C for 2 hours. The percentage of fat/oil was calculated using the formula: $\text{Crude Fat } (\%) = \frac{W\,(g) \times 100}{\text{Sample }(g)}$

The Kjeldahl method determined the crude protein contents of triplicate (10g) samples of raw and roasted para rubber seed meals.

The metabolizable energy content of the feed samples was calculated using the formula: ME (kcal/kg DM) = (21.26 X % DM) + (47.13 X % fat) + (35.85 X % CF) (NRC 1994). The hydrogen cyanide content of four experimental diets, treated and untreated full-fat para rubber seed kernel, was determined qualitatively and quantitatively at Mycotoxin and Food Analysis Laboratory of Food Science and Technology Faculty, Kwame Nkrmahno University of Science and Technology (KNUST) Kumasi Ghana using (AOAC 2008) procedures. The mineral composition was assessed through a process of dry-ashing the samples at 550°C in a furnace, followed by dissolution in 10% hydrochloric acid (HCl) and subsequent filtration. Calcium and magnesium levels were quantified using an Atomic Absorption Spectrophotometer, as per the (Page et al. 1992) guidelines. Additionally, sodium and potassium concentrations were determined employing a flame photometer (Allen, et. al. 1974). Also, Iron, Copper and Zinc were determined using Atomic Absorption Spectrophotometer (FAO 2008).

Haematology and Blood Serum Collection Procedure

The albino mice were allotted into four treatments or groups with two replicates per group in a clean standard white plastic cage in the Laboratory. After completing the feeding period, the blood samples were obtained from the orbit using a terminal standard operating procedure that requires no sedation and mice were immediately euthanized humanely by cervical dislocation. Mice were chosen randomly from each cage to collect blood for haematological and serum analyses.

Blood obtained was transferred into sets of two different vacutainer tubes, one containing serum separator gel as coagulant and the other containing EDTA as anticoagulant. Subsequently, the vacutainer tubes were stored in a chilled container with ice packs and transported to the laboratory for analysis. Within the laboratory, the samples underwent a quality assessment before the actual examination.

Blood samples for biochemistry were centrifuged at 3000 rpm for 5 min to separate the serum. Serum was transferred into a clean tube and content well mixed for subsequent analysis in a BS 240 Clinical Chemistry Analyzer. The rest of the samples were stored in a refrigerator for subsequent analysis.

For hematological studies, blood in EDTA tubes was gently inverted multiple times immediately after the sample was obtained and before using the blood to ensure thorough and consistent mixing.

Tests such as packed cell volume (PCV), hemoglobin (Hb) concentration, total white blood cell (TWBC) counts, red blood cell (RBC) indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were carried out. The packed cell volume (PCV) was determined using the microhematocrit centrifugation method, while the concentration of hemoglobin (Hb) was measured using a spectrophotometer. Red blood cell (RBC) and total white blood cell (TWBC) counts were manually performed using a haemocytometer, following the procedure outlined in the Merck Veterinary Manual ((Fraser 1986). The differential white blood cell (WBC) count was obtained from air-dried thin blood smears stained with Giemsa stain, as described in the same manual. Additionally, the red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated using established formulas.

MCV (fL) = (PCV/RBC) x 10;
MCH (pg) = (Hb/RBC) x 10;
MCHC (g/dL) = (Hgb/PCV) x 100

Histological Studies

After the blood samples collection, the mice were immediately euthanized humanely by cervical dislocation. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. Liver and kidney samples were harvested and fixed in 10% formalin solution for a week and processed by standard histological procedures. Tissue samples were washed 24 hours under slow-speed running water, dehydrated in graded concentrations of ethanol, thus 70% (6 hours), 80% (10 hours), 95% I (2 hours), 95% II (2 hours), 100% I (2 hours), 100% II (2 hours), and immersed in ethanol - xylene 1:1 (10 minutes), xylene for clearing (3 minutes), xylene – paraffin1:1 (10 minutes), paraffin wax I (2 hours), paraffin wax II (2 hours), paraffin wax III (1 hour), and finally embedded in paraffin wax. 5 µm thick slices were subsequently cut from each paraffin block sample onto a glass slide, and stained with haematoxylin-eosin (HE) routinely. Tissue structure was then observed under the Hund H600 microscope, and photomicrographs were taken as images for presentation.

Total Cost of the Expenditure Made during the Study

Throughout the study, numerous expenditures were made to acquire materials necessary for conducting the experiment. The details of each item, providing descriptions, quantities, unit prices, and total costs. Additionally, the costs of both conventional and alternative feeds to facilitate a cost analysis between the two. This comparison aims to assess the practicality of using or substituting either feed.

Ethical Considerations

In conducting this study, the researcher applied for ethical clearance from the University of Cape Coast Institutional Review Board (UCCIRB). The researcher received Ethical Clearance with the reference ID UCCIRB/CANS/2023/13, which granted the researcher approval to conduct this study. The ethical approval by UCCIRB indicates that this study meets ethical standard.

Statistical Analysiss

one-way ANOVA analysis using Generalised Linear Model of GenStat Discovery Edition (VSN, 2012) was used in the study. Differences in means were separated using the least significant difference test at 5% level of significance.

CHAPTER FOUR

This study investigated the influence of para rubber seed kernel incorporated feed on the growth performance, haematology and blood biochemistry, liver and kidney tissue histology of ICR albino mice. Chapter Four of the study focused on the results and discussions. Forty (40) albino mice were used for the study, grouped into four treatments, two replicates per treatment. The study conducted a proximate analysis of full-fat para rubber seed kernels meals which was treated and untreated. Additionally, One-way ANOVA (analysis of variance), as outlined by the generalized linear model of the GenStat discovery edition (VSN, 2012), was applied in the analysis of the variables (growth performance, haematology and blood biochemistry).

Nutrient Composition of Para Rubber Seed Kernel

Research Question 1: What is the nutrient composition of para rubber seeds kernel?

This research question aimed to evaluate the nutrient composition of para rubber seed kernel. Table 6 provides the results of the analysis of proximate composition.

Table 7: Proximate Composition of Raw Para Rubber Seed Kernel (PRSK) and Roasted Para Rubber Seed Kernel (RPRSK).

	RPRSKM		RoPRSKM	
	Mean	Std. Deviation	Mean	Std. Deviation
Dry matter content	94.1	.0	73.4	40.3
Moisture content	5.8	.0	2.8	.6
Ash content	3.2	.1	3.2	.0
Crude protein content	23.8	.1	24.5	.2
Oil/Fat content	45.1	.0	46.6	.1
Crude fibre content	9.6	.1	9.1	.0
Carbonhydrate content	18.0	.0	16.3	.0

Source: Field Data (2023)

RPRSK means raw para rubber seed kernel meal and RoPRSKM means roasted para rubber seed kernel meal.

Table 7 indicates the proximate composition of the raw para rubber seed kernel meal and the roasted para rubber seed kernel meal. The table provide that the raw para rubber seed kernel meal dry matter has a of 94.1% with no standard deviation. while in the roasted para rubber seed kernel meal it has a mean of 73.4 with a standard deviation of 40.3. This results suggest that there is a higher variability in dry matter content within the RoPRSKM as compared with RPRSKM.

Additionally, Table 7 shows the moisture content in the RPRSKM which has a mean of 5.8 with no standard deviation, while in RoPRSKM, it has a mean of 2.8 with a standard deviation of 0.6. These findings indicate that RoPRSKM tends to have lower Moisture content on average and less variability than RPRSKM. Furthermore, the crude Protein and Oil/Fat content results show that the mean and standard deviation are higher in RoPRSKM than in RPRSKM. This implies that RoPRSKM tends to have higher average Protein content and more consistency in Protein levels than RPRSKM.

Table 8: Mineral Composition of Raw Para Rubber Seed Kernel (RPRSK) and Roasted Para Rubber Seed Kernel (RoPRSK)

	Sample	
Parameter	RPRSKM	RoPRSKM
Phosphorus ug/g	3,182.4	3292.3
Potassium ug/g	6950.9	6617.0
Sodium ug/g	458.7	524.9
Calcium%	1.6	1.4
Magnesium%	0.52	0.32
Zinc ug/g	396.7	344.6
Copper ug/g	267.5	136.5
Iron ug/g	228.4	215.8
Hydrogen Cyanide mg/kg	0.81±0.02	0.26±0.00

RPRSK means raw para rubber seed kernel meal and RoPRSKM means roasted para rubber seed kernel meal.

Table 8 indicates the mineral and hydrogen cyanide contents of both Raw Para Rubber Seed Kernel (PRSK) and Roasted Para Rubber Seed Kernel (RPRSK). It compares various parameters for these two samples. The roasted para rubber seed kernel (RPRSK) had a slightly higher phosphorus and sodium concentrations than the raw para rubber seed kernel (PRSK). On the contrary, potassium, other minerals concentration and hydrogen cyanide content were slightly higher in the raw para rubber seed kernel (PRSK) compared to the roasted kernel (RPRSK).

Table 9: Proximate Composition of experimental diets (D1, D2, D3, D4).

Parameter	T1		T2		T3		T4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry Matter	89.4	.0	89.4	.0	90.4	.0	90.5	.0
Moisture Content	10.5	.0	10.5	.0	9.5	.0	9.4	.0
Ash Content	6.5	.1	6.2	.1	6.7	.3	6.4	.1
Protein Content	18.6	.0	20.5	.6	20.5	.2	21.6	.1
Oil	3.4	.0	4.6	.0	6.5	.0	7.8	.0
Fibre	8.3	.2	8.1	.0	8.8	.0	8.7	.1
CHO	62.9	.0	60.3	.6	57.3	.3	55.3	.1
Hydrogen Cyanide mg/kg	1.41	0.03	1.52	0.01	1.61	0.01	1.66	0.03

T1 means treatment one, T2 means treatment two, T3 means treatment three and T4 means treatment four.

Incorporation of RoPRSKM in to the control diet (D1) to replace 5, 10, and 15% ME in diets D2, D3 and D4 led to increased dry matter, crude protein, oil or fat and hydrogen cyanide content in the D2, D3 and D4 respectively. This might have resulted from the high levels of crude protein, crude oil or fat and hydrogen cyanide in the roasted para rubber seed kernel compared to the levels in the control diet (D1) whose ME content was replaced at 5, 10, 15% respectively to obtain four equi caloric diets (D1, D2, D3 and D4).

Table 10: Mean growth performance in of ICR Albino Mice on diets with and without roasted para rubber seed kernel

	Experimental Diets				
Parameter	D1	D2	D3	D4	P Value
Feed intake / Animal /d/g	7.3±0.153[a]	7.3±0.004[a]	6.1±0.00566[b]	6.6± 0.269[ab]	0.005
Water intake/Animal/d	8.6±1.365	9.4±1.047	8.8± 0.544	8.2±0.509	0.675
Weight gain / g / animal/period/ g	3.7± 1.411	2.5± 0.748	1.7± 0.392	2.2± 0.1.031	0.347

D1 means control diet, D2 means diet two, D3 means diet three and D4 means diet four.

Table 10 shows the growth performance of ICR Albino Mice fed different experimental diets, (D1, D2, D3 and D4). Albino mice on D3 had a significantly lower feed intake (6.190 grams) as compared to the others. Average feed intake on D4 also was lower than D1 and D2 but was higher than that of D3 (6.6991 grams). There are no significant differences in water intake and weight gain among the four diets.

Table 11: Haematology test result of ICR Albino Mice on diets with and without roasted para rubber seed kernel meal

Test	T1 Mean ± SD	T2 Mean ± SD	T3 Mean ± SD	T4 Mean ± SD	P Value	Range/Ref
WBC (K/uL)	7.85 ± 3.1	9.80 ± 4.2	6.33 ± 0.8	9.75 ± 0.6	0.4130	1.06 - 56.10
LYM (K/uL)	4.15 ± 1.6	2.20 ± 0.4	2.53 ± 0.3	6.85 ± 2.3	0.0457*	0.12 - 23.50
RBC (M/uL)	8.25 ± 0.7	7.25 ± 1.2	8.47 ± 0.2	8.85 ± 0.0	0.2074	3.60 - 15.20
HB (g/dL)	12.30 ± 1.1	12.20 ± 1.1	12.57 ± 1.0	14.30 ± 0.1	0.2306	6.10 - 21.70
HCT (%)	43.05 ± 3.0	43.55 ± 2.0	43.07± 3.2	46.45 ± 1.0	0.5552	16.70 - 69.80
MCV (fl)	52.50 ± 0.7	53.50 ± 0.7	50.00 ± 3.6	52.50 ± 0.7	0.4469	39.00 - 90.80
MCH (pg)	14.95 ± 0.0	15.85 ± 0.2	14.57 ± 1.1	15.80 ± 0.4	0.2665	12.60 - 31.00
MCHC (g/dL)	28.55 ± 0.4	28.00 ± 1.2	28.70 ± 0.3	29.75 ± 0.7	0.2358	27.00 - 37.60
PLT (K/uL)	567.00 ± 25.4	441.00 ± 209.3	554.00 ±92.0	406.00 ± 200.8	0.5942	59.00 - 263.00
MPV (fl)	6.20 ± 0.0	5.80 ± 0.4	5.97 ± 0.1	5.70 ± 0.1	0.2320	5.20 - 13.10

WBC (K/uL) means white blood cell, LYM (K/uL) means Lymphocytes, RBC (M/uL) means red blood cell, HB (g/dL) means haemoglobin, HCT (%) means Hematocrit, MCV (fL) means Mean Corpuscular Volume, MCH (pg) means mean corpuscular haemoglobin, MCHC (g/dL) means Mean corpuscular haemoglobin concentration, PLT (K/uL) means Platelets and MPV (fL) means Mean Platelet Volume.

Table 11 presents the results of various haematological (blood-related) tests conducted on ICR Albino Mice fed four different dietary conditions, (with and without roasted para rubber seed kernel meal). This shows that the haematological parameters measures, except lymphocyte count which showed significant differences as indicated by the p-valune of 0.04, had no significant difference. The haematological parameters of these albino mice remained within the normal references for albino mice. In addition, Animals fed without and with para rubber seed kernel meal diet showed higher platelet count (567.00 ± 25.45, 441.00 ± 209.30, 554.00 ± 92.05, 406.00 ± 200.81)than the normal reference range of a albino mice. Despite the higher counts there were no significant differences among treatments.

Table 12: Liver function (Biochemistry) test results on albino mice fed roasted full-fat para rubber seed kernel meal

Test	T1 Mean ± SD	T2 Mean ± SD	T3 Mean ± SD	T4 Mean ± SD	P Value	Range / Reference
(GOT) AST U/L	167 ± 49.1	210.5 ± 35.9	228.30±93.7	183.75 ± 11.9	0.429	57-329
GPT (ALT) U/L	63.8 ± 26.4	89.3 ± 41.7	81.00 ± 45.6	60.75 ± 15.4	0.594	7-227
Alkaline Phos. U/L	309.5±123.6[b]	569.0 ±64.6a	468.00±95.3[ab]	634.80±116.2[a]	0.005*	40 – 390
Total Protein g/L	61.00 ± 4.2[a]	56.00 ± 2.9[a]	57.33 ± 1.5a	48.50 ± 1.0[b]	0.000*	62 – 83
Albumin g/L	32.50 ± 1.0	34.75 ± 1.7	34.00 ± 1.0	33.25 ± 0.5	0.089	37 – 53
Globulin g/L	28.50 ± 4.2[a]	21.25±2.9[bc]	23.33 ± 2.3[ab]	15.25 ± 0.9[c]	0.001*	20 – 35
Bilirubin-Total (SI) umol/L	42.69±14.2	68.10±44.8	77.50 ± 18.3	84.00 ± 41.1	0.375	3.42 - 25.65
Bilirubin - Total mg/dl	2.49 ± 0.8	3.99 ± 2.6	4.53 ± 1.0	4.91 ± 2.4	0.375	0.20 - 1.50
Bilirubin-Direct (SI) umol/L	23.51 ± 8.5	34.5±23.0	39.39 ± 9.0	33.3 ± 29.6	0.765	0.00 - 10.26
Bilirubin-Direct mg/dl	1.34 ± 0.4	2.02 ± 1.3	2.30 ± 0.5	1.95 ± 17.3	0.767	0.00 - 0.60
Bilirubin-Indirect (SI) umol/L	19.20 ± 5.9	33.6 ± 21.8	38.13±9.3	50.70 ± 19.7	0.106	1.71 - 22.23
Bilirubin - Indirect mg/dl	1.12 ± 0.3	1.97 ± 1.2	2.20 ± 0.5	2.97 ± 1.1	0,106	0.10 - 1.30

T1 mean treatment one (control), T2 mean treatment two, T3 mean treatment three and T4 mean treatment three.

This table presents the results of a liver function (biochemistry) test conducted on albino mice fed diets with and without roasted full-fat para rubber seed kernel meal. The table provides various liver-related parameters, including the means and standard deviations (SD) for each parameter, p-values, and reference or range values where applicable. No significant differences existed in Albumin, Alanine Aminotransferase (ALT or GPT) and Bilirubin (Total, Direct, and Indirect) levels among the four groups, as indicated by the p-values. The mean Aspartate Aminotransferase (AST or GOT) levels were highest in group T3 (228.30 U/L) and lowest in group T4 (183.75 U/L). However, none of the differences in AST levels among the groups were significant, as indicated by the p-value (0.429).

Alkaline Phosphatase (Alkaline Phos.) of group T2 had significantly higher alkaline phosphatase levels than the other groups (a). The p-value (0.005*) suggests a statistically significant difference. Total Protein in group T1 was significantly higher compared to the other groups (a). ICR albino mice group T4 had significantly lower total protein levels than the other groups (b). The p-value (0.000*) indicates a highly significant difference. ICR albino mice group T1 had significantly higher globulin levels than the other groups (a). ICR albino mice group T2 had significantly lower globulin levels than the other groups. ICR albino mice group T3 had intermediate globulin levels. ICR albino mice group on T4 had the lowest globulin levels. The p-value (0.001*) suggests a highly significant difference.

Table 13: Kidney function (Biochemistry) test results on albino mice fed roasted full-fat para rubber seed kernel meal

Test	T1 Mean ± SD	T2 Mean ± SD	T3 Mean ± SD	T4 Mean ± SD	P Value	Range / Reference
Creatinine (mg/dl)	0.50 ± 0.2	1.73 ± 1.1	0.83 ± 0.0	0.4 ± 0.2	0.107	0.6 - 1.4
Sodium (Na+) (mmol/L)	165.00± 3.0	163.37 ± 1.2	163.05 ± 0.9	161.80 ± 0.0	0.208	145 - 175
Urea (mmol/L)	8.10 ± 1.8	8.27 ± 0.9	7.63 ± 0.4	6.53 ± 0.7	0.290	1.7 - 8.3
Chloride (Cl-) (mmol/L)	200.00 ± 0.0	197.00 ± 3.0	154.5 ± 0.0	200.00 ± 0.0	0.113	96 - 110
Potassium (K+) (mmol/L)	9.24 ± 0.3	9.24 ± 0.9	9.29 ± 0.4	8.736 ± 0.6	0.506	6.5 - 9.5
eGFR (ml/min)	219.00 ± 43.2	139.00 ± 33.9	169.00 ± 5.2	247.00 ± 61.6	0.102	60 -
Creatinine (SI) (umol/L)	44.20 ± 23.3	153.23 ± 105.4	73.67 ± 5.1	35.33 ± 23.3	0.107	53.0 - 123.8

The table displays the results of kidney function (Blood biochemistry) tests conducted on albino mice fed without and with roasted full-fat para rubber seed kernel incorporated as meal. These tests are related to kidney function, and the table compares the mean values and standard deviations (SD) of the test results across four ICR albino mice groups on different dietary treatments (T1, T2, T3, and T4). Additionally, it provides p-values and reference ranges for each test. The Creatinine levels for ICR albino mice group T2 was higher compared to the other groups. However, the p-value of 0.107 suggests that the difference was not significant. All ICR albino mice groups' values were within the reference range for Creatinine. Sodium levels in all groups were higher than the reference range, but the p-value suggests that the differences among values for ICR albino mice on the four diets were insignificant. Urea levels were within the reference range for all groups, and the differences were insignificant (p-value = 0.290). The levels of Potassium in the groups were higher than the reference range, but the p-value suggests that the differences were not statistically significant. Similar to the Creatinine levels in mg/dl, the Creatinine levels in umol/L show no significant differences between groups.

Liver and Kidney Histology of Albino Mice

The liver and kidney histology aspect of the study addressed Research Question 4.

Research Question 4. How does para rubber seed kernel meal affect albino mice liver and kidney tissue histology?

This research question sought to investigate the liver and kidney tissue histology of albino mice fed a diet containing para rubber seed kernel meal.

Histology of ICR albino mice liver when fed on non-roasted para rubber seed kernel meal incorporated diet (D1 and roasted para rubber seed kernel meal incorporated diet (D2, D3, D4) was normal. Similar normal trend was observed for kidney histology when kidney structures of ICR albino mice were compared under the microscope. The arrangements of kidney and liver cells and tissue architecture of the respective organs did not show any differences compared to their control groups. These experimental diets had enrichment ranging from T1 to T4. T1 was the control diet, which did not contain any PRSKM. T2 was an experimental diet that included 5% PRSKM. T3 was another experimental diet which contained 10% PRSKM and T4, an experimental diet with highest concentration of 15% PRSKM. The images in Figures 1 and 2 depict H&E stained photomicrographs of liver and kidney sections of the albino mice fed with various enriched diets.

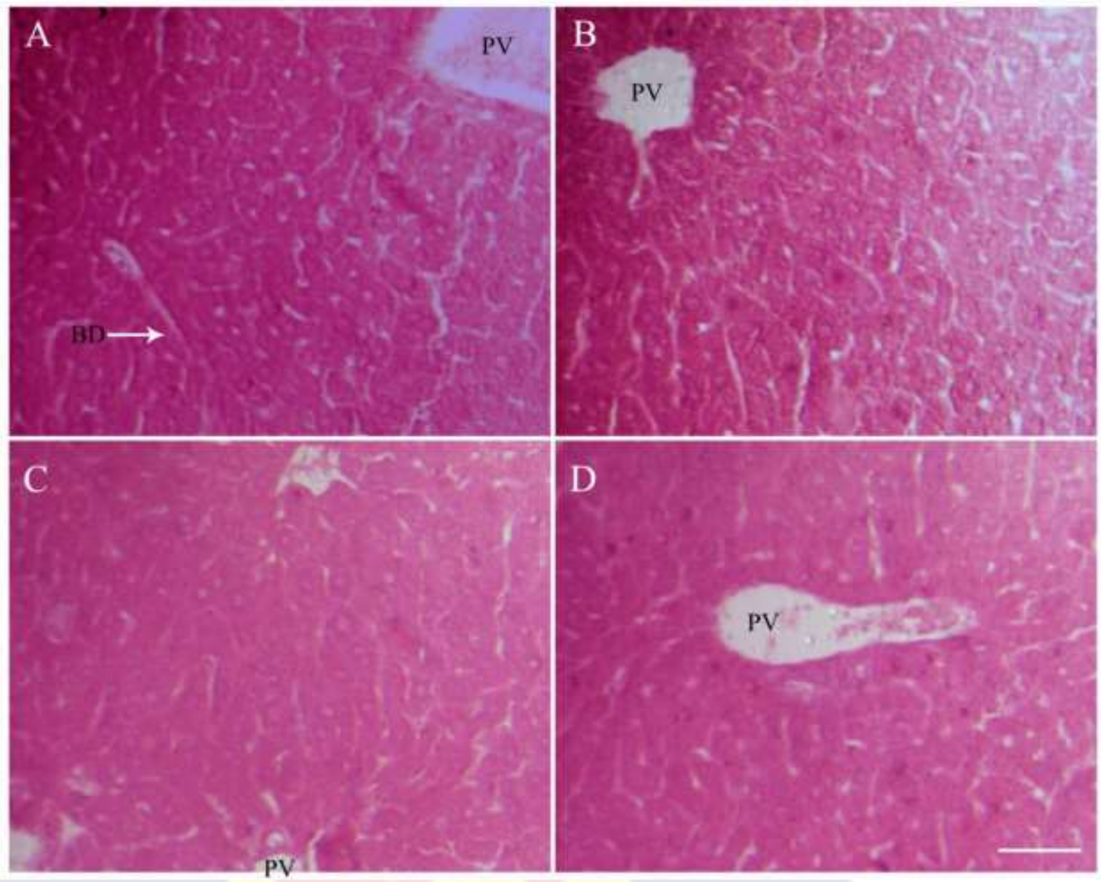

Figure 1. H&E stained (100×) liver sections of male albino mice showing (A) T1 control containing No PRSKM, (B) T2 containing 5% PRSKM, (C) T3 containing 10% PRSKM, and (D) T4 containing 15% PRSKM. Liver tissue histology for all treatment groups appeared normal. There were no areas of inflammatory infiltration or cell death in tissue structure. Portal Vein (PV), and Bile Duct (BD)

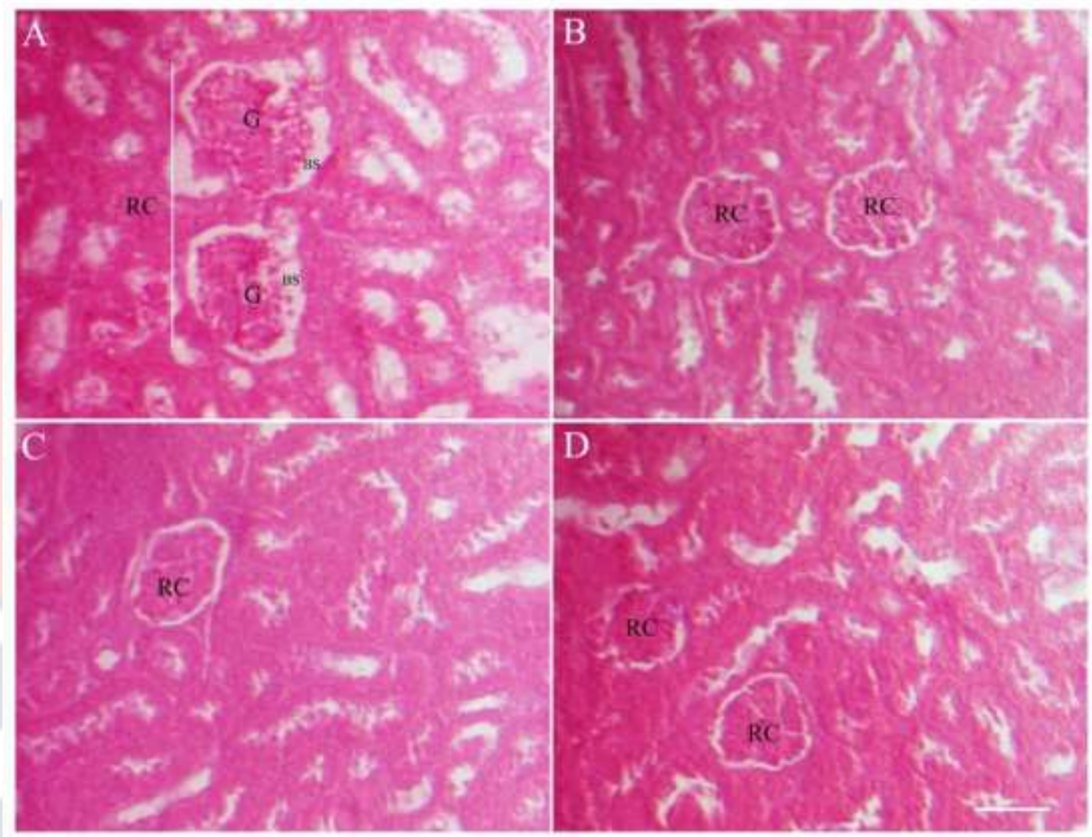

Figure 2. H&E stained (100×) kidney sections of male albino mice showing (A) T1 as control containing No PRSKM, (B) T2 containing 5% PRSKM, (C) T3 containing 10% PRSKM, and (D) T4 containing 15% PRSKM. Kidney tissue histology for all treatment groups appeared normal. There were no areas of cell degeneration or death in tissue structure. Renal Corpuscle (RC), Bowman's Space (BS), Glomerulus (G)

Table 14**: Total Costs of the Study**

Item	Description	Quantity	Type of Diet	Unit Price (USD $)	Total Price per item (USD $)
Purchase of Cages and Drinkers from China	Purchase made for the Cages and Drinkers from China for the experiment	10 sets (10 Cages and 10 drinkers)	-	58	580
Import Tax for Cages	Tax paid for importation of the cages from China	10 sets	-	-	235
Albino Mice	Purchase of Albino Mice	40	-	2	80
Albino Mice Transportation	Payment made for mice transportation from Accra	40	-	-	30
Koudjis Chicks Starter	Control diet purchase	8 KG	Controlled diet	0.625	5
Para rubber seed kernel meal	Experimental Diet	10 KG	Experimental Diet	-	-
Pelleting the feed	Pelleting the feed for control diet	2 KG	Control diet	-	5.25
Pelleting the feed	Pelleting the feed for Experimental Diet	6 KG	Experimental	5.25	15.75
Tub	It was used for washing of the cages and the water bottles during the experiment	2	-	4.75	9.5
Towel	It was use for drying of the cages during the experiment	1	-	1.5	1.5
Plastics	It was used for storage of feed and collection of leftover feed	1.2 pack	-	5.15 per pack	6.18
Glove	It was used for safety of the researcher	1 pack	-	5	5

Brush	It was used to wash the drinkers bottles during the experiment	2 pieces	-	0.6	1.2
Proximate analysis Cost	Money used to analysis the raw and roasted para rubber seed meal	-	-	-	20
Hydrogen cyanide and the prepared diet	Analysis of the prepared diet and hydrogen cyanide	-	-	-	23
Blood Analysis	To analyse the blood of albino mice at the hospital	-	-	-	267.5
Chemical	Purchase of chemical for histology analysis	1	-	100	100
EDTA Tube	It was used for blood biochemistry collections	2	-	25	50
Glass Slide and Ethanol	It was used for histology Analysis	-	-	-	39.5
Total Cost					1,474.38

Table 14 demonstrates that a total expenditure of 1,474.38 United States Dollars was incurred for materials and other essential items necessary to conduct the experiment. The table clearly shows that 0% of the experiment's cost was attributed to alternative feeds. In contrast to the conventional feed (Koudjis Chicks Starter), which is priced at 0.625 USD per KG, of the alternative feed, where as Para rubber seed kernel meal was obtained at no cost.

Discussion

This aspect of the chapter discussed the results following the research questions and objectives. Four research questions guide this study.

Research question: 1 What is the nutrient composition of para rubber seeds kernel?

The results of the proximate composition analysis of raw and roasted para rubber seed kernel is presented in Table 7. The moisture content for raw full-fat para rubber seeds kernel meal was higher with the unprocessed seed. However, the treatment on the full-fat para rubber seed kernel reduced the moisture content in the rubber seed meal. This is important because moisture content in feed ingredients can lead to mold and bacterial growth, decreased shelf life and nutritional value.

The present results agrees with those earlier reported by Farr (2015), Phaobenga (2016) & Farr *et al.* (2019). The moisture content of the roasted product was within the range reported by Eka et al. (2010), Udo et al. (2016), Aguibe (2017) & Oluodo et al., 2018. This implies that the moisture content of the roasted para rubber seed kernel meal was low enough to be less susceptible to microbial attacks and also exhibit longer shelf-life characteristics.

Protein is essential for the growth and development of muscles and tissues in animals. It provides the building blocks (amino acids) necessary for synthesizing proteins vital for muscle growth and repair. Adequate protein intake is essential for proper reproductive performance in animals. It ensures the development of healthy embryos and supports lactation in female animals. Proteins are essential for the proper functioning of the immune system. They help produce antibodies and other immune factors that protect animals from diseases and infections. Protein is required to maintain and repair various body tissues, including skin, hair, feathers, and internal organs. Without enough protein, animals may experience slow growth, poor coat or feather quality, and reduced overall health.

The crude protein content of the fresh para rubber seed meal (PRSM) was (23.8%) in and this fit in the reference range suggested by (Eka et al., 2010), and of Udo et al. (2016). With the roasted full-fat para rubber seed kernels meal used in this study, the crude protein (CP) values of 23 .8 – 24.5 % were within the range of 23.31 reported by Udo et al. (2016), 25.4 % by Sharma et al. (2014), 22.3 % by Onwurah et al. (2010), 21.9% Oyewusi et al. (2007), Oluodo et al., &Udo, et al. (2016). However, it differs from the reported literature (Farr, 2015; Farr et al., 2019; Nath, 2015; Mmereole, 2008; Eka et al.,2010; Phaobenga 2016) & 21.87 Suprayudi (2015).

Crude fibre consists mainly of cellulose, hemicellulose, and lignin, complex carbohydrates that monogastric digestive enzymes cannot break down. They pass through the digestive system largely intact, providing bulk and aiding in various digestive processes. The crude fibre in raw para rubber seed kernel meal and roasted para rubber seed kernel meal was similar in this study. The crude fibre 9.2 – 9.6% was in the range of 9.45% reported by previous studies (Farr, 2015; Farr et al., 2019). However, the results contradict the reported value by (Eka et al., 2010), Deng et al. 2015). It was found that oil/fat content is higher in unprocessed RPRSKM than in processed RoPRSKM.

Some chemical changes may have occurred during processing, leading to the breakdown or alteration of carbohydrates. For example, heat treatment can cause the Maillard reaction, reducing the available carbohydrates or changing their chemical composition. Additionally, it may be the lower moisture content because oil/fat often binds to water molecules, which may result in a lower percentage of carbohydrates by weight. The oil/fat content of 45 – 46% observed in this present study was close to value of 45.5 reported by Lalabe et al. (2017) and is within the range 49.3 reported by Suprayudi et al. (2015), 42.5 Onwurah et al., (2010). But the fat level was lower than value of 68.53 reported by Eka et al. (2010).

However, the oil/fat content of this study was higher than value of 6.4% Oyewusi (2007), 10.12 Mmereole (2008), 11% Hossain et al. (2015), 22% Aguibe et al. (2017), 38.47 Udo et al. (2016). Carbohydrates are organic compounds composed of carbon, hydrogen, and oxygen atoms, serving as a fundamental energy source for all living organisms. When consumed, carbohydrates are broken down during digestion into glucose and other simple sugars. These sugars are then absorbed into the bloodstream and transported to cells throughout the body, which are used for energy production. In this study, the value reported for raw para rubber seed kernel meal is in alignment with Farr (2015), who reported that raw para rubber seed kernel contains (16%) carbohydrate. With the roasted para rubber seed kernel meal, the reported carbohydrate value in this study contravenes that of Farr et al (2019),

Mineral Composition of Para Rubber Seed Kernel Meal

The Raw Para Rubber Seed Kernel (PRSK) had a slightly higher calcium and magnesium content than the Roasted Para Rubber Seed Kernel (RPRSK). The reduced calcium and magnesium content after roasting could be due to heat-induced changes or leaching during the process.

The mineral content in the untreated seeds was less than those reported by Udo et al. (2018), specifically 1740 and 2066 mg/kg for calcium and magnesium, respectively. Similarly, the mineral content of raw rubber seeds meal (RRSM) was observed to be higher than that obtained by Far et al. (2019). In addition, The Roasted Para Rubber Seed Kernel (RPRSK) has a slightly higher phosphorus concentration than the Raw Para Rubber Seed Kernel (PRSK). The slightly higher phosphorus concentration increase might be due to changes in the chemical composition during the roasting process. The phosphorus value in raw para rubber seed in this study disagreed with that of (Farr, 2015) & Farr et al. (2019).

Furthermore, the Raw Para Rubber Seed Kernel (PRSK) has a slightly higher potassium concentration than the Roasted Para Rubber Seed Kernel (RPRSK). The decrease in potassium content after roasting might be attributed to the alteration of the structure of the kernel during the roasting process. The Roasted Para Rubber Seed Kernel (RPRSK) has a higher sodium concentration than the Raw Para Rubber Seed Kernel (PRSK). The roasting process might result in the release or absorption of sodium, leading to the differences in values between the current work and previous results by Farr (2015), (Udo et al. (2018), (Farr et al. (2019).

The Raw Para Rubber Seed Kernel (PRSK) has a higher zinc concentration than the Roasted Para Rubber Seed Kernel (RPRSK). The roasting process might affect the bioavailability or solubility of zinc in the kernel as explained by Farr (2015) and Farr et al. (2019). The Raw Para Rubber Seed Kernel (PRSK) has a significantly higher copper concentration than the Roasted Para Rubber Seed Kernel (RPRSK). Similar to zinc, changes in bioavailability or solubility may contribute to this difference (Farr 2015), and (Farr et al. (2019). The roasting process appears to influence the mineral composition of the rubber seed kernel. phosphorus increases, potassium, calcium, magnesium, zinc, and copper decrease concentration.

Hydrogen cyanide is a gas or liquid characterised by an almond-like odour. It can interfere with the body's ability to use oxygen. It may cause damage to the brain, heart, blood vessels, lungs, and liver (National Institute for Occupational Safety and Health (NIOSH, 2023). When the hydrogen cyanide-containing feed resources are subjected to various processing methods like ensiling, sun-drying, fermentation, boiling, roasting, and soaking can reduce or remove the hydrogen cyanide content (Adejoh et al., (2020).

In addition, if the feed ingredients are kept for two months, it helps to reduce or remove the HCN from the feed ingredient and makes it safe for animal consumption (Oluodo, et al., 2018). Para rubber seed kernels meal is commonly described as having high cyanide content (Akinsanmi et al., 2018), and its anti-nutritional factors contents are generally considered to be heat labile. The cyanide content in rubber seed kernels can be reduced or removed to make the kernel safe for animal consumption. The HCN level established is less than 249 to 315 mg/kg earlier reported by Okafor and Anyawu (2006), 60.5 mg/kg (Farr, 2015; Farr et al., 2019), (Udo et al., 2018).

The difference is that the sample for this study was taken from the germplasm, which has been stored for over eight months. Studies have shown that storing the para rubber seed kernel for two to four months at an ambient temperature effectively reduces anti-nutritional factors (Oluodo, et al., 2018). The storage process significantly reduces the hydrogen cyanide concentration in the seed kernel.

Research question: 2 How do para rubber seeds kernel meal incorporated feed influence the growth performance of albino rats?

The growth performance of the albino mice fed on diets with and without roasted full-fat para rubber seed kernel meal (RSM) is shown in Table 10. Feed intake in animal production refers to the amount of feed (dry matter) an animal consumes over a specific period. It is a critical parameter in animal agriculture and is closely monitored and managed to ensure the animals' health, growth, and productivity.

Feed intake is a critical component of an animal's overall nutrition, and it can vary widely depending on factors such as the animal's species, age, weight, physiological state, and environmental conditions. Feed intake influences an animal's growth rate, body condition, reproductive performance, and overall health. In this present study, the feed intake by the albino mice on experimental diets (D3 and D4) decerased significantly as compared to those fed on diet with 5% and 10%level of roasted para rubber meal inclusion (D1 and D2). The reduced in consumption might be caused by increased fat level, as full-fat para rubber seed kernel meal replaced ME (metabolizable energy) in the test diet. Increased fat has the tendency to reduce feed flow through the GIT and results in reduced feed intake.

The present results agree with that of (Aguihe et al., 2017 and Khatun et al., 2015). The finding shows that inclusion 10 - 20% rate of para rubber seed meal did not damage the physiological system regarding feed consumption, weight gain, and meal conversion ratio. Ijaiya et al. (2011) also reported that adding 75% of rubber seed meal to broiler chickens' diet could significantly improve feed intake because of the high fibre content in rubber seed meal, increasing the total fibre content and diluting other nutrients.

Water is essential for the transportation of nutrients within an animal's body. It helps in the digestion, absorption, and distribution of nutrients from feed to body tissues, ensuring proper growth and development. Animals often rely on evaporative cooling through sweating or panting to regulate their temperature. Sufficient water intake is necessary for this cooling mechanism to function effectively, especially in hot weather.

In this present study, water intake per albino mice was not affected by dietary treatments on roasted para rubber seed kernel meal as compared to the control group. This means that the albino mice properly utilized the water to transport nutrients within the body and maintain evaporation during the hot temperature.

Intake of feed dry matter is generally controlled by age, size or weight and environment. The present results did not show any significant difference between albino mice fed the control diet and those fed with the experimental diets of the inclusion of 5%-15% of full-fat para rubber seed kernel in terms of weight gain.

The results of this present study concurs with the findings of Deng et al., (2015). This observation is in line with that of Alegbeleye et al. (2004), who reported that Nile tilapia (Oreochromis niloticus) fed with the inclusion of 208 g kg− RSM gave the best result with the highest growth rate and feed utilization. This observation also agrees with Ijaiya et al. (2011), who noted that a diet with a 50% inclusion level of rubber seed meal positively affects broiler chickens' weight gain and nutrient digestibility.

However, it disagrees with Ahaotu (2018), who observed that higher dietary fibre depressed weight gain in broiler chickens. The average feed intake per albino mice is around 5g/ day. However, it will differ by more than two (2) fold depending on feed palatability, fat and sugar contents of the feed or strain of albino mice because fat content of the diet should be around a maximum of 8% or less (University of North Carolina 2023). The high feed intake above the normal 5g/day in this study is as a result of the strain and age of the mice.

Research Question: 3. How does para rubber seed kernel meal impact the haematology and blood biochemistry of albino rat fed para rubber seed kernel meal incorporated diets?

Table 11 indicates the haematological test results of mice fed roasted para rubber seed kernels meal (RoPRSKM) with their reference range. Utilizing alternative ingredients in livestock feed formulation and feeding without a comprehensive understanding of their nutritional content, anti-nutrient properties, and impact on animal consumption, digestion, and well-being can pose health risks to the animals (Obasoyo et al. 2005 & Amel et al. 2006). It is imperative to thoroughly assess these feed ingredients to ascertain their effects on the animals' health.

In addition to evaluating the animals' physiological state, blood analysis can also be employed to gauge their health status and prognosis when fed different protein-based feed components (Obasoyo et al. 2005; Amel et al. 2006). Etim et al. (2013) indicated that changes in blood parameters of an animal correlates to a change in physiological condition. The various components of blood can be used as indicators to determine an animal's health condition. They also help evaluate the quality of protein and amino acid levels in feeds fed to an animal (Harper et al., 1999).

Furthermore, haematological parameters are crucial in monitoring feeds' potential toxicity, according to NseAbasi et al. (2014) emphasised. In addition, these parameters enable the assessment of adverse impact of external substances on the blood of albino mice, as demonstrated by Odeyemi et al. (2009). These blood elements can be seen at a specific level when the animal is normal.

Elevated levels of haemoglobin (Hb) and red blood cells (RBC) in animals suggest dehydration. While, lower levels indicate anaemia, as Aboderin et al. (2006) and Ruben (2018) stated. Haemoglobin plays a vital physiological role in conveying oxygen to the animal's tissues, facilitating the oxidation of ingested food to release energy for various bodily functions. Additionally, according to Ugwuene (2011), Omiyale et al. (2012), and Soetan et al. (2013), it aids in transporting carbon dioxide out of the animal's body.

This study showed no significant differences in RBC count among the ICR albino mice on various dietary groups. However, ICR albino mice on diet T4 were found to have a slightly higher hemoglobin level than those on the other diets, but this difference was not statistically significant. In other words, while there was a numerical difference in hemoglobin levels, it was not strong enough to be considered statistically meaningful.

This study's findings are inline with Udo, et al (2018). In addition, the RBC and HB values of ICR albino mice on T1 8.25 ± 0.78, T2 7.25 ± 1.20, T3 8.47 ± 0.25 and T4 8.85 ± 0.07, (12.30 ± 1.13 12.20 ± 1.13 12.57 ± 1.07 14.30 ± 0.14) found are within the acceptable range (3.57 - 15.2), (3.57 - 15.2) and (6.1 – 21.7) values for healthy mice reported by McBean (2016-2017).

White blood cell (WBC) is a component of the immune system that fights infections and diseases. When white blood cell counts are higher or lower than expected, it suggests the body has been infected by infection (National Institute of Health, 2022). In this study, the results indicated that there was no significant difference in WBC count among ICR albino mice on the different dietary groups (T1, T2, T3, and T4). The p-value of 0.4130 suggests that the variations observed in WBC counts were not statistically significant. In simple terms, the different diets did not substantially impact the WBC count in the mice.

The findings concurs with (Farr, 2015), (Fawole et al., 2017) and (Akinsanmi et al., 2020). The WBC values (T1 7.85 ± 3.18, T2 9.80 ± 4.24, T3 6.33 ± 0.81 and T4 9.75 ± 0.64) reported in this study for mice fed on control and experimental diets were within the reference ranges (1.06 - 56.8) for normal mice reported by (McBean 2016-2017). If the iron-rich protein is lower than the normal levels, it indicates a sign of anemia.

Hematocrit tests evaluate the amount of space red blood cells occupy in the body. If the levels are too high, the body may have been dehydrated. When it is lower than average, it indicates a sign of anaemia (NIH, 2022). In addition, Hematocrit (HCT) serves as an index for reducing blood toxicity, and abnormal levels indicate the presence of a toxic factor that significantly impacts blood formation (Oyawoye & Ogunkunle, 1998). The results show no significant differences in hematocrit values among ICR albino mice on the dietary groups (T1, T2, T3, T4). The data presents mean values for each group, which are largely consistent with those reported by Farr (2015), Farr et al. (2019), and Akinsanmi et al. (2020). The observed HCT values for ICR albino mice on diets (T1 43.05 ± 3.04, T2 43.55 ± 2.05, T3 43.07 ± 3.27, and T4 46.45 ± 1.06) reported in this study for mice fed on control and experimental diets were within the reference ranges (16.7 - 69.8) for healthy mice reported by McBean (2016-2017). This suggests that the incorporation of RoPRSKM ME at 5%, 10% and 15% into that of the test diet did not influence significantly the hematocrit levels in the blood of consuming mice.

Mean Corpuscular Volume (MCV) evaluates the average size of blood cells. When the levels are lower than usual, it indicates a sign of anaemia or thalassemia (NIH, 2022). The observed MCV values for ICR albino mice on diets (12.30 ± 1.13, 12.20 ± 1.13, 12.57 ± 1.07, and 14.30 ± 0.14) reported for mice fed with and without para rubber seed kernel meal incorporated diets in this study were within the reference ranges (39 - 90.8) for normal mice reported (McBean, 2016-2017). Mean corpuscular hemoglobin concentration (MCHC) represents the total amount of hemoglobin in a single red blood cell (RBC) in relation to the cell's volume. It measures red blood cells' function and health status for signs of anaemia and other blood disorders (Kamfrath, 2022). When MCHC is high, it indicates too much haemoglobin in the red blood cell and when it is low, it suggests anaemia (Ruben, 2018). The MCHC values for ICR albino mice on diets (T1 28.55 ± 0.49, T2 28.00 ± 1.27, T3 28.70 ± 0.35 and T4 29.75 ± 0.78) reported for mice are within the acceptable range (27 - 37.60) for normal MCHC of albino mice reported (McBean 2016-2017).

Platelets are blood cell fragments that respond to a blood clot. Lower or higher Platelet levels than usual indicate a sign of clotting or bleeding disorder (NIH, 2022). Platelet Count and Mean Platelet Volume (MPV) for ICR albino mice did not show significant differences for mice on the dietary groups. ICR albino mice on diet T1 showed a slightly higher mean platelet volume than those on diet T3, but this difference was insignificant. As indicated in Table 10, the platelet count and Mean Platelet Volume for mice fed with and without para rubber seed kernel meal incorporated diets were in the normal reference ranges (59 - 2633) and (5.2 – 13.1) reported for mice (McBean, 2016-2017). None of the parameters assessed (including red blood cells, white blood cells, mean cell volume, mean corpuscular hemoglobin concentration, hematocrit, packed cell volume, platelets, and lymphocytes) showed significant differences compared to the control group. These findings are consistent with those of Onyimonyi et al.

(2012), who reported that feeding albino rats with para rubber seed kernel meal had no significant effect on these blood parameters.

However, the current study also revealed that lymphocyte counts in the T4 group were significantly higher than those in the other groups. Lymphocytes provide antibodies to attack invading bacteria, viruses, and toxins. When lymphocyte cell count is higher than average, it indicates infection (Ruben, 2018). This means that there may be an infection in the blood. Diet T4 had a significantly higher lymphocyte count than the other diet groups (T1, T2, and T3). The p-value of 0.0457 shows that the difference is statistically significant. The specific diet represented by T4 may have impacted the mice's lymphocyte count, making it higher than the other diets. However, in this study, the LYMPH valumes for mice fed with and without para rubber seed kernel meal incorporated diets, as indicated in Table 10, were in the normal reference range (0.12 – 23.46) reported for mice (McBean 2016-2017).

This results contravenes the report of Farr's (2015) report that the dietary treatment had a slight effect on the haematological parameters of the rats, except white blood cell count, MCV and lymphocyte cell. This also disagrees with Esonu et al. (2001), who found that feeding rubber seed kernel meals to albino rats decreased their red blood cell count, haemoglobin concentration, and hematocrit value.

Blood Biochemistry

The liver blood biochemistry of ICR albino mice on diets with or without RoPRSEKM is shown in the Table11. The liver is a multifunctional organ that plays a vital role in metabolism, detoxification, digestion, and other processes essential for an animal's overall health and survival (Baquerre, et al., 2021). Its functions are interconnected and essential for maintaining homeostasis within the body. The results presented in the table are from liver function (biochemistry) test conducted on albino mice that were either fed with or without roasted full-fat para rubber seed kernel meal. The result indicates no significant difference in albumin levels among the four groups.

Albumin is a blood protein with crucial functions: it maintains blood vessel osmotic pressure, transports hormones, fatty acids, and drugs, regulates blood pH, and prevents fluid leakage. Low levels signal malnutrition, chronic inflammation, hepatitis, or severe liver damage, while high levels suggest dehydration or reduced blood volume (Washington & Hoosier, 2012). The lack of significant differences suggests that the different diets did not have a notable impact on albumin production in these mice. This study's results conform to that of Udo et al. (2018), who noted that with the inclusion of para rubber seed meal at 10% to 30% of the diet or ration in the feed, various parameters of the blood samples were not significantly different between treatments except for white blood cell and cholesterol levels.

Additionally, the results showed no significant differences in Alanine Transaminase (ALT) levels across the groups. ALT is an enzyme that, when elevated, can indicate liver damage or stress. However, in this study, the p-values suggest that the different diets did not cause significant variations in ALT levels (Udo et al., 2018). Similarly, there were no significant differences in total bilirubin, direct bilirubin, and indirect bilirubin levels among the groups. Bilirubin is a substance produced during the breakdown of red blood cells (hemoglobin) and is processed by the liver. Elevated bilirubin levels can signal liver dysfunction or hemolysis, but the p-values indicate that this was not the case in this study.

Aspartate Aminotransferase (AST) is an enzyme present in various body tissues, with the highest concentrations found in the liver and heart. While low AST levels are generally not a concern, extremely low levels may suggest severe liver dysfunction or a vitamin B6 deficiency, which is vital for AST function. Elevated AST levels in the blood often indicate liver or heart damage (Washington & Hoosier, 2012; Botros & Sikaris, 2013). However, in this current study, The AST levels were highest in ICR albino mice group on T3 diet and lowest in group T4 diet. However, the p-value (0.429) suggests that these differences in AST levels among the groups were not statistically significant. AST is another enzyme often used to assess liver function, and where there is some variability, it does not appear to be related to the different diets.

ICR albino mice group on T2 diet had significantly higher alkaline phosphatase levels than the other groups, as indicated by the p-value (0.005*). Elevated alkaline phosphatase levels can indicate bone growth (Washington & Hoosier, 2012). The increased ALP level among each treatment (replicates) group may not indicate liver damage. It may derive from the bone growth. In addition, it may also derive the fighting that took place among the treatments group. Fighting can affect an animal's ALP levels, mainly if the animal sustains injuries during the fight; chronic stress caused by fighting can affect liver function over time, leading to changes in enzyme levels as reported (Mota-Rojas et al., 2021).

Total protein refers to the measurement of all the proteins that the level produces, which are present in the blood. High total protein levels can be influenced by dehydration or a metabolic disturbance. While low total protein levels are the result of chronic malnutrition, impaired absorption of proteins, or protein depletion stemming from kidney or liver issues (Washington & Hoosier, 2012). ICR albino mice on group T1 diet had significantly higher total protein levels than the other groups, while ICR albino mice group on T4 diet had significantly lower total protein levels. The highly significant p-value (0.000*) indicates that the differences in total protein are noteworthy. Although total protein was highly significant, the rest of the group fell within the acceptable range of albino mice. These findings suggest that the ICR albino mice group on T1 diet may have led to increased protein production, while the group on T4 diet may have experienced a decrease.

Furthermore, increased total protein levels may be caused by dehydration, and the lower total protein level found in the ICR albino mice group on diet T4, which may result from the constant fighting or impaired absorption of proteins. Animals constantly fighting may have higher cortisol levels, a stress hormone that can affect protein metabolism and lead to lower protein levels in blood biochemistry measurements (Mota-Rojas et al., 2021). The study results agree with results of Farr (2015) and Farr et al., (2019). They reported that including para rubber seed meal in rats' diets can slightly affect the rat's total protein, albumin and globulin. In addition, Adedokun et al. (2017) examined broiler chicken-fed cassava composite meals at the inclusion levels of 0, 5, 10,15 and 20% in the diets and found that the serum biochemistry parameters differed significantly in all parameters measured.

ICR albino mice group on T1 had significantly higher globulin levels than the other groups, and ICR albino mice group on T4 had the lowest globulin levels. ICR albino mice group on T2 diet had significantly lower globulin levels than the other groups, whilst ICR albino mice group on T3 diet had intermediate globulin levels. The highly significant p-value (0.001*) suggests substantial differences in globulin levels. Globulin is often measured as part of the total protein level and can be associated with immune function and liver health. These results suggest that diet significantly impacted globulin levels in these mice. The study results agree with earlier research results of Farr (2015) and Farr et al. (2019). Bahman (2011) noted that blood analysis provides a quick method for evaluating animals' clinical and nutritional health conditions in feeding trials.

Generally, the hematology and blood biochemistry measurements obtained in this study could serve as evaluation of the ICR albino mice clinical and nutritional conditions (Bahman 2011). With much of the measurements taken falling within the reference ranges given for albino mice (McBean 2016-2017) it can be inferred that incorporation of full-fat para rubber seed kernel meal into standardized 2825 kcal ME / kg DM of commercial chick starter crumble produced by Kuodjis Limited company at Tema, Ghana did not significantly influence the ICR albino mice general performance.

The kidney function tests conducted on albino mice fed without and with roasted full-fat para rubber seed kernel meal. The kidney play multiple roles in the body. They filter waste and excess blood fluids to produce urine, eliminating toxins and maintaining proper fluid balance (Ogobuiro & Tuma, 2023). Additionally, the kidney regulates electrolyte balance, including sodium, potassium, and calcium, which are vital for nerve and muscle function (Ogobuiro &Tuma, 2023).

Kidneys also help control blood pressure through fluid regulation and hormone production. Furthermore, the kidney produces erythropoietin, stimulating red blood cell production essential for oxygen transport. Lastly, they contribute to the body's acid-base balance by managing hydrogen and bicarbonate ions (Ogobuiro & Tuma, 2023). There are many biochemistries used to regulate normal kidney function. These biochemistries include Sodium, Potassium, glomerular filtration rate, Creatinine, and Chloride.

Sodium is a substance cells need to work and helps uphold the right balance of fluids in the body. It allows an animal's nerves and muscles to work. When sodium concentration is higher than usual, it decreases live body weight gain, daily body weight gain (DBWG), and feed and water intake, reducing the digestibility of animal nutrients (Fang et al., 2018). In this study, albino mice fed both with and without roasted full-fat para rubber seed kernel meal maintained sodium levels within the normal reference range for albino mice, indicating that their electrolyte balance was preserved. Despite differences in diet, no significant differences were observed among the treatment groups.

The findings of the current study align with those of Akinsanmi et al. (2020) and Udo et al. (2018), who reported that para rubber seed kernel meal or cake does not have a significant effects on poultry birds health status. However, these results contrast with the findings of Ahamefule et al. (2007), who observed that incorporating sun-dried, ensiled, and fermented cassava peel-based diets in rabbits can impact blood serum.

Potassium’s significant role in an animal body is to help maintain a standard range of fluid inside the animal cells, and it also helps activate various cell and nerve functions (Weir, 2023). When potassium is low in an animal, it indicates it is suffering from hypokalemia. Hypokalemia is characterized by a low blood concentration of potassium. Common causes include long-lasting kidney failure and severe vomiting. Other, less common causes can include diabetes mellitus, diarrhea, diuretic use, and malnutrition (Weir, 2023).

The current study result shows that the Potassium levels remain within the reference range for all groups, indicating that the mice maintain normal potassium homeostasis despite dietary variations. This finding aligns with the results of Akinsanmi et al. (2020), who reported that para rubber seed kernel meal or cake does not have significant impact on poultry birds health status.

The glomerular filtration rate is a measure of how well the kidneys are filtering waste from the blood (Schwartz & Furth, 2007). When eGFR is high, it indicates that the kidneys are filtering blood at a faster rate, which can be a sign of good kidney function (Schwartz & Furth, 2007). Conversely, when eGFR is low, it suggests that the kidneys are not filtering blood as effectively, which may indicate kidney damage or dysfunction (Schwartz & Furth, 2007).

In this study the albino mice fed without and with para rubber seed kernel meal, all values are above the lower limit of the reference range (60 ml/min), suggesting adequate filtration capacity in all mice. This finding agrees with Akinsanmi et al., (2020) and Udo, et al. (2018), who reported that para rubber seed kernel meal or cake had no significant effects on the health status of poultry birds who reported that para rubber seed kernel meal or cake does not have significant impact on poultry birds health status.

Creatinine is a waste product generated from the breakdown of creatine phosphate in muscles (Patel et al., 2013). It is filtered from the blood by the kidneys and excreted in urine. Creatinine levels can reflect kidney function: elevated levels may indicate impaired kidney function, whereas low levels might suggest muscle depletion (Patel et al., 2013).

The Creatinine levels in SI units of albino mice fed without and with para rubber seed kernel meal full within the reference range across treatment groups, with T2 showing the highest mean value (1.73 ± 1.1 mg/dl) and T4 showing the lowest (0.4 ± 0.2 mg/dl). However, all values fall within the reference range, suggesting normal kidney function regarding creatinine clearance. The findings align with Akinsanmi et al., (2020) & Udo, et al. (2018), who reported that para rubber seed kernel meal or cake have no significant effects on poultry birds health status.

Urea, a waste product from protein breakdown in the liver, eliminates excess nitrogen (Pundir, et al., 2019). High levels suggest kidney issues, dehydration, or excessive protein breakdown. Low levels indicate liver disease, malnutrition, or low protein intake, causing weakness and impaired immune function. Monitoring urea helps diagnose and manage animal health (Pundir, et al., 2019). The current study revealed that the albino mice fed without and with para rubber seed kernel meal urea levels show slight variability but all values fall within the reference range of normal albino mice. This suggests that the diet does not significantly affect kidney function in terms of urea clearance. The finding concurs Udo et al., (2018) who found that the boiled rubber seed kernel meal inclusion in WAD (West African dwarf) goats does not affect the various parameters of the blood samples except for white blood cell and cholesterol levels.

Chloride levels vary notably among the treatment groups, with T3 showing a considerable decrease compared to the reference range. The Elevated chloride levels observed in this present study may cause dehydration due to disproportionate water loss compared to chloride loss. Although there are some fluctuations in certain parameters, kidney function remains largely within normal ranges in mice fed roasted full-fat para rubber seed kernel meal. The current finding concurs with Fawole et al., (2017) finding that rubber seed protein inclusion Isolates in fish diets did not cause oxidative-induced stress.

Histology of Albino Mice Liver and Kidney

This aspect of the study discussed the result of the liver and kidney histopathology of albino mice. The discussion is guided by Research Question 4.

Research Question 4. How does para rubber seed kernel meal affect albino mice liver and kidney tissue histopathology?

This research question sought to investigate the liver as well as kidney tissue histology of ICR albino mice fed a diet containing para rubber seed kernel meal. Histological testing is the preferred method for examining tissues for research and diagnostic purposes; it enables the evaluation of inflammation, healing progress, and the existence and supply of degraded meal in the nearby tissue (Ragamouni et al., 2013). The kidney and liver histology of the mice fed with and without roasted para rubber seed kernel meal photomicrographs are shown in Figures 1 and 2.

The portal vein is a crucial liver blood vessel that carries nutrient-rich, oxygen-poor blood from the digestive organs (e.g., stomach, small intestine, colon, and spleen) to the liver from the gastro-intestinal (GI) tract. Such unique system enables the liver to process and regulate nutrients like sugars, amino acids, and fats, as well as detoxify the blood before it enters the general circulation (Kalra et al., 2023). The liver's role in metabolizing these substances and removing toxins is essential for maintaining overall metabolic and homeostatic functions. Blood entering the liver via the portal vein is distributed through smaller vessels within the liver, where it undergoes processing, detoxification, and storage, making the portal system a vital component of liver function. Blockages or damage to the portal vein can lead to increasing pressure in the portal venous system, which is referred to as portal hypertension (Kalra et al., 2023).

Bile ducts are thin, tube-like structures in the liver and other parts of the digestive system that transport bile from the liver and gallbladder to the small intestine. Bile is a greenish-yellow fluid that is more essential in digestion and the absorption of fats in the small intestine. It is formed by the liver and kept in the gallbladder until wanted (Kalra et al., 2023). The bile duct system is essential for digestion and removing waste products from the body. Blockages or issues with the bile ducts can lead to digestive problems and liver-related health conditions (Kalra et al., 2023). However, the study's findings showed no areas of inflammatory infiltration or cell death in tissue structure of the liver of the mice fed with RoPRSKM as compared with control groups.

Also, based on feed dry matter intake, the hydrogen cyanide intake of ICR albino mice on study diets D1, D2, D3, and D4 ranged from 0.0010mg/kg for animals on diet one (1) and three (3) to a maximum of 0.0011mg/kg for diets two (2) and four (4) which was far below the tolerable level for hydrogen cyanide level of mammals (0.5mg/kg body weight (WHO, 2012). The cyanide content in the diets were low, making it harmless to the liver of the ICR albino mice (mammals) under current study. This finding concurs with Manyelo et al. (2019), who established that the broiler histological measurements did not change when the animals were fed either 75% or 100% sorghum bicolor substituted for Zea mays in the diets. In addition, the finding agrees with the finding of Fawole et al. (2017), who fed Labeorohita fingerlings with para rubber seed protein isolates substituting soybean meal at varying inclusion levels, RPI 25, RPI 50, RPI 75, and RPI 100. They found that the liver did not show signs of changes. However, the result contradicts the findings of Oyedele. et al. (2021), who found that feeding Dry Distilled Cassava with Soluble in broiler chickens' diet at the inclusion levels of 4, 8, and 12% results in liver, kidney, small intestine, and large intestine changes.

The renal corpuscle is a specialized structure within the kidney responsible for the initial stage of blood filtration in urine formation. The renal corpuscle filters the blood, removes waste products and excess substances, and helps maintain the body's fluid and electrolyte balance. This process is vital for regulating the composition of bodily fluids and excreting waste products from the body in the form of urine (Ogobuiro & Tuma, 2023).

Bowman's space is a critical anatomical structure within the renal corpuscle of the nephron, and its primary function is to serve as the initial site for the filtration of blood to form a precursor of urine. It plays a vital role in the kidney's ability to regulate the composition of bodily fluids and remove waste products from the bloodstream, ultimately contributing to overall health and bodily functions (Ogobuiro & Tuma, 2023).

The glomerulus is a crucial component in the kidney responsible for filtering blood. It consists of a network of capillaries fed by the renal artery, with high pressure forcing small molecules and solutes (e.g., water, electrolytes, waste products, small proteins) out of the blood and into Bowman's space, the initial part of the nephron. (Ogobuiro & Tuma, 2023).

This process creates the glomerular filtrate, a mix of dissolved substances that will undergo further processing in the renal tubules. Large molecules like blood cells and most proteins are retained in the bloodstream. The diameter of the afferent and efferent arterioles can influence the glomerular filtration rate (GFR), which has implications for blood pressure and the body's fluid balance (Ogobuiro & Tuma, 2023). The study found that various areas of the kidney tissue showed no areas of cell degeneration or death in tissue structure. This result implies that kidney carried out its normal function. The diet did not interfere with kidney function and regulations.

This study result implies that para rubber seed kernel meal is an appropriate alternative feed resource or ingredient to replace some expensive conventional energy feed resources or ingredients. The results align with Manyelo et al. (2019), who reported that the broiler histological measurements did not change when the animals were fed either 75% or 100% sorghum bicolor substituted for Zea mays in the diets. However, it is contrary to the finding of Oyedele. et al. (2021), who established that feeding Dry Distilled Cassava with Soluble in broiler chickens' diet at the inclusion levels of 4, 8, and 12% results in liver, kidney, small intestine, and large intestine changes.

Total Costs Analysis of the Study

This analysis suggests that using Para rubber seed kernel meal as energy replacement in the diet incurs no expenses. Because The para rubber kernels were received free. If it had not been used as animal feed it would have gone rotten.

At least its use in animal feed would at least reduced the high feed cost and help to increased livestock farmers income.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Overview

This study investigated the influence of para rubber seed kernel incorporated feed on the growth performance, blood composition, and liver and kidney tissue histology of ICR albino mice. This chapter contains the summary, conclusion, recommendation and suggestions for future studies. Additionally, it provided the method employed in conducting the research.

Summary of the Findings

This experimental study investigated the influence of para rubber seed kernel incorporated feed on the growth performance, blood composition, and liver and kidney tissue histopathology of ICR albino mice. The high cost of feed, driven by competition between humans and animals for the same types of feed, prompted this study. It sought to evaluate the nutrient and proximate composition of para rubber seeds kernel; assess the growth performance of albino mice fed para rubber seeds kernel meal; examine the blood composition (hematology, blood biochemistry) of albino mice fed on para rubber seeds kernel meal; and investigate the liver and kidney tissue histopathology of albino mice fed diet containing para rubber seed kernel meal.

The proximate analysis was done at the nutrition laboratory of the Animal Science Department of the School of Agriculture, following the procedures recommended by AOAC (2008). The evaluation of the nutrient and proximate composition of para rubber seeds kernel findings showed that moisture content tended to be high in raw full-fat para rubber seeds kernel meal; however, it decreased when the full-fat para rubber seed kernel meal is treated. Additionally, the findings showed that crude protein content increased in full-fat para rubber seeds kernel meal when it is treated. Furthermore, it was found that the ash content (3.3%) and the treated para rubber seed kernel (3.2%) were slightly different. The Oil/Fat, crude fibre,and total carbohydrate compositions were found to be high when the para rubber seed kernel is unprocessed, compared to when the para rubber seed kernel is processed.

Assessment of the growth performance of albino mice fed para rubber seeds kernel meal findings indicated that feed intake of the albino mice fed on control (D1) and when 5% ME in diet D1 was replaced with para rubber seed kernel meal (D2) had similar average feed intake values of 7.358 g\d and 7.325 g/d which were higher. In comparison, ICR albino mice fed on diet (D3) and (D4) 10% and 15% of ME in D1 was replaced with RoPRSKM had lower feed intake of 6.190 g / d and 6.6991 g / d respectively. However, the weight gain and the water intake showed no statistically significant differences among the ICR albino mice on the four diets.

The haematological findings indicated that all albino mice that were fed with para rubber seed kernel meal did not have any detrimental effects on their Red Blood cells, white blood cells, mean cell volume, mean corpuscular haemoglobin concentration, hematocrit, packed cell volume, platelets and lymphocytes compared to value for ICR albino mice fed with control diet (D1).

In the blood serum (liver and kidney) function test, no significant differences were observed in Albumin, Alanine Aminotransferase (ALT or GPT), and Bilirubin (Total, Direct, and Indirect) levels among the ICR albino mice groups on the four dietary treatments, as indicated by the p-values. Mean Aspartate Aminotransferase (AST) levels obtained varied across groups, with group / ICR albino mice on T3 diet having the highest (228.30 U/L) and ICR albino mice group on T4 diet the lowest (183.75 U/L), but these differences were not statistically significant (p-value = 0.429). Notably, Alkaline Phosphatase (Alkaline Phos.) levels for ICR albino mice in group T2 were significantly higher than in the other groups (p-value = 0.005*), indicating a statistically significant difference. Total Protein levels in Group T1 were significantly higher than in the other groups (p-value = 0.000*). ICR albino mice group on T4 had significantly lower total protein levels than the other groups (p-value = 0.000*), suggesting highly significant differences. Regarding globulin levels, ICR albino mice group on T1 diet had significantly higher levels than the other groups (p-value = 0.001*), while ICR albino mice group on T2 diet had significantly lower levels (p-value = 0.001*). ICR albino mice group on T3 diet had intermediate levels, and ICR albino mice group on T4 had the lowest levels. These findings suggest highly significant differences in globulin levels among the groups.

In the kidney function test carried out in the study, although Creatinine levels in ICR albino mice group on T2 were higher than in the other groups, the p-value of 0.107 suggests that this difference was not statistically significant. Importantly, all groups' values for all ICR albino mice fell within the reference range for Creatinine, indicating normal levels. Both Creatinine measurements in mg/dl and umol/L showed no significant differences between ICR albino mice groups on the four dietary treatments. Sodium levels were higher than the reference range in all ICR albino mice groups studied but the p-value suggested that these differences were not statistically significant. Similarly, blood urea levels for ICR albino mice groups studied were within the reference range for all groups, and the differences were deemed insignificant (p-value = 0.290). The potassium in the entire groups were higher than the reference range, but the p-value suggested that these differences were not statistically significant. The lack of statistical significance implies that the observed variations may not be clinically meaningful despite being outside the reference range. The histological examination of ICR albino mice fed with and without roasted para rubber seed kernel meal revealed no changes in the liver and kidney tissues. This lack of histological alterations was consistent with the biochemical indicators, which fell within reference values. The study also determined that the hydrogen cyanide intake for the mice on various diets was well below the tolerable level for mammals, indicating that it should not adversely affect the liver and kidney tissues of the ICR albino mice.

Conclusions

This research examined the para rubber seed kernel mealrelative to its impact on the nutrient composition of albino mice. The proximate analysis results show that collecting the para rubber seeds kernel during its season and storing it before subjecting the seeds to treatment (roasted) can help reduce the hydrogen cyanide content and improve the seed's meal nutritional value.

In addition, the growth performance of the albino mice fed without and with para rubber seeds kernel meal was assessed. The finding suggested that the albino mice fed without and with para rubber seeds kernel meal incorporated diets were able to utilize the feed. However, water intake and weigh gain had no significant difference between the albino mice on the controlled diet and the one on the test diets.

Furthermore, the Blood parameters were measured, and found that the albino mice fed without and with the roasted para rubber seed kernel meal were unaffected by the para rubber seed kernel meal diet except_lymphocytes which shows a significant difference. However, they all fall into the normal reference range of albino mice lymphocyte counts

Moreover, Liver function tests showed no significant differences in most parameters, except for alkaline phosphatase and total protein, which varied significantly between dietary groups.

Creatinine, sodium, urea, and potassium levels were generally within the reference range for all groups, with no statistically significant differences. Finally, Histological evidence indicated lack of significant impact on the kidney and liver tissues of the mice, and hydrogen cyanide levels were considered safe.

Recommendations

Based on the findings, summary and conclusion, the following recommendations are made.

1. Livestock and para rubber farmers should stockpile para rubber seed kernels during its production season to help reduce the hydrogen cyanide

in the seed and improve the seed's meal nutritional value for their animal consumption.

2. Para rubber seed kernel meal should be included in the formulation and compounding of poultry and livestock diets as a protein and energy feed ingredient. This may help reduce some expensive protein and energy feed resources.
3. Animal farmers should be educated on using para rubber seed kernels as alternative feed to replace expensive conventional feed ingredients.

Suggestions

A study should be conducted where layer or broilers chickens should be fed para rubber seed kernel meal incorporated feed; because layer or broilers chicken production take longer duration. Such a study will determine whether the remaining HCN in the RoPRSKM has effects on the animals after storage and roasted. Reason being that insufficient exposure to the experimental diet or a short duration might not have led to observable changes in the animal.

Also, the level of metabolizable energy from the roasted para rubber seed kernel meal should be increased above the 15% of test diet used in current study to establish the level of tolerance of the meal in poultry and other livestock feeds.

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APPENNDIX A. Dietary Treatments

Control Diet (Treatment 1)

Experimental Diet (Treatment 2)

Experimental Diet (Treatment 3)

Experimental Diet (Treatment 4)

APPENDIX B. Collection of Haematology and Blood Biochemistry

Mice dissection

APPENDIX C. Histology Processing Procedures


FOR LAB USE ONLY
70% Ethanol
80% Ethanol
95% Ethanol I
95% Ethanol II