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EFFECT OF COMPOSITE FOOD POWDER ON VITAMIN A LEVELS, ANAEMIA, GROWTH STATUS AND TOXICOLOGICAL EFFECT IN SPRAGUE DAWLEY RATS

BY

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DECLARATION

I, Akosua Nyame Adom Frempong, author of this thesis "Effect of composite food powder on vitamin A levels, anaemia, growth status and toxicological effect in Sprague Dawley rats," do hereby declare that this thesis is the result of my work carried out at the Department of Nutrition and Food Science, Faculty of Science, University of Ghana, under the supervision of Prof. Matilda Steiner-Asiedu, Dr Godfred Egbi and Dr Samuel Adjei. All references to other works have been duly acknowledged.

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DEDICATION

This work is dedicated to God and my cherished family for their immense love and support as I placed this work together.

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ABSTRACT

Introduction: Animal experiments play essential roles in research and help alleviate new diseases or existing conditions. Animal models have helped in the advancement of our nutritional knowledge. Since malnutrition, especially micronutrient deficiencies, are a significant public health concern, animal studies can advance understanding in this area. Animal studies can assess the toxicity and effect of micronutrient powders before human consumption. This study was conducted to determine the effect of the Composite Food Powder on vitamin A levels, anaemia, growth status and toxicological effect in Sprague Dawley rats. The powder contains anchovies, carrot, eggplant and soybean. These foods combined will be rich in iron and vitamin A, making them an excellent strategy to treat micronutrient deficiencies.

Methodology: Composite Foods Powder (CFP) (100g) was made with dried and powdered 15g anchovies, 50g turkey berry, 10g carrot and 25g soybean. The Association of Official Analytical Chemists (AOAC, 2005) standard procedure was used to determine the proximate composition of the CFP. β-carotene and iron analyses were determined by HPLC and Atomic Absorption Spectrophotometry methods, respectively. For the animal experimentation, twenty Sprague-Dawley rats were assigned to 2 groups, control (6) and experimental (14), for 28 days of investigation. The animals were weighed weekly. The length was measured on days 0, 7 and 15. After the animal was euthanised correctly, blood was taken by cardiac puncture on the 29th day for analysis. The kidneys and livers of the animals were isolated and processed for histopathological examination.

Weight, length, haematological indices, serum retinol and biochemistry, were presented as means ± standard deviations. The histopathological analysis was presented in microphotographs. The significant difference in means between the groups was determined by independent t-test.

Results: The blood analysis shows that the CFP contains 7.2mg/g β-Carotene and 5.28 iron, respectively. The mean weight of the control group was 161.37 ± 8.83 g, while that of the experimental group was 166.77 ± 7.91 g. The mean length for the control group was 19.03 ± 1.28 g, while that of the experimental group was 19.13 ± 1.00 g. No significant difference was observed across the control, and experimental groups for red blood cell count, haemoglobin, haematocrit, mean cell volume and mean cell haemoglobin values. No significant difference was observed across the control and experimental groups for Lymphocyte per cent and number, Eosinophil per cent and number, monocyte number and per cent, neutrophil per cent and number and basophil number and basophil per cent. The serum retinol concentration was generally low among the experimental and control groups. The control group had 0.002 ± 0.001 10^3 µg, and the experimental group had 0.002 ± 0.001 10^3 ug. No significant difference was observed across the control and experimental groups for urea, creatinine, direct bilirubin, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, total protein, and albumin, except for total bilirubin. There was a significant difference between the control and experimental groups for total bilirubin.

Conclusion: This study revealed that CFP made up of locally available food products, contains iron and β- carotene that could meet the micro nutritional needs of infants. The CFP was found to have no significant effect on the SD rats' growth indices and serum retinol. The CFP was shown to have no toxicity regarding haematological and biochemical markers and very few histopathological alterations in isolated organs.

LIST OF ACRONYMS AND ABBREVIATIONS

CFP	Composite Foods Powder
SD rats	Sprague Dawley rats
Hb	Haemoglobin
RDW	Red Cell Distribution Width
RBC	Red Blood Cell Count
Lymph#	Lymphocyte number
Lymph%	Lymphocyte %
Eos%	Eosinophil %
MCHC	Mean Cell Haemoglobin Concentration
Eos#	Eosinophil number
WBC	White blood cell count
Mono%	Monocyte %
MCH	Mean Cell Haemoglobin
Mono#	Monocyte number
MCV	Mean cell volume
Neut#	Neutrophil number
Neut%	Neutrophil%
Baso %	Basophil%
Baso#	Basophil number
µg	Microgram
RDA	Recommended Daily Allowance
g/dL	Gram per decilitre
µg/g	Microgram per gram
cm	Centimetre
RE	Retinol Equivalent
g	Gram
UNICEF	United Nations Children‘s Fund
HPLC	High Performance Liquid Chromatography
P-value	Probability value
VAD	Vitamin A deficiency

mg	Milligram
ml	Millilitre
SD	Standard Deviation
WHO	World Health Organization
Aas	Atomic absorption spectroscopy
NMIMR	Noguchi Memorial Institute for Medical Research,
SPSS	Statistical Package for Social Sciences

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CHAPTER ONE

1. 0 Introduction

1.1 Background Information

Malnutrition is a public health concern in many developing countries, particularly sub-Saharan Africa. It comprises micronutrient deficiencies, undernutrition, overweight and obesity (Bhutta et al., 2013). A child's growth is affected by their nutrition and genetic material. Children's known causes of growth failure are insufficient energy and nutrient intake and recurrent infections (Martorell, 2012). Micronutrient deficiencies also contribute to this growth failure through anorexia, increased morbidity and multiple simultaneous deficiencies associated with micronutrient deficiencies (Martorell, 2012). Micronutrient deficiencies occur when the body lacks essential minerals and vitamins for growth. It causes adverse health consequences and increases the risk of morbidities and comorbidities (Bhutta et al., 2013; Clark et al., 2020).

In 1990, several international organizations, heads of state and governments had a world summit for children where goals such as decreasing micronutrient deficiencies, mainly iron deficiencies and vitamin A were established (Sachs, 2015). In 2015, 17 sustainable development goals were set to transform the world. Goal 3 aims to End hunger, achieve food security, improve nutrition, and promote sustainable agriculture by 2030 (Madeley, 2015). However, hidden hunger (micronutrient deficiencies) is still predominant in children, causing public health problems associated with long-term effects on human resources and economic growth (Sachs, 2015).

Micronutrient deficiencies are not only caused by an inadequate amount of food and high morbidity rates (Clark et al., 2020). They are also caused by diets of low quality, which are common in many developing countries. Most people's diets in developing countries usually contain insufficient vegetables, fruits and animal products and mainly comprise legumes,

tubers and cereals. This diet is typically low in vitamins and minerals and has poor bioavailability (Clark et al., 2020).

Iron deficiency anaemia (IDA) is a haematological disorder characterized by reduced red blood cells (RBC) and haemoglobin in the blood as a result of insufficient iron (Deloughery, 2017). It is a common micronutrient deficiency in developing countries, affecting about 3.5 billion people. In most developing countries, iron deficiency anaemia is caused by insufficient consumption of bioavailable iron and infections with intestinal parasites. Having a haemoglobin concentration of 10 - 10.9 g/dl is considered mild anaemia. 7 - 9.9 g/dl is considered moderate, while 7.0g/dl is considered severe anaemia (WHO, 2011a). In Ghana, the overall prevalence of anaemia is 66% among children aged 6–59 months (Ewusie et al., 2014). Also, in the Ghana demographic health survey, 66% of children aged 6-59 months have a form of anaemia, 27% have mild anaemia and 37% have moderate anaemia, with 2% being severely anaemic (GSS et al., 2015). In 2017, it was reported that anaemia prevalence in Ghana was 35.6% (University of Ghana et al., 2017). Anaemia is associated with growth retardation, poor cognitive and motor development, decreased physical activity and impaired immune function outcomes in children. Resulting in educational losses and increased morbidity in children (Balarajan et al., 2011).

Vitamin A deficiency occurs when a diet is regularly inadequate in vitamin A. Therefore, the body cannot meet its physiological needs (WHO, 2011). Retinol is a form of vitamin A that predominately circulates in the blood. Retinol levels in the serum show the amount of vitamin A stored in the liver. Serum retinol levels below 0.70umol/g liver are considered severely depleted vitamin A stores, and 1.05umol/g liver is considered extremely high vitamin A stores (WHO, 2011b). It is associated with childhood blindness, anaemia, reduced immunity and death due to diarrhoea. Although vitamin A deficiency is declining, it still affects a third of all children between zero and nine months (WHO, 2009). In 2013, 29% of children

between 6 and 59 months in low-middle-income countries experienced vitamin A deficiency. South Asia and Sub-Saharan Africa had the highest prevalence globally, 48% and 44%, respectively (Stevens et al., 2015).

A strategy may be the fortification of stews and soups with composite food powder (CFP). This powder is very nutritious, and its constituents are readily available. Anchovies, carrots, eggplant, and soybean are ingredients in CFP; the food category of fish, animals and animal products includes anchovies; the fruits and vegetable food group includes carrots and turkey berries; the food group of beans, oils, and nuts contain soybeans. The combined foods will be rich in iron and vitamin A, making them an excellent strategy for treating anaemia. However, documented data in Ghana about the effect of the composite food powder on vitamin A levels, haemoglobin levels growth, and anti-anaemic properties is scant.

Animal studies aid in our understanding of diseases even though they can't determine what will happen in humans with certainty. However, they complement human trials (Van der Worp et al., 2010). This research, therefore, seeks to investigate the effects of the composite foods powder on growth status, vitamin A, anaemia status and toxicological effects in SD rats as an animal experimentation model before a trial involving humans.

1.2.0 Rationale

According to the 2020 Global nutrition report, 149 million (21.9%) children under five years are stunted. 30% of all children under five years of age have vitamin A deficiency. Between 1993 and 2005, anaemia prevalence was about 1.62 billion, about 24.8% (approximately ¼) of the world population. In 2010, it was estimated that 2.2 billion people were anaemic, approximately 32.9% of the world population (Micha et al., 2020). It was also assessed in 2013 that 29% of children between 6-59 months in low-middle-income countries experienced

vitamin A deficiency (Stevens et al., 2015). Hence children under five years are at risk of stunting, vitamin A deficiency and anaemia. Stunting, iron-deficiency anaemia and vitamin A deficiency are global health concerns affecting preschool-aged children and women. They affect child and maternal mortality rates, physical performance, cognitive and motor development and many more. They also harm the socio-economic development of the country (Micha et al., 2020).

In Ghana, vitamin A and anaemia are public health problems, resulting in a high burden of morbidity and mortality among children and women throughout their lives. 66% of children aged 6–59 months and 42% among women of reproductive age are anaemic. The Ghana demographic health survey, 19% of children under the age of 5 years are stunted. Also, 20.8% and 19% of children between 6 and 59 months have vitamin A deficiency and stunting (SPRING and Ghana Health Service, 2016). In the Northern belt (predominantly low-income households), 31% of children under 5 are vitamin A deficient, while those in wealthier families are 9%. Also, 53.2% of children under 5 in the northern belt are anaemic, while the southern and middle belts are 32.3% and 28.2%, respectively.

It has been estimated that undernutrition and micronutrient deficiencies cost $2.1 trillion annually (Panel, 2016). Hence the adverse effects of undernutrition and micronutrient deficiencies (especially stunting, vitamin A deficiency and anaemia) need to be addressed. Numerous interventions have been conducted to reduce these health problems and the cost involved. Studies have shown that investing in food fortification can help reduce the cost of undernutrition and micronutrient deficiencies (Panel, 2016). These studies have led to the development of micronutrient powders. These powders are used to fortify the diet. Green leafy vegetable powders could alleviate anaemia and vitamin A deficiency, according to a study by Egbi et al. (2018). Therefore, it is essential to conduct more studies on micronutrient

powders that are readily available and affordable in the Ghanaian market to help improve growth, vitamin A and anaemia status.

This study will, therefore, assess the effect of the composite food powder on growth, anaemia and vitamin A status in SD rats. The CFP contains anchovies, carrots, turkey berries and soybean. The combined foods will be rich in iron and vitamin A, making them an excellent strategy for treating anaemia, aiding growth and reducing vitamin A deficiency. Findings from this formative research will guide intervention planning in humans. It will also significantly contribute to research, particularly in sub-Saharan Africa, where the prevalence of stunting, wasting, vitamin A deficiency, and anaemia remains high.

1.3.0 Objectives

Main Objective

1. To assess the effect of the Composite Food Powder on vitamin A levels, anaemia, growth status and toxicological effect in Sprague Dawley rats.

Specific Objectives

1. To determine the iron and vitamin A content of the composite food powder.
2. To determine the effect of consumption of the composite food powder on:
    a. Serum retinol concentration of the SD rats.
    b. On the growth of the SD rats.
3. To determine toxicological effects of Composite Food Powder using haematological, biochemical and histopathological methods.

1.4.0 Hypothesis

1. The CFP will significantly affect vitamin A levels, anaemia, growth status and toxicological effect in Sprague Dawley rats.

CHAPTER TWO

2.0.0 Literature Review

2.1.0 Importance of animal studies

Animal studies are analogous to humans, but this theory has not been refuted or proved. However, animal experiments play important roles in research and help alleviate new diseases or existing conditions (Van der Worp et a., 2010). Experiments with laboratory animals are used in biomedical, agricultural, nutritional, industrial research and many more. It allows for the discovery of nutrient-nutrient interactions, diet-related diseases, the development and testing of innovative veterinary and medicine, the improvement and development of surgical materials and procedures and the investigation of experimental diseases and pathology (Shanks et al., 2009; Gallagher, 2003).

Rodents are the most prevalent and widely used animals in biomedical research around the world. Due to its broad strengths and applications, the rat model has long been regarded as the ideal experimental model for studying human diseases. It remains the leading animal model for risk assessment of nearly many therapies and chemical toxicities. (Hickman et al., 2017; Bryda, 2013). Several studies have used animal models to assess malnutrition interventions (Castro-Rodriguez, 2020; Laus et al., 2011; Fall, 2013). Sprague-Dawley and Wistar rats are the most widely employed in scientific investigations and are the best animal model for many experiments due to their low cost. Animal studies help further our nutrition knowledge (Chalvon-Demersay, 2017). In 2006, at an Experimental Biology (History of Nutrition) symposium, an outstanding assessment as to how research with food and animals has led to our understanding of nutrition theories and principles in energetics, lipids, carbohydrates, proteins, growth and body composition was presented (Baker, 2008).

2.2.0 Malnutrition

Melvin (2006) explained that all the processes such as digestion, ingestion, absorption, transport and metabolism of nutrients found in food by living things are nutrition. It is a critical part of life. Good nutrition is vital for growth, adequate organ formation, a robust immune system and economic and social growth. Well-nourished people are more productive and can break the cycle of hunger and malnutrition in their lives. (Hoseini et al., 2015). Malnutrition is an increased or decreased nutrient and energy intake of a person's requirements to maintain normal body functions (Sheet, 2012). WHO (2014) describes malnutrition as inadequate consumption of nutrients and the inability of the body to absorb or use these nutrients. Malnutrition affects a child's physical growth, cognitive development and IQ (Intelligence quotient), resulting in higher absenteeism, delayed enrolment and poor performance in class (Nyaradi et al., 2013). Malnutrition is classified into two: undernutrition and overnutrition. Undernutrition is underweight, wasting, stunting, and deficient in micronutrients. Diet-related non-communicable diseases, obesity and overweight are all examples of overnutrition. Underweight, wasting, and stunting are growth indicators that indicate a nutritional imbalance that leads to malnutrition (WHO, 2016a).

2.2.1 Underweight

An underweight person has a body weight less than the recommended weight for age, height, and build. Chronic and acute malnutrition are both included in the term "underweight." Childhood underweight is associated with a high risk of infectious diseases during adulthood. When left untreated, it also affects cognitive development, causes long-term intellectual impairments, and reduces adult work capacity (WHO, 2016a).

In 2011, around 101 million children under the age of five were categorized as underweight. Between 30 and 59 million young children were underweight in Sub-Saharan Africa and the Southern Hemisphere (Little, 2016; WHO & UNICEF, 2013). The prevalence of underweight has been steadily decreasing in Ghana throughout the years. The prevalence of being underweight has reduced from 18% in 2003 to 14% in 2008 and 11% in 2014 (GDHS, 2014).

2.2.2 Wasting

The proportion of children under the age of five who are below -2SD of the median weight-for-height (WHZ) Z-score is known as wasting. This population includes children who are too thin for height. Wasting is a symptom of acute malnutrition, which occurs when an individual's diet is deficient in energy, resulting in an increased risk of infectious infections. As a result of wasting, the immune system's ability to operate can be harmed. Approximately 7.4% of the world's population is affected by wasting, with 50 million young children and another 16 million seriously wasted. The majority of them live in Asia and Africa, with 34.3 million (68%) and 13.9 million (28%), respectively (UNICEF/WHO/WBG, 2015). In Ghana, wasting affects 5% of children under five, with less than 1% severely wasted (GDHS, 2014).

2.2.3 Stunting

Stunting is a symptom of long-term growth deficits and shortness of growth. It's linked to stunted development and growth because of poor nutrition, recurrent infections, and a lack of psychological stimulation. Poor cognitive and educational performance, low adult income, and lost production are all consequences of stunting. There is a more significant risk of nutrition-related chronic diseases later in life when stunting is accompanied by substantial weight gain.

Stunting is characterized as a height of -2SD below the WHO Child Growth Standards median height-for-age (HAZ) Z-score (WHO, 2006a). Stunting is a widespread and pervasive problem among children under five in underdeveloped nations, especially in Sub-Saharan Africa, with a prevalence rate of 40.1% (Ricci et al., 2019). In 2016, around 155 million stunted children lived in developing nations, accounting for a third of Africa (WHO, 2006a). In Ghana, the prevalence of childhood stunting was 19 per cent in 2014, albeit there were regional differences, with the Greater Accra region having the lowest prevalence of 10% and the Northern region having the highest incidence of 33%. Stunting was significantly more common in rural areas, with 22 per cent compared to 15 per cent in urban areas (Ewusi et al., 2014).

2.2.4 Costs of Malnutrition

Malnutrition, in all its forms, impacts individuals, households and nations (Nugent et al., 2020). It costs the world $3.5 trillion annually, while each person is $500. In most countries, this expense has slowed economic growth. Childhood and early adult mortality due to hunger and diet-related non-communicable diseases have also resulted in the loss of human capital (Fanzo et al., 2019; Panel, 2016). Undernutrition in children and mothers accounts for about 10% of the worldwide illness burden. Undernutrition is responsible for 45% of preventable childhood deaths. These deaths impact the nation's human capital and families (Panel, 2016). Malawi's GDP was roughly US$600 million in 2012, and undernutrition cost them more than 10%, with wasting as the main problem. Wasting also accounted for almost 13% of all fatalities among preschool children worldwide (Webb et al., 2018). According to cross-country data, stunting also produced a 1% loss in potential height, which resulted in a 2.4% reduction in wages (Panel, 2016).

On a national level, it was projected that stunting-related height loss might cost the country around 12% of GDP. In Tanzania, a simulation of lifetime earnings revealed that, if stunting is eliminated, a person's lifetime wages will increase by $539. (Panel, 2016). In Ghana, the National Development Planning Commission launched "The Cost of Hunger in Africa: The Social and Economic Impact of Child Undernutrition on Ghana's Long-Term Development (COHA)". According to the report, Ghana's economy lost $2.6 billion – or 6.4 of its GDP – due to child malnutrition. It has increased healthcare expenditures, pressures on the school system, and poorer productivity in Ghana's workforce, costing the country a lot of money (National Development Planning Commission 2012).

2.2.5 Causes of Malnutrition

The causes of malnutrition are diverse and related mainly to health, caring practices and food. Three levels of malnutrition causality have been identified: basic, underlying, and immediate; these levels are interconnected. Basic causes include resources that are accessible and the political, social, ideological, and economic setting. Immediate causes include inadequate diet and disease. The underlying causes are inadequate household food security, inadequate care, inadequate health services, and an unhealthy household environment (Blossner et al., 2005). Several studies in Africa show that micronutrient powders help alleviate malnutrition on the intermediate and underlying levels. Malnutrition is relieved by improving infant and young child feeding practices and household security through micronutrient powder use (Kyei-Arthur, 2020; McLean, 2019; Tam, 2020). This present study uses CFP, a micronutrient powder, to address malnutrition. Below is a causal framework for child malnutrition adapted from UNICEF (1990).

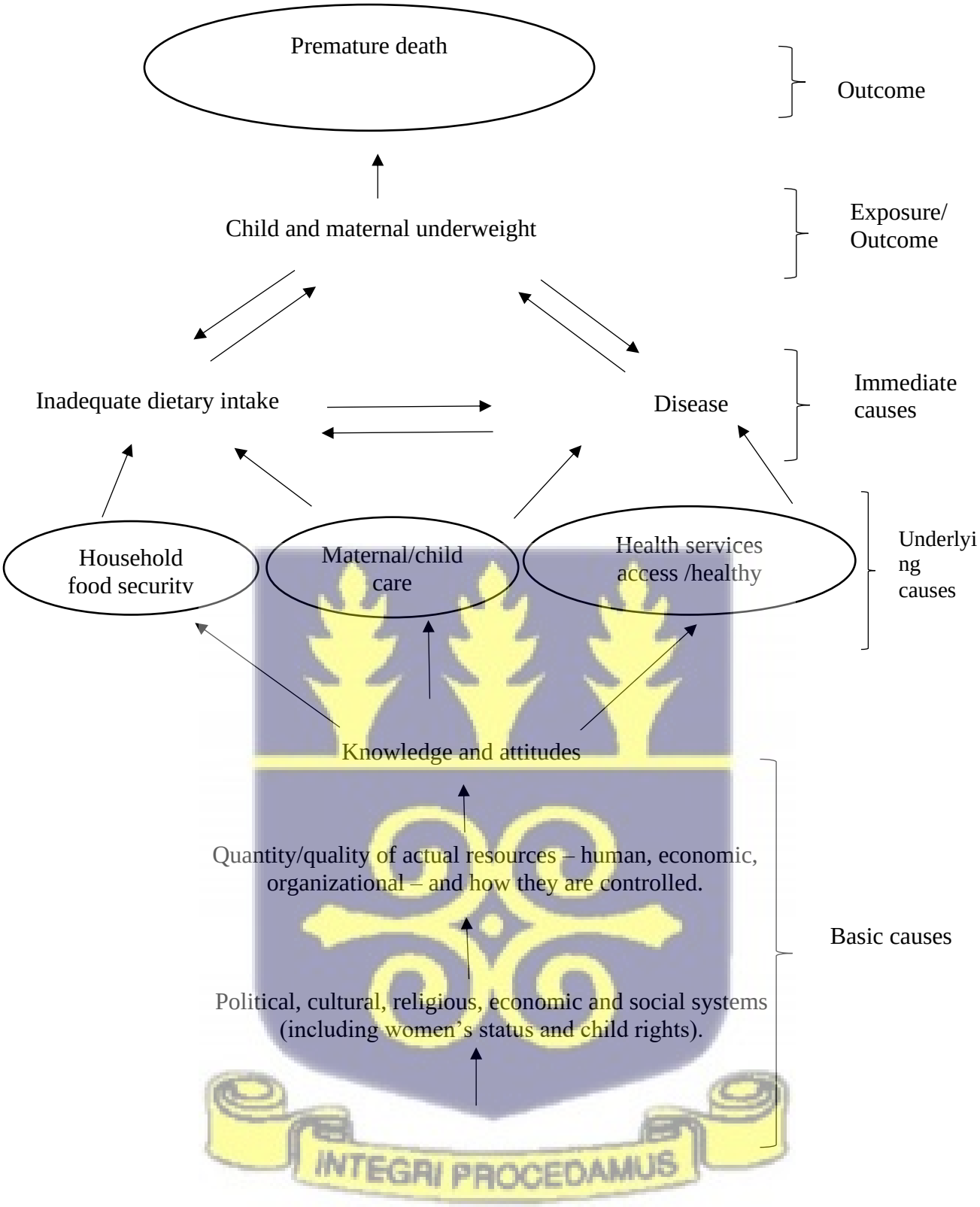

Figure 1: Causal framework for child malnutrition adapted from UNICEF (1990).

2.2.6 Micronutrient deficiencies

Micronutrients are nutrients needed in small quantities. They help produce hormones and enzymes required for the immune system's development, growth, and functioning. Micronutrient deficiencies usually occur concurrently and occur due to lack or insufficient consumption of nutrients, absorption and use of one or more minerals or vitamins (Gernand et al., 2019)

Iodine, vitamin A, zinc and iron deficiency are micronutrients of concern globally. When an individual suffers mild-to-moderate forms of these micronutrient deficiencies, they experience adverse effects on their well-being, such as poor vision, suboptimal growth, poor intellectual development and morbidity (Bailey et al., 2015). For example, adequate consumption of vitamin A in preschoolers reduces mortality due to infectious diseases (most notably measles, malaria and diarrhoea) by 23-35% (Imdad et al., 2017)

The 2020 Global nutrition report, 30% of all preschoolers have vitamin A deficiency. Between 1993 and 2005, anaemia prevalence was about 1.62 billion, about 24.8% (approximately ¼) of the world population. In 2010, it was estimated that 2.2 billion people were anaemic, approximately 32.9% of the world (Micha et al., 2020). It was also assessed in 2013 that 29% of children between 6 and 59 months in low-middle-income countries experienced vitamin A deficiency (Stevens et al., 2015). In Ghana, the overall prevalence of anaemia is 66% in children aged 6–59 months (Ewusie et al., 2014). Also, in the Ghana demographic health survey, 66% of children 6-59 months have anaemia, 27% have mild anaemia, 37% have moderate anaemia and 2% are severely anaemic (GSS et al., 2015). In 2017, it was reported that anaemia prevalence in Ghana was 35.6% (University of Ghana et al., 2017).

2.2.7 Addressing micronutrient deficiencies

Micronutrient deficiencies can be addressed. These include home fortification (point-of-use fortification), General fortification, Education to encourage a varied consumption of food, supplementation and promotion of public health measures. In point-of-use fortification, micronutrients are added to processed or cooked foods to increase the consumption of micronutrients of a specific group. The intervention has been proven to be cost-effective. It is effective for children 6-23 months because their stomach size is small, hence challenging to meet their nutrient needs. Also, their complementary foods are usually inadequate in micronutrients needed for their growth (Dary & Hurrell, 2006). Micronutrient powder is generally used in home fortification programs. It improves the micronutrient quality of diets by increasing nutrient availability. The combination of the existing diet and the micronutrient powder usually meets the individual's daily Recommended Daily Allowance (RDA) (Dary & Hurrell, 2006).

2.2.8 Micronutrient powders

They comprise a blend of several micronutrients used to prevent micronutrient deficiencies, especially among preschoolers and school-age children, through school feeding programs (Sheet, 2012). These powders were initially made to treat anaemia in young children since they struggle to swallow folic acid and iron tablets. Syrups stain the teeth, making them bulky and easily over-dosed. They are added to semi-solid or solid foods after preparation and before eating and are usually distributed in small sachets. They are used as food or food substitutes. The powders are typically odourless, tasteless, dissolvable in most warm foods, do not mix well with liquids and are colourless. The purpose of these foods is not for energy but for the recommended dietary allowance of the micronutrients per dose (Pelletier & DePee, 2019; WHO, 2016).

Evidence shows that micronutrient powders have a positive impact on nutritional anaemia. Since most people suffer from multiple micronutrient deficiencies, it has been assumed that micronutrient powders positively impact other micronutrient deficiencies. They have also been shown in specific contexts to impact stunting positively (WHO, 2016).

2.3.0 Composite food powder

In Sub-Saharan Africa, especially in poor societies, their diet comprises starchy staples, coarse grains and indigenous vegetables. Their staple foods are typically low in fat and high in fibre, contributing to people's health, although they are limited in variety. African native vegetables, like some nuts and legumes, are inexpensive and widely available sources of vitamins, particularly vitamin A, minerals, and proteins. These vegetables can be grown in the in-home gardens or wild and consumed in Ghana. In Ghana, the consumption of these vegetables is affected by the season. There are two main seasons in Ghana, rain and dry seasons. More vegetables are available for consumption during the rainy season; however, consumption reduces in the dry season. Due to climate changes in Ghana, the rainfall has diminished. Therefore, micronutrient powders like composite foods powder can be made during the rainy season for consumption during the dry season (Owusu & Waylen, 2013).

CFP comprises indigenous vegetables and legumes grown and easily found in Ghana. The powder contains dry anchovies, carrots, turkey berries and soybean; anchovies belong to the food group of fish, animal and animal products. Carrot and Turkey berries also belong to fruits and vegetables food groups. Soybean belongs to the legumes, oil and nuts food group.

2.3.1 Carrots

Carrot (*Daucus carota*) is a root vegetable cultivated in almost every part of the world and consumed in processed and raw forms. It is an excellent source of iron, dietary fibre, complex carbohydrates, pectin vitamins such as β-carotene and minerals. Carrot has moisture content

of 88%, protein (1%), fat (0.2%), carbohydrate (7%), crude fiber (3%), total ash (1.1%), iron (2.2 mg/100 g) and carotenes (5.33 mg/100 g). (Ergun, & Süslüoğlu, 2018; Gopalan et al., 2014). Dried carrot also contains 8.78±0.17% moisture, 5.05±0.32% ash, 6.16±0.06% protein, 2.43±0.12% and 24.66±0.83% crude fibre (Gazalli et al., 2013). Carrots have been shown to have anti-inflammatory, anti-diabetic, hypoglycemic, anticancer, and anti-oxidative effects. It also contains beta-carotene, considered provitamin A (Ergun & Süslüoğlu, 2018).

2.3.2 Soybean

Soybean (*Glycine max*) is a legume that provides most of the essential amino acids (except cysteine and methionine). It provides the least expensive form of protein. It contains essential fatty acids, such as monounsaturated and polyunsaturated fatty acids, which reduce the risk of cardiovascular diseases. Soybean is rich in minerals and vitamins (such as iron, vitamins B1, B2 & B6). Soybean is hematinic and essential in the formation of red blood cells (Hassan, 2013). The moisture content of dried soybean is 5.2g/100g; protein is 36.18g/100g, crude fat is 24.1g/100g, crude fibre is 4g/100g, 5.41g/100g and carbohydrate 25.31g/100g and ash is 4.41g/100g (Rani et al., 2008). Another study showed that dried soybeans contain 37.69% protein, 28.2% crude fat, 4.29% ash, 8.07% moisture, 5.44% crude fibre, 16.31% carbohydrates and 16.4mg/100g (Etiosa, 2017).

2.3.3 Turkey berry

Solanum torvum is known as turkey berry, a bushy perennial plant that grows up to a height of 5m. Turkey berry is known as 'Kwahu Nsusua' or 'Abedru' or 'Kantose' in Ghana. It is usually consumed with palm nut soup, light soup, 'kontomire' stew, and many other stews in Ghana. Turkey berry prevents and treats anaemia (Agbemafle, 2019). It is an important source of vitamin C, iron, zinc, selenium, phosphorus and vitamin A, which helps treat

anaemia. It has pain-relieving, antimicrobial, antiviral, antiulcer and antioxidant (Ndebia et al., 2007; Sivapriya and Leela, 2007). Turkey berry contains 86% water, 2.3% protein, 0.28% fat, 7.03% carbohydrate, 3.99% fiber, 0.14% ash, 76.87mg/kg iron, 0.078mg/100g β-carotene (Akoto et al., 2015). Dried turkey berry contains 6.11% moisture, 12.95% protein 1.8% fat, 12.66% dietary fibre, 6.19% ash content, 0.0550mg/100ml β-carotene and 5.67mg/100g iron (Sivakumar et al., 2021).

2.3.4 Anchovies

Anchovies (*Engraulidae*) are small fishes found in saltwater that grow up to 20 cm. In Ghana, it is referred to as Amobi, Keta schoolboys, Abobi or Amoni. They have an excellent complement to the essential amino acid, lysine. Combined with cereals and legumes, they provide a nutritional balance (Sabo, 2018). They also are significant sources of other nutrients, such as Vitamin A, D, E, manganese, iron, calcium, zinc and iodine. Anchovies contain 59.72% to 70.94% moisture, 1.52% to 7.60% ash, 12.05% to 17.52% protein, 10.02% to 23.90% crude fat and 76.64mg/kg to 400.52mg/kg iron (Gencbay & Turhan, 2016). Dried anchovies have moisture content between 19.16% to 22.7%, protein 63.12 to 67.34%, ash 8.77% to 12.33% and fat 2.64% to 3.73% (Ahmad, 2018).

2.4.0 Proximate composition and micronutrients of interest

Proximate analysis is carried out on foods to obtain the major nutrient content (Md Noh et al., 2020). The macronutrients include moisture, protein, carbohydrate, fat and ash. Micronutrients are also essential in foods; their content is critical, especially when managing hidden hunger. The section contains briefs on iron and β-carotene (pro-vitamin A) micronutrients of interest.

2.4.1 Moisture

Water can be found in nearly all foods. The amount of water present is vital for several reasons. The microbial life and most enzymatic activities require water, which impacts shelf life and food stability. The amount of water in the product affects storage capacity, bulk, and transportation costs. Because water is relatively inexpensive, its quantity, particularly cheap products, is commercially significant, and regulations exist for this and other reasons (Isengard, 2001; Mathlouthi, 2001). There are two analytical methods of determining moisture in foods. These include the direct and indirect methods. The direct techniques remove water from the samples through instruments such as vacuum driers, oven driers, freeze driers and others. The air oven method is the most typical method used to determine moisture. It uses the principle of weight loss under specified conditions to assess moisture content (Isengard, 2001; Mathlouthi, 2001; Vogl & Ostermann, 2006).

2.4.2 Ash

Ash is the inorganic substance found after the organic matter has been incinerated. The ash content indicates the quality of the food sample. The higher the ash content, the higher the food quality, and the lower the ash content, the lower the quality of the food sample.

There are several methods of determining ash in food samples. The two main methods include dry ash and wet ash. Dry ash is a common and standard method of determining ash content. The food sample is ignited in a muffle furnace at 550-600℃. Then all organic compounds, volatile compounds and water evaporate or burn after this process. The ash is determined by the weight lost after ignition. Wet ash is made by oxidizing organic matter with acids and oxidizing agents, or a mixture of the two. Vegetables, for example, are frequently dried before ashing because of their high moisture content. Food with high-fat

content, like beef, will need drying and fat extraction before the product can be ashed. Ash content is expressed in two ways: wet and dry. (Ismail, 2017; Nielsen, 2017).

2.4.3 Crude fibre

It is a carbohydrate that the small intestine cannot digest. It consists of lignin, cellulose, hemicellulose, pectin and more. The fibre in the proximate analysis is represented by the cell material left after defatting the sample with petroleum ether, boiling with 1.25% acid, filtering and washing with water, boiling with 1.25% alkali, filtering and washing in alcohol again. Then the sample is ashed and weighed to determine the fibre (Nielsen, 2017).

2.4.4 Proteins

They are essential for the maintenance and growth of the body. Proteins aid in protein transport, enzyme activities and other cellular activities. Many analytical procedures have been developed to determine proteins in food (Salanta, 2020). However, there are two main methods of protein determination in foods, and these are the direct and indirect methods. The indirect method includes the Kjeldahl, modified Lowry, and Bradford methods. The nitrogen content in food is determined using the Kjeldahl method. A protein-to-nitrogen ratio for the food under consideration is used to assess the protein content.

This technique has three stages which are digestion, distillation, and titration. The first step is digestion, in which nitrogen bonds are broken and converted to ammonium at 370 °C in the presence of a catalyst. The catalysts that can be used include mercury, selenium, copper and titanium. The digested food sample is made alkaline with sodium hydroxide and then distilled for nitrogen to be removed as ammonia. In a boric acid solution, the ammonia is "entrapped." The amount of nitrogen in the solution is determined by titration with a standard hydrogen chloride solution. The nitrogen content can then calculate the protein (Nielsen, 2017).

2.4.5 Crude fat

Fat is a collection of compounds that are only slightly soluble in water but have a wide range of solubility in organic solvents. Fat is removed with an organic solvent, the fat extraction procedure's principle. Heating causes the solvent to evaporate, causing it to condense. To extract the fat, the solvent goes to the sample and soaks it. The solvent is moved to the heating flask every 30 minutes to restart the process. The amount of fat removed or lost determines the amount of fat (Nielsen, 2017).

2.4.6 Carbohydrate determination

This can be calculated by using the formula below to calculate the per cent remaining after all other components have been measured (Md Noh et al., 2020).

% Carbohydrate = 100 – (% protein + % fat + %moisture +% fibre + % ash)

2.4.7 Iron determination

Iron is determined by many methods such as the phenanthroline method for ferrous iron, phenanthroline method for total iron, ferrozine method for total iron, thiocyanate method for total iron, 2,4,6-tripyridyl-s-triazine method for total iron, colourimetric methods and more. The spectrometer is a photometer with increased accuracy and sensitivity that measures the light intensity of a solution as a function of the wavelength of light. This principle determines iron (Fidanza, 2013; Nielsen, 2017).

2.4.8 β-carotene determination

There are several methods of determining β-carotene in foods. These include high-performance liquid chromatographic (HPLC), thin-layer chromatography and mass spectrometry. HPLC is a sensitive, reliable and fast method for determining β-carotene. The food sample is saponified, and β-carotene is extracted and separated from other carotenoids on a reversed-phase HPLC column. β-carotene is then determined by measuring the absorbance at 445 nm on HPLC (Gupta, 2015).

2.5.0 The role of vitamin A and iron in nutrition and health

2.5.1 Vitamin A

Vitamin A is a general term for compounds structurally similar to retinol and performs retinol's critical functions and biological activity. Vitamin A and other occurring compounds are lipid-soluble (Russell et al., 2001). It causes xerophthalmia, bitot's spot, anorexia, a compromised function of innate and adaptive immunity and growth retardation.

These can lead to many health problems in vulnerable groups, such as young children and pregnant women, who are at the highest risk (Green & Fascetti, 2016). The sources of vitamin A are divided into two groups preformed vitamin A and provitamin A. Provitamin A (precursor forms) refers to the carotenoids that can be bio-converted to retinol in the intestines. Carotenoids are grouped into two; xanthophylls and carotenes. The carotenes are described as nonpolar hydrocarbons, including β-carotene, lycopene and α-carotene. They usually occur in yellow and green fruits and vegetables, except citrus fruits. In the diet, preformed vitamin A is found only in animal-based foods; fish, eggs, milk, breast milk and fortified foods (Gester, 1997; Rosa, 2010).

Vitamin A activity of carotenoids and retinol are different; therefore, the retinol equivalent (RE) concept was introduced. This relationship was established:

1 µg retinol = 1.0 µg RE

1 µg □-carotene = 0.167 µg RE

1 µg other provitamins A carotenoid = 0.084 RE

Hence, 1/6 of retinol is equivalent to the retinol activity of B-carotene. However, in 2000 US institute of medicine changed it to 1/12. (Russell et al., 2001).

β-carotene has the highest vitamin A activity compared to other carotenoids. Bioavailability could be one of the reasons for the variation in carotenoids. It is the amount of ingested nutrients available for utilization under normal body functions. However, bioconversion, now separated from bioavailability, is the amount of bioavailable nutrients converted to the active form of the nutrient. A new term called bioefficacy emerged. It is the amount of ingested nutrients available for use and converted to an active form for use by the body. For example, the amount of provitamin A required to yield 1 µg retinol after consumption is bio-efficacy (Green & Fascetti, 2016; Russell et al., 2001).

2.5.1.1 Vitamin A deficiency

The isotope dilution test, which measures liver vitamin A stores, is a gold standard for assessing vitamin A deficiency; the technique requires a liver biopsy (WHO, 2011). However, serum retinol concentration is usually used to measure vitamin A deficiency. When liver stores are exhausted, serum retinol is elevated and linked to vitamin A insufficiency functional outcomes. It is assessed using blood from the veins. High-pressure liquid chromatography is usually used to determine vitamin A deficiency due to its high sensitivity and specificity. Others include ultraviolet spectrophotometry and fluorescence (WHO, 2012). Serum retinol is affected by infection, liver function, and nutritional deficiencies, such as

protein and zinc deficiencies. Serum retinol concentration is a poor indicator for people with marginal to sufficient vitamin A status. It does not reflect liver vitamin A stores until the reserve drops to toxic levels. Hence, serum retinol determination should not be used in populations where inflammation or infections are common in vitamin A assessment. Although some low retinol may be attributed to other diseases, it may overstate vitamin A deficiency (WHO, 2012).

2.5.2 Iron

Humans have recognized the vital role of iron in health and disease. Since ancient times, Hindus, Egyptians, Romans, and Greeks knew iron had medicinal uses. Iron was used to treat iron deficiency, known as chlorosis, in the 17th century. It was in 1932 that evidence of the need for iron for haemoglobin synthesis was found (Liberal et al., 2020).

Iron is part of the haemoglobin and myoglobin structure needed to transport oxygen into the body. It is also required to form enzymes involved in transferring electrons, oxidation and reduction processes in the body.

Iron in the body is usually found in haemoglobin in circulating red blood cells. In food, iron is in two forms; heme and non-heme (Liberal et al., 2020). The major sources of haem iron are found in fish, poultry and meat. In contrast, non-heme iron is found in pulses, cereals, fruits, legumes and vegetables. Haem iron is highly bioavailable (15%-35%) compared to non-heme iron (2%-20%). However, non-haem iron is strongly influenced by other food components. Non-heme iron provides more iron nutrition than heme iron, despite its reduced bioavailability (Moustarah & Mohiuddin, 2019). Several dietary factors influence iron absorption. These include; polyphenols, phytic acid, peptides and calcium from partially digested proteins. Ascorbic acid and muscle tissue enhance iron absorption by reducing ferric

iron to ferrous iron and binding it to insoluble complexes for absorption (Abbaspour et al., 2014; Moustarah & Mohiuddin, 2019).

2.5.2.1 Iron deficiency

It is a condition where the body has depleted iron stores with signs of a compromised supply of iron to tissues (Abbaspour et al., 2014). It exists with or without anaemia. Iron deficiency without anaemia may cause functional changes, but the change1s may occur with the development of anaemia. Even mild forms of iron deficiency anaemia are associated with cognitive development, immunity and human resource. Iron deficiency and anaemia are also associated with learning capacity, morbidity, comorbidities and mortality (Abbaspour et al., 2014; WHO, 2001). Iron deficiency during childhood is the root cause of neurological defects (Cusick et al., 2018). Iron deficiency occurs when the body demands increase through pregnancy, excess blood loss, menstruation, rapid growth and pathologic infections. Also, iron deficiency occurs when there is inadequate consumption of iron, malabsorption, low consumption of bioavailable iron, etc.

This deficiency affects 20-50% of people globally, hence the most common nutritional deficiency in the world. It can cause low birth weight in babies, premature birth, increased infections and death in children. It contributes to 20% of all maternal deaths globally (WHO, 2001).

2.5.1.2 Nutrient-nutrient interactions

Vitamin A and Iron: Vitamin A and iron deficiencies usually coexist in low- and middle-income countries. Many nutritional studies have observed a close association between the two deficiencies. In most of the studies, the participants have low consumption of vitamin A

and iron or one or the other. They also show a high prevalence of anaemia and vitamin A deficiency in the same population. Vitamin A deficiency is involved in the inhibition of erythrocyte production. It can also increase the severity of infections and decrease iron absorption in the intestines, increasing the risk of anaemia. Studies show that addressing vitamin A deficiency also reduces nutritional anaemia; however, the mechanism is unclear. The dietary sources that protect against vitamin A deficiency and nutritional anaemia are similar. The sources include some breastmilk, fish, liver, green leafy vegetables, orange and yellow fruits except citrus fruits (Balarajan et al., 2011; Kraemer & Zimmermann, 2007; Semba & Bloem, 2002).

Vitamin C and iron: Ferric iron, which is substantially less accessible than ferrous iron, is the most common type of iron found in meals. Apart from animal tissue, vitamin C is the only food component that has been shown to aid iron absorption. Iron is absorbed mainly in the duodenum and upper jejunum, where ferrous iron can be transferred into mucosal epithelial cells of the small intestine. Iron is always oxidized to the ferric state when ingested orally from its original form. To be dissolved sufficiently for absorption, it requires an acidic stomach environment. Vitamin C can make the stomach more acidic, which prevents ferrous iron from oxidizing into ferric iron (Beck et al., 2014; Teucher et al., 2004; Zijp et al., 2000).

2.5.3 Factors that influence iron deficiency

Inadequate dietary intake in children can lead to weight loss, failure to grow, and depletion of nutritional reserves. Under these circumstances, infections are more likely to occur, be more severe, and last longer (WHO, 2011a). In impoverished nations, malaria and intestinal parasites are two of the most common causes of anaemia. In Sub-Saharan Africa, malaria is connected to a high rate of anaemia in young infants. In Sub-Saharan Africa, malaria infected

24 million children in 2018. Among these children, there were 1.8 million with severe anaemia and 12 million with mild anaemia (WHO, 2019).

Helminths: Hookworm infestation, for example, can cause iron-deficiency anaemia. Capillary blood loss occurs due to fourth-stage larvae and adult helminths eating the gastrointestinal mucosa (Bethony, 2006; Loukas et al., 2016). The hookworms appear to establish themselves as chronic infections that mount throughout the host's lifetime, resulting in cumulative blood loss and iron deficiency anaemia in the infected individual. (Bethony, 2006).

Malaria: Malaria is the most common parasitic disease that affects humans. In tropical locations, malaria is a leading cause of anaemia. Malaria infection results in haemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis, which slows anaemia recovery.

Malaria and intestinal parasites are two of the most predominate causes of anaemia in developing countries (White, 2018). Malaria is linked to a high incidence of anaemia in children in Sub-Saharan Africa. Malaria infected 24 million children in Sub-Saharan Africa in 2018. There were 1.8 million children with severe anaemia and 12 million with mild anaemia among these children (WHO, 2019). Ghana is one of the world's 15 countries with the highest malaria burden, accounting for 4% of global cases and 7% of all malaria cases in West Africa.

Dietary factors: Iron absorption might be influenced by various dietary variables. Ascorbic acid, meat, fish, and chicken are absorption enhancers; plant components in vegetables, tea and coffee (e.g., polyphenols, phytates), and calcium are absorption inhibitors. Ascorbic acid is an essential factor in increasing nonheme iron absorption. It reduces ferric iron (Fe 3+) to

ferrous (Fe2+) iron and prevents the production of insoluble and unabsorbable iron complexes.

Meat also improves non-heme iron absorption (Dasa & Abera, 2018). Zhang et al. (1990) suggested that meat enhances iron absorption through various stomach acidity and chelation processes. Meat may boost non-heme iron absorption at first by increasing gastric acid production and hence enhancing iron solubilization in the stomach. Fish and poultry are examples of meat products and have an enhancing effect on non-heme iron absorption. Plants produce bulk phenolic chemicals in leaves, stems, and flowers. They are reactive chemicals that produce iron-phenolic complexes in the intestine, making iron absorption more difficult. Tannins are big polyphenols that can cause protein precipitation. These phenolic chemicals can be found in various foods, including coffee, tea, beans, 'kontomire', and garden eggs.

2.6.0 Anaemia and types of anaemia

The World Health Organization (2011a) defines anaemia as a state in which there is a reduction in red blood cell concentration, and the blood subsequently can no longer circulate oxygen well, making it impossible for the body to meet its physiological needs. Anaemia affects about 2.2 billion people, with a global prevalence of 32.9% (Kassebaum et al., 2014). It is estimated that of the 273 million children who suffer from anaemia, 83 million are from sub-Saharan Africa (Simo et al., 2020; McLean et al., 2009). In less developed countries, nutritional disorders and infections account for most cases of anaemia. Dietary factors contributing to anaemia are iron, vitamins A and B12, folate, ascorbic acid and zinc deficiencies (Woyengo & Nyachoti, 2013). Anaemia is classified as microcytic, normocytic and macrocytic.

2.6.1 Microcytic anaemia

The red blood cells are smaller than usual in this form of anaemia. The mean corpuscular value in this type of anaemia is less than 80fl, which is not normal. It reflects the defects in haemoglobin synthesis. The causes include thalassemia, anaemia of inflammation, iron-deficiency anaemia and sideroblastic anaemia. Thalassemia is a disease of the lack of globin in the red blood cells; it requires no specific therapy. In anaemia of inflammation, iron delivery is restricted to the haem of the haemoglobin. It is treated by addressing the underlying cause. Sideroblastic anaemia is associated with defects in the synthesis of the heme group (Chulilla et al., 2009; DeLoughery, 2014).

2.6.2 Normocytic anaemia

In normocytic anaemia, the red blood cells have typical shapes and sizes, but cell production is decreased, and destruction has increased. However, it appears because of the impaired production of red blood cells, which usually accompanies chronic diseases. The causes of normocytic anaemia include endocrine deficiencies such as pituitary insufficiency, hypothyroidism and more, anaemia of inflammation, renal disease, blood loss, iron, copper, folate and vitamin B12 (Brill & Baumgardner, 2000; Cascio & DeLoughery 2017).

2.6.3 Macrocytic anaemia

This anaemia is associated with DNA synthesis and red blood cell membrane defects. Defects in the red blood cell membrane occur in the presence of hypothyroidism or liver disease. Also, the presence of reticulocytes can cause macrocytic anaemia. It is associated with aplastic anaemia, reticulocytosis, vitamin B12 deficiency, folate deficiency, renal and thyroid disease. (Cascio & DeLoughery 2017; Tefferi, 2005). Table 2.1 shows the categories of anaemia, diagnosis and complete blood count clues.

Table 2.1: Categories of anaemia, diagnosis and complete blood count clues

Categories of anaemia	Diagnosis	CBC clue
Microcytic	Iron deficiency anaemia	Thrombocytosis
		Increased RDW
	Thalassemia	Normal or elevated RDW
		Normal or elevated RBC count
	Anaemia of chronic disease	Normal RDW
Normocytic	Nutritional anaemia	Increased RDW
	Anaemia of renal insufficiency	Normal RDW
	Hemolysis	Normal or elevated RDW
		Thrombocytosis
	Anaemia of chronic disease	Normal RDW
Macrocytic	Nutritional	Marked or mild Monocytosis Increased RDW

2.7.0 Determining anaemia using complete blood count (CBC)

In many hospitals worldwide, CBC is the most often utilized test. It determines the number of blood cells and their kinds; red blood cells (RBCs), white blood cells (WBCs), and platelets. The test's primary goal is to identify anaemia, infection, and blood hemostasis. White blood cell count, platelet count, red blood cell count, haemoglobin, and hematocrit value are some of the most common assays. (Doig & Zhang, 2017). The WBC help the body fight off infections. Monocytes, eosinophils, basophils, lymphocytes and neutrophils are types of white blood cells. The absolute count of each type in the blood has clinical significance. For example, the absolute neutrophil count helps identify the specific number of white blood cells that fight infection (Doig & Zhang, 2017; Tefferi, 2005). RBC transports oxygen and carbon dioxide around the body.

The RBC contains haemoglobin, which is a protein that makes red blood cells red. The HCT value measures the amount of blood made up of RBC. Platelets aid in the prevention of bleeding by clumping together to produce blood clots that "plug" cuts (Doig & Zhang, 2017; Tefferi, 2005).

2.7.1 Complete blood count indices

2.7.1.1 Red blood cell count (RBC), haemoglobin and hematocrit

RBC, haemoglobin and hematocrit below the reference values are known as anaemia. Acute or chronic bleeding, RBC destruction, nutritional deficit (e.g., vitamin B_{12} or folate deficiency, iron deficiency), bone marrow abnormalities or damage, and chronic inflammatory disease can all contribute to this phenomenon. In comparison, red blood cell count, haemoglobin and hematocrit above the reference values are known as polycythemia. It is caused by dehydration, Lung (pulmonary) disease, a tumour of the kidney, smoking, living at a high altitude, and genetic factors are all contributing factors (altered oxygen sensing, abnormality in haemoglobin oxygen release) (Turner et al., 2021).

2.7.1.2 Mean cell volume (MCV) and mean cell haemoglobin (MCH)

MCV and MCH levels below the reference values indicate the RBCs are smaller than usual (microcytic), which might be caused by anaemia or thalassemia. RBCs are larger than usual if MCV and MCH readings are higher than the reference values (macrocytic). Anaemia (lack of vitamin B12 or folate), myelodysplasia, liver illness, and hypothyroidism are all causes of this (Maner, & Moosavi, 2019; Turner et al., 2021).

2.7.1.3 Mean cell haemoglobin concentration (MCHC)

When MCV is low, MCHC may also be low; hypochromia is evident in disorders like iron deficiency anaemia and thalassemia. High MCHC readings (hyperchromic) are seen in autoimmune hemolytic anaemia, burn victims, and hereditary spherocytosis, a rare congenital illness in which haemoglobin is highly concentrated inside the red cells (Chaudhry & Kasarla, 2017).

2.7.1.4 Red cell distribution width (RDW)

A low RDW indicates RBC size uniformity. A high RDW suggests a mixture of small and large RBCs; juvenile RBCs are often larger. For example, high variation (anisocytosis) in RBC size (together with variation in shape–poikilocytosis) causes an increase in the RDW in iron deficiency or folate or pernicious anaemia (Scher et al., 2021; Zöller et al., 2014).

2.7.1.5 White blood cell count (WBC)

Bone marrow problems or injury, autoimmune ailments, severe infections (sepsis), lymphoma or other cancers that disseminate to the bone marrow, nutritional deficiencies, and immune system diseases are all reasons for low WBC. Infection, most usually bacterial or viral, inflammation, leukaemia, myeloproliferative neoplasms, allergies, asthma, tissue death (trauma, burns, heart attack), intense exercise, or severe stress are all causes of high WBC (Scher et al., 2021; Turner et al., 2021).

2.7.1.6 Neutrophil count and per cent

Neutropenia is defined as a low absolute neutrophil count or per cent neutrophils, and several things can cause it, including a severe, overwhelming infection (sepsis), autoimmune disorders, dietary deficiencies, drug-induced reactions, immunodeficiency, myelodysplasia, Damage to the bone marrow (e.g., chemotherapy, radiation therapy), bone marrow cancer. Acute bacterial infections, a high absolute neutrophil count, and high per cent neutrophils are known as neutrophilia. Inflammation, trauma, a heart attack, burns, stress, strenuous exercise, and Cushing syndrome, some leukaemias are all possible causes (Scher et al., 2021; Turner et al., 2021).

2.7.1.7 Lymphocyte count and per cent

Autoimmune illnesses, infections, bone marrow destruction and corticosteroids are all causes of low absolute lymphocyte count and per cent lymphocytes. Acute viral infections (e.g., chickenpox, herpes, rubella), certain bacterial infections (e.g., pertussis, tuberculosis), toxoplasmosis, a chronic inflammatory disorder, lymphocytic leukaemia are causes of high absolute lymphocyte count and per cent also known as lymphocytosis (Scher et al., 2021; Turner et al., 2021).

2.7.1.8 Monocyte count and per cent

The absolute monocyte count and the percentage of monocytes in the blood are usually not medically necessary. Low counts regularly may suggest bone marrow damage or failure, as well as hairy cell leukaemia and aplastic anaemia.

High absolute monocyte count and per cent monocytes are caused by infections that last a long time (e.g., tuberculosis, fungal infection), collagen vascular disorders, monocytic or myelomonocytic leukaemia (acute or chronic) (Scher et al., 2021; Turner et al., 2021).

2.7.1.9 Eosinophil count and per cent

The absolute eosinophil count and per cent of eosinophils in the blood are generally low. A single low number, or even a few, is usually not considered medically serious. Asthma, allergies, drug reactions, parasitic infections, inflammatory disorders, some cancers, specific acute or chronic leukaemias or lymphomas, Addison disease, and connective tissue disorders are all causes of a high absolute eosinophil count (Scher et al., 2021; Turner et al., 2021).

2.7.1.10 Absolute basophil count and per cent

As with eosinophils, the absolute basophil count and per cent of basophils in the blood are usually low and not medically necessary. Allergic reactions (hives, food allergies), inflammation (rheumatoid arthritis, ulcerative colitis), certain leukaemias, and uremia are all causes of a high absolute basophil count and per cent basophils (Scher et al., 2021; Turner et al., 2021).

2.7.1.11 Platelet count

Viral infection (hepatitis, measles, mononucleosis), platelet autoantibody, drugs (such as sulfa drugs and quinidine), cirrhosis, autoimmune disorders, leukaemia, lymphoma and myelodysplasia are some causes of low platelet.

High Platelet Count, also known as thrombocytosis, causes include cancer (lung, gastrointestinal, breast, ovarian, lymphoma), rheumatoid arthritis, inflammatory bowel disease, lupus, iron deficiency anaemia, hemolytic anaemia, myeloproliferative disorder (Scher et al., 2021; Mumford et al., 2019).

2.7.1.12 Mean platelet volume

A low MPV implies that platelet size is small on average; older platelets are generally smaller than younger platelets. A low MPV could indicate that a disorder impacts platelet creation by the bone marrow. High mean platelet volume suggests a presence of a considerable number of larger and younger platelets in the blood, indicating that the bone marrow is actively generating and releasing platelets into circulation. Table 2.2 shows rats' sex-specific reference intervals for CBC analytes (Mumford et al., 2019).

Table 2.2: Gender-specific reference intervals for CBC analytes.

Analyte	Sex	Reference interval
Haemoglobin (g/dL)	Male	10.4-16.5
	Female	8.6-15.4
Hematocrit	Male	42-49
	Female	40-46
Red Blood Cell Count (10^{12} cells/L)	Male/Female	6.39-8.1
Red Cell Distribution Width %	Male	13.03-16.57
	Female	12.23-14.57
Mean Cell Volume (Fl)	Male	29.41-123.07
	Female	15.15-119.44
Mean Cell Hemoglobin (Pg)	Male	18.37-36.98
	Female	13.07-41.57
Mean Cell Hemoglobin Concentration (g/dL)	Male	25.4-80.6
	Female	21.2-95.0
Platelet Count (10^9 cells/L)	Male	170-557
	Female	148-615
Mean Platelet Volume (fL)	Male/Female	6.7-8.10
White Blood Cell Count (10^9 cells/µL)	Male	3-9.22
	Female	2.58-7.34
Neutrophil Number (10^9/L)	Male	0.28-1.43
	Female	0.19-0.91
Lymphocyte Number (10^9/L)	Male	2.45-7.66
	Female	2.09-6.39
Monocyte Number (10^9/L)	Male	0.17-0.76
	Female	0.08-0.43
Lymphocyte Percent	Male	69.68-86.89
	Female	71.77-89.94
Monocyte Percent	Male	3.77-10.82
	Female	2.10-9.34
Eosinophil Percent	Male	0.54-3.39
	Female	0.64-0.94
Basophil Percent	Male/Female	0-2
Eosinophil Number (10^9/L)	Male/Female	0.03-0.21
Basophil Number (10^9/L)	Male/Female	0-0.02
Neutrophil Percent	Male	6.14-22.95
	Female	4.27-18.48

Source: Delwatta et al., 2018 & He et al., 2017

2.8.0 Serum biochemistry

Proteins, enzymes, lipids, hormones, and other compounds are in the serum. Testing for these compounds can reveal information about an animal's organs and tissues and its metabolic status. If a test result falls outside of the reference ranges, it could suggest the presence of disease. Further examination of the test findings could reveal which organ system is impacted, as well as the kind and degree of the condition (Weatherby & Ferguson, 2002).

2.8.1 Proteins (total protein, albumin)

Albumin and globulin are the two primary forms of protein in the blood. These proteins can be analyzed separately or as part of a total protein test, which analyzes all of the samples' proteins. Albumin levels can reveal whether an animal or a person is dehydrated and information about the liver, kidneys, and digestive tract (Bobbarala et al., 2020). Dehydration, chronic liver disease, chronic active hepatitis, and cirrhosis can all cause high total protein levels in the blood. Pregnancy, cirrhosis, persistent alcoholism, prolonged immobilization, heart failure, starvation, malabsorption or malnutrition, hyperthyroidism, burns, severe skin disease, and other chronic conditions can all cause low total protein levels in the blood (Bobbarala et al., 2020).

2.8.2 Liver and muscle enzymes

There are many distinct liver enzymes, but alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are the two that occur in most tests (ALP). The first enzyme is usually seen when the liver cells are stressed or injured. The second enzyme is often enhanced when bile flow in the liver is diminished. In many mammals, elevated levels of the liver enzymes ALT and aspartate transferase (AST) suggest hepatocellular injury.

AST is more liver-specific in rats but can also be in skeletal muscle. ALP is a leaking enzyme mainly found in bone, the intestines, and the liver. Elevations can develop due to bone, intestinal, or hepatic disease or due to young animals' bone growth (Lim et al., 2007; McClatchey, 2002).

2.8.3 Bilirubin

Bilirubin is a pigment primarily produced in the liver and linked to the breakdown of haemoglobin from red blood cells. It is a component of bile that is kept in the gall bladder. Increased bilirubin levels are linked to a higher rate of RBC breakdown or a reduction in bile flow through the liver. Bilirubin results from haemoglobin metabolism in rodents; hence its presence can signify haemolysis or liver damage (Lim et al., 2007).

2.8.4 Urea and creatinine

Urea (blood urea nitrogen or BUN) and creatinine are the two most prevalent components used to monitor kidney function. It is a waste product of protein digestion formed in the liver and expelled by the kidney. Dehydration, gastrointestinal bleeding, heart disease, or primary renal disease can all cause an increase in urea. Urea decreases are linked to dehydration, liver failure, and a lower protein intake in the diet. Creatinine is a waste product of muscle metabolism eliminated by the kidney. Increased creatinine levels suggest a decline in renal function. (Walker et al., 1990). It is important to note that the liver synthesizes urea from ammonia during deamination. The quantity of urea produced depends on surplus amino acids from the diet not required for protein synthesis (Thomas & Gillham, 2013).

2.9.0 Histology of liver and kidney

2.9.1 Liver

The liver is a three-dimensional structure composed of epithelial and mesenchymal components arranged in a repeating pattern in microscopic units. It is made up of both parenchymal and non-parenchymal cells. Hepatocytes represent the parenchyma. Kupffer, satellite cells, and the endothelial cells that line the blood sinusoids are non-parenchymal. The liver cells are organized into lobules on a microscopic level. The liver's structural units are these. The central veins drain the anatomical lobules, which are hexagonal. Each hexagon's periphery comprises three buildings collectively known as the portal triad, which include the following: A branch of the hepatic artery that enters the liver is known as an arteriole. A branch of the hepatic portal vein that enters the liver is called a venule. (Krishna, 2013).

The liver performs various activities, including albumin, lipoproteins, glycoproteins, prothrombin, fibrinogen formation, vitamin storage and modification, iron storage and metabolism, drug and toxin degradation, bile production, carbohydrate metabolism, and more. The following are the most important features of protein metabolism in the liver: Amino acid deamination and transamination, followed by glucose or lipid conversion of the non-nitrogenous component of the molecule. Several enzymes involved in these pathways (such as alanine and aspartate aminotransferases) are routinely measured in serum to determine liver damage (Lim et al., 2007).

2.9.2 Kidney

The kidney's "functional units", the nephrons clean the blood of toxins and balance the constituents of the circulation to homeostatic set points through the processes of filtration, reabsorption, and secretion. The nephrons regulate blood pressure, red blood cell production, and calcium absorption. A tubule is a part of the nephron with a specific network of channels that runs through each nephron. In each nephron's glomerulus, a high-pressure capillary bed is served by an afferent arteriole. The glomerulus filters blood to generate filtrate, which the nephron tubule collects. The Bowman's capsule is the tubule's proximal end, which surrounds the glomerulus and collects filtered fluid. Filtered fluid (filtrate) collected by the Bowman's capsule passes through the rest of the tubule to the proximal convoluted tubule (PCT), the loop of Henle, and distal convoluted tubule (DCT), in that order, before emptying the nephron into common collecting ducts shared by numerous nephrons (Thomas & Gillham, 2013; Lim et al., 2007).

2.9.3 Kidney and liver tissue processing

Tissue processing refers to the steps involved in preparing an animal or human tissue for microtome section cutting, from fixation to thoroughly infiltrated with the proper histological wax (Shields & Heinbockel, 2018).

2.9.3.1 Fixing

The tissue is dissected and trimmed to approximately 1-2cm in length/width. The tissue is fixed with a formaldehyde solution known as a liquid fixing agent. The substance progressively penetrates the tissue, causing physical and chemical changes that will harden, protect and preserve the tissue from further processing. Few reagents are suitable for fixing since they must possess specific characteristics.

The most common fixative for preserving tissues that will be processed to generate paraffin slices is formalin, generally in the form of a phosphate-buffered solution. Tissues should be fixed for a long enough time for the fixative to infiltrate every part of the tissue and then for another long enough for the fixation chemical reactions to reach equilibrium (fixation time). Following fixation, the tissue sample is transferred to a tissue cassette (Dey, 2018; Shields & Heinbockel, 2018).

2.9.3.2 Dehydration

Due to the hydrophobic nature of melted paraffin wax, most of the water in the tissue must be removed before the wax can be infiltrated. In any quantity, ethanol is miscible with water. As a result, the tissue is immersed in increasingly concentrated ethanol solutions until pure, water-free alcohol is achieved. Ethanol gradually replaces the water in the tissue (Dey, 2018; Hewitson & Darby, 2010).

2.9.3.3 Clearing

A solvent entirely miscible with ethanol and paraffin wax is utilized to remove the remaining ethanol. The solvent will displace the ethanol in the tissue, which will then be replaced by molten paraffin wax. This is referred to as "clearing," and the reagent is referred to as a "clearing agent." The clearing agent removes a considerable amount of fat from the tissue, which would otherwise pose a barrier to wax penetration. A typical clearing agent is xylene (Dey, 2018; Hewitson & Darby, 2010).

2.9.3.4 Wax infiltration

The tissue can now be injected with an appropriate histological wax. Paraffin wax-based histology waxes are the most used, even though many alternate reagents have been researched and employed for this purpose throughout the years. The wax is liquid at 60°C and can penetrate the tissue; it is cooled to 20°C, where it solidifies to a consistency that allows for regular cutting of the pieces. Pure paraffin wax and various additives, such as styrene or polyethene resins, are used to make these waxes. The physical features of these wax compositions allow wax-infiltrated tissues to be sectioned down to a thickness of at least 3µm (Dey, 2018; Hewitson & Darby, 2010).

2.9.3.5 Embedding or blocking out

The tissue is shaped into a "block" that can be clamped into a microtome for section cutting once it has been extensively penetrated with wax. This is done in an "embedding centre," where molten wax is poured into a mould, and the specimen is placed within. On top of the mould, a cassette is inserted, which is then filled with more wax and set on a cold plate to harden. The block can now be removed from the mould and its corresponding cassette and set for microtomy. (Hewitson, & Darby, 2010; Shields & Heinbockel, 2018).

2.9.3.6 H&E staining

The paraffin wax must be removed before tissue staining may begin. As a result, deparaffinization is the first step in H&E staining. The paraffin can be removed effectively with xylene or xylene substitutes used in tissue processing. In most cases, three stations of clearing for three minutes each are enough to prepare the tissue for hydration and staining with aqueous haematoxylin.

Because clearing agents are insoluble in water, they are followed by anhydrous alcohol and then diluted alcohols before reaching the water. Hematoxylin and Eosin (H&E) stain cause the biopsy portion to turn pink and blue, allowing distinct sections of the cells to be identified (Feldman & Wolfe, 2014).

CHAPTER THREE

3.0 Methodology

This section describes the materials and methods used in this study.

3.1 Composite foods powder

This section discusses the materials used in preparing the food for feeding the rats and the methods in the food preparation.

3.1.1 Source of materials

Fresh samples of carrots (*Daucus carota*), turkey berry (Solanum torvum), anchovies (*Engraulidae*) and soybeans (*Glycine max*) were bought from local markets, specifically Madina and Agbobloshie markets in Accra. These local markets were chosen conveniently.

3.1.2 Samples and sample preparation

Turkey Berries: The turkey berries were sorted, manually discarding blemished ones and fresh ones grouped for processing. The berries were then washed with tap water to eliminate physical contaminants. The berries were placed in cold water, covered and boiled at (100°C) with a gas cylinder burner weighing approximately 5kg for 5 minutes, and the water was drained with a stainless-steel basket strainer. The draining lasted for about 30 minutes. The sample was allowed to dry in a hot air oven at 50 - 55°C for 10 hours and milled with a hammer mill. The dried samples were sealed using a 220V 180W impulse heat sealer machine in plastic (polyethene) bags. The sealed samples were then stored in ice chests.

Carrots: The carrots were sorted manually, discarding blemished ones. They were then washed with water and cut into smaller sizes before processing. The samples were placed in cold water and were covered and boiled at (100°C) with a gas cylinder burner weighing approximately 5kg for 5 minutes, and the water was drained with a stainless-steel basket strainer. The draining lasted for about 30 minutes. The sample was allowed to dry in a hot air oven at 50 - 55°C for 10 hours and milled with a hammer mill.

The dried samples were sealed using a 220V 180W impulse heat sealer machine in plastic (polyethene) bags. The sealed samples were then stored in ice chests.

Soybeans: The soybeans were sorted manually, removing blemished ones. They were then washed with water to eliminate any physical contaminants before processing. The samples were placed in cold water and were covered and boiled at (100°C) with a gas cylinder burner weighing approximately 5kg for 5 minutes, and the water was drained with a stainless-steel basket strainer. The sample was allowed to dry in a hot air oven at 50-55℃ for 10 hours and milled with a hammer mill. The dried samples were sealed using a 220V 180W impulse heat sealer machine in plastic (polyethene) bags. The sealed samples were then stored in ice chests.

Anchovies: Dried anchovies from the market were sorted, cleaned and the head with the gut removed. The sample was then milled with a hammer mill. The dried samples were sealed using a 220V 180W impulse heat sealer machine in plastic (polyethene) bags. The sealed samples were then stored in ice chests.

3.1.3 Preparation of composite food powder

CFP (100g) was made with dried and powdered 15g anchovies, 50g turkey berry, 10g carrot and 25g soybean. The quantities were determined based on each food item's iron and β-carotene content. The CFP was intended to meet at least half the recommended dietary allowance for an infant. The recommended dietary intake for children between 1 to 3 years is iron (7 mg) and β-carotene (600ug). Soybean, turkey berry, anchovies and carrots were mixed using a food mixer for about 5 minutes to ensure even distribution.

The samples were then placed in plain polythene and sealed using a 220V 180W impulse heat sealer machine for storage in food flasks.

3.2.0 Proximate analysis of the composite food powder

3.2.1 Moisture determination

The samples' moisture was determined in duplicate; 2 grams of the samples were weighed. An electronic balance was used to weigh the samples into covered moisture cans previously dried at 98-100℃ in an air oven and cooled in a desiccator. The cans were heated at 100℃ for 12 hours. Then cooled in a desiccator and weighed with an electronic balance. The loss in weight was expressed as a percentage.

$$\text{Moisture\%} = \frac{\text{Loss in weight on drying (g)} \times 100}{\text{Initial sample weight (g)}}$$

3.2.2 Crude fat determination

The fat content was determined in duplicate by the gravimetric Werner-Schmid process. A fat extraction thimble and a round bottom flask were weighed using a weighing balance. About 2g of the samples were weighed into a fat extraction thimble and plugged with fat-free cotton wool. The thimble with its contents was placed in an extraction tube for filtration into the round bottom flask. The Soxhlet flask was filled with petroleum ether, a little over half its volume inside the fume chamber. The extractor was assembled on the rack, the heater was turned on, and the water condenser was checked for water leaks. Reflux lasted for 3 hours. After that, the extractor was disconnected, and tongs were used to lift the thimble out of the Soxhlet flask before draining. The apparatus was reconnected to claim the ether. The round bottom flask was dried in an oven for 60℃ overnight, cooled in a desiccator and weighed.

$$\text{Petroleum ether extract (\%)} = \frac{\text{Weight of extract (g)} \times 100}{\text{Weight of dry sample (g)}}$$

3.2.3 Protein determination

The macro Kjeldahl method was based on the AOAC (2005) method. The samples were determined in duplicates. A standard 300ml Kjeldahl digestion flask was obtained, and about 2g of the sample and 30ml of concentrated sulphuric acid was weighed into the flask, respectively. The flask was placed onto the digestion rack and heated gently to prevent vigorous charring and frothing. The flask and contents were then subjected to vigorous heating for about 2 hours to obtain a clear digest. Next, the digest was cooled and transferred quantitatively into a 100ml standardized flask, and distilled water was added to make up for the mark. 10ml of this digest was pipette into a semi-micro Kjeldahl-Markham distillation apparatus, and the remaining was discarded. After which 10ml distilled water and 30ml 40% NaOH solution was added.

Evolved ammonia was steamed and distilled into a 50ml conical flask containing two drops of the double indicator and a 10ml solution of 4% boric acid. The tip of the condenser was put into the boric acid double indicator solution, and the distillation continued until there was a colour change and about three times the original volume was obtained and of the original content of the conical flask. The distillate was titrated with standard 0 in the hydrochloric acid solution until an end-point was reached.

$$\% \text{ Nitrogen(N)} = \frac{14 \times 0.1 \times \text{titre value} \times \text{dilution factor} \times 100}{1000 \times \text{wt of sample}}$$

Crude protein was determined by;

% Crude protein = % N X 6.25.

3.2.4 Dietary Fibre

The crude fibre was determined according to the methods of AOAC (2005).

Acid Digestion: Fat-free material from the Soxhlet thimble was moved into a 600ml beaker, 0.5g celite was added, and 200ml of boiling 1.25% sulphuric acid was added. The beaker was heated to a boiling point for 30 minutes. The beaker was covered with a watch glass, and distilled water was added in intervals to maintain the volume.

Filtration: By the use of a suction pump, the contents of the beaker were filtered via a Buchner funnel. The celite aids in preventing the sample from adhering to the filter. The residue was acid-free after being rinsed with hot water.

Base Digestion: The residue was transferred quantitatively into the 500ml beaker, and 200ml of boiling 1.25% (W/V) NaOH was added. The beaker with its contents was stirred while being heated for 30 minutes. A Buchner funnel was used to filter the contents of the beaker. Hot water was used to wash the filter until it was free from sodium hydroxide. Finally, the residue was rinsed with hot water and 100mL of 1% hydrochloric acid. The residue was then put quantitatively into a porcelain crucible and dried overnight at 105°C. The crucible was weighed after cooling in a desiccator.

Ignition: The weight of the residue was then recorded. The crucible and its contents were then placed in a 550°C furnace. The weight of the ash left after igniting was measured. The crude fibre content was calculated using the weight loss of the crucible and its contents after burning.

Weight of sample = a

Weight of crucible + oven-dried sample = b

Weight of crucible + ash = c

Fibre = b-c

% Crude fibre = b- c X 100

3.2.5 Ash content

Porcelain crucibles were cleaned, dried, marked, heated at 600℃ for 10 minutes in the muffle furnace and cooled in a desiccator. The crucibles were then weighed. Two grams of the sample were accurately weighed in duplicates. The porcelain crucibles and their contents were covered and ignited in a muffed furnace at 600℃ overnight until greyish-white ash resulted. The crucibles and their contents were weighed after cooling in a desiccator.

$$\text{Ash\%} = \frac{\text{Weight of ash (g)}}{\text{Weight of original sample (g)}} \times 100$$

3.2.6 Carbohydrate

Carbohydrate was determined by accounting for ash, crude fibre, protein and crude fat (Muller and Tobin, 1980), and results were recorded in grams (g).

% Carbohydrate = 100 – (% protein + % fat + %moisture +% fibre + % ash)

3.3.0 Iron content determination

The Atomic Absorption Spectrophotometry at the Ecological laboratory, University of Ghana, Legon, was used. A wet digestion method using concentrated nitric acid and hydrogen peroxide (AOAC 2000) was used to remove all organic material from the samples before analysing the individual minerals.

One gram (1g) of the material was weighed into a 250ml beaker, which was then filled with 25ml concentrated nitric acid and covered with a watch glass. In a fume chamber, the material was digested on a hot plate until all organic material was oxidized. At this stage, the solution turned yellow. 1ml of 70% perchloric acid was added with care after the sample was cooled. Digestion continued till the solution was colourless. When the solution turned black,

the heat source was removed and cooled, and then 5ml of HNO_3 and 2ml of $HCLO_4$ were added.

After digestion, 30ml distilled water was added and boiled for 10 minutes. The solution was then filtered while hot with a Whatman paper into a 100ml volumetric flask. The beaker and filter paper was washed well with distilled water. The flask was cooled, and the solution was transferred into a glass stopper bottle before analysis with atomic spectroscopy.

$$\text{Mineral (e.g. Iron)} = \frac{AAS\ reading}{1000} \times \frac{100}{1000} \times \frac{1}{\text{weight of samples}}$$

3.4.0 β-carotene determination

β-carotene analyses were determined by HPLC using revised standard operating procedures of the department of nutrition NMIMR. Two grams (2g) of the sample were weighed, and 1g of pyrogallic was added for easy grinding. About 50ml of acetone was added and mixed. The mixture was then filtered with a Whatman filter paper. 25ml of petroleum ether was also added. After that, 2ml 80% potassium hydroxide was added and kept in the dark for 12 hours. The sample was washed 11 times using a separating funnel. Sodium sulphate anhydrous was used to drain the extract into a conical flask and dried it with nitrogen. Approximately 1ml hexane was added to the dried sample and filtered through a micropore (0.45um membrane) into a vile. Approximately 20ul of the filtrate was injected into HPLC for analysis.

$$\text{Conc. of β-carotene standard (ng/µl)} = \frac{\text{Absorbance} \times 10000}{\text{Coefficient of extinction of standard}}$$

$$\text{Conc. of β-carotene (ng/µl) in each extract} = \frac{\text{Area of each extract} \times \text{Conc. of standard (ng/20µl)}}{\text{Area of standard}}$$

$$\text{Conc. of β-carotene (ng/ml) in each extract} = \frac{\text{Conc. β-carotene (ng/µl) in extract} \times \text{Recons. Vol}}{\text{Volume of extract injected into HPLC}}$$

Conc. of β-carotene (ng/ml) in each extract = Conc. of β-carotene (ng/ml) in extract×Total Vol.

$$\text{Conc. of β-carotene (ng/g)} = \frac{\text{Conc. of β-carotene (ng/ml) in each extract}}{\text{Sample weight (g)}}$$

Conc. of β-carotene (µg/g) = Conc. of β-carotene (ng/g) × 0.001

3.5.0 Animal experimentation

Sprague-Dawley rats Hsd: SD stock, either sex, age 6 - 7 weeks, 150-200 g weight were obtained and maintained in the Department of Animal Experimentation, NMIMR. The 20 rats were housed in plastic cages with softwood shavings used as bedding and fed with rodent feed pellets (AGRIFEEDS, Kumasi). The rats were given water ad libitum and maintained a temperature of 25±2 ºC, relative humidity of 60-70 per cent, and a 12:12 light: dark cycle.

3.5.2 Feeding period

The experimental design used was post-test only with a control group. The study was performed to mimic approximately 40g CFP being fed to children between 2 to 5 years. The animals were acclimatized for a week. This period helped the animals adjust to the new environment, handling and restraining techniques. All the animals were fed with the rodent feed pellet for 28 days. The rats were assigned to 2 groups comprising 6 control and 14 experimental animals. It was assumed that the CFP given to animals was toxic and the animals may die during experimentation. Hence, more animals were used in the experimental group compared to the control group. The controls were fed with rodent pellets only. Approximately 0.4g of the CFP was mixed with 12g of the mashed rodent feed for the experimental group. The feed was given *ad libitum*, the rats could choose when, how often

and how long they wished to eat, but the quantity of feed was restricted. Also, water was given to the animals *ad libitum* in their cages.

The rodent feed was weighed into a metallic feed bowl daily. The leftover was discarded, and the bowl was cleaned before the new sample. The amount of feed consumed was calculated as Feed intake = Feed given - Feed leftover. A 200ml water bottle was filled with water for the rats. The amount leftover was discarded weekly. The amount of water consumed was calculated as Water intake= Initial water given – water leftover.

The animals were weighed weekly by lifting them by the tail from their cages onto the weighing scale. The length was measured by gripping the loose skin behind the ears of the rat with a less dominant hand and holding the tail with the other hand. At the same time, an assistant measured the length from the snort to the anus. The length of the animals was measured on days 0, 7 and 15. The different groups were biologically evaluated by determining food consumption and body weight gain. The rats were humanely euthanized on day 29 using chloroform. All ethical protocols were observed during euthanization. Each animal was introduced into a plastic container containing cotton wool soaked in chloroform. Blood was collected by cardiac puncture after the animal had been euthanized. The liver and kidney were isolated and preserved in 10 % formalin for histopathological analysis. Table 3.2 shows rat groupings by experimental diet.

Table 3.2: Rat groupings by experimental diet.

Group	Number of rats	Dosing
Control	6	Rodent feed
Experimental	14	CFP (0.4g per 200g body weight)

40g of the composite foods powder will be given to children from 2 to 5 years. The average weight of a child within this age group is 20kg. Rats used in the study are between 150g to 200g. Therefore, 40g of the composite foods used for feeding children will be converted to an equivalent for the rats.

40g = 20kg child

0.4g =200g rat

3.6.0 Haematology and biochemical analysis

3.6.1 Haematology analysis

Blood was collected into EDTA-2K tubes for haematological analysis with the SYSMEX haematology auto-analyzer (Kobe, Japan). Haemoglobin, Haematocrit, Red Blood Cell Count, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration, Platelet Count, Mean Platelet Volume, White Blood cell count, Lymphocyte number, Lymphocyte per cent, Eosinophil per cent, Eosinophil number, Monocyte per cent, Monocyte number, Neutrophil number, Neutrophil per cent, Basophil number and Basophil per cent were determined.

3.6.2 Biochemical analysis

Blood was collected into plain tubes. Blood samples were centrifuged at 3 000 rpm for 5 minutes. The serum was collected for assays.

3.6.2.1 Serum biochemistry

The SELECTRA JUNIOR Version 04 autoanalyzer (Vital Scientific, Spankeren, The Netherlands) was used to perform the following biochemical assays: Aspartate aminotransferase, total protein, alanine aminotransferase, albumin, alkaline phosphatase, total bilirubin (TBIL), direct bilirubin (DBIL), urea (URE), creatinine (CR)

3.6.2.2 Serum retinol

Frozen serum samples were thawed for a minute in the dark. 120ul of serum was pipette into an Eppendorf tube, and the same volume of methanol was added. The tube with its contents was vortex for 30 seconds to denature the proteins. 500ul of hexane was added to the mixture using an Eppendorf dispenser after the vortex. The mixture was vortex again for 2 minutes to solubilize all fat-soluble compounds.

The mixture was centrifuged for 2 minutes at 1000 ×10rpm in an Eppendorf micro-centrifuge. The supernatant was then picked into another Eppendorf tube and evaporated slowly under nitrogen gas to dryness. The residue was then reconstituted with 120ul of methanol by vortexing for 15 seconds. The sample was then injected into an HPLC for analysis. The concentration of retinol was determined by comparing generated peak areas for unknowns against those of known amounts of purified standards. The standards were prepared by a series of dilutions with different quantities of the standards. The dilutions were injected into the HPLC column, where peak signals were generated to obtain the peak areas. A standard curve was generated by plotting the amount of each standard dilution on the x-axis and the corresponding peak area on the y-axis (Noguchi Memorial Institute for Medical Research, NMIMR, 1997).

3.7.0 Histopathological Examination of Liver and kidney

The isolated livers and kidneys were preserved in formalin. The samples were grossly examined, and the tissue was dissected and trimmed to approximately 1-2cm in length/width. The tissues were then placed in cassettes in a 10% neutral buffered formalin solution. The tissue samples were transferred to a tissue cassette. The tissue was immersed in a series of ethanol solutions (70% ethanol for 15 min, 90% ethanol for 15 min,100% ethanol for 15 min, 100% ethanol for 15 min, 100% ethanol for 30 min and 100% ethanol for 45 min) of increasing concentration until pure, water-free alcohol is obtained. Xylene was used to clear the ethanol by immersing the tissue in a series of solutions (xylene for 20 min, xylene for 20 min and xylene for 45 min). The tissue was embedded in paraffin. After embedding, the tissues were sectioned at 4µm using a rotary microtome. They were then mounted onto microscopic slides and then dried overnight.

The slides were identified with codes written on the frosted sides of the slides. The slides were deparaffinized and stained with hematoxylin and eosin (H&E) dyes. The slides were later observed under a light microscope.

3.8.0 Data capture and analysis

The Proximate composition of CFP, length, weight, haematological data, feed and water consumed by the animals were measured in duplicate and entered and cleaned in excel. Analysis of variance (ANOVA) using Statistical Package for Social Sciences (version 20, SPSS Incorporated, Chicago, USA) was used to determine the significance of data at 5%. The data were presented as means ± standard deviations.

Average feed and water consumed were measured in grams and millilitres, respectively. The continuous variable obtained was normally distributed. The data were presented as means ± standard deviations and analyzed with an independent t-test to determine the statistical significance between groups.

Proximate composition was determined from the CFP through laboratory proximate analysis. Using Microsoft Excel software, data from the moisture, fat, fibre, ash, iron and β-carotene were represented as mean ± standard deviation.

Weight was measured in grams; the length was measured in centimetres for each animal. The continuous variable obtained was normally distributed and presented as means± standard deviations. The significant difference in means between the groups was determined by independent t-test.

There were 19 haematological indices measured for each rat in this study. The continuous variable obtained was normally distributed. The data were analyzed with an independent t-test to determine the statistical significance between groups.

Vitamin A status was determined using data from serum retinol. Data obtained from the serum retinol analysis were continuous variables analyzed as the haematological indicators.

There were eight serum biochemical parameters measured for each rat in this study. The continuous variable obtained was normally distributed. The data were analyzed with an independent t-test to determine the statistical significance between groups.

CHAPTER FOUR

4.0 Results

4.1 The iron and β-carotene content of food items for the CFP

The CFP contains anchovies, turkey berries, carrots and soybean. Table 4.1 shows that carrot has the highest amount of β-Carotene (2.3mg), followed by turkey berry (0.026mg). The β-carotene content for anchovies and soybean was not detected. Anchovies (6.2mg) had the highest iron content, followed by soybean (1.2mg), turkey berry (0.8mg) and carrot (1.2mg).

Table 4.1 Iron and β-carotene content of food items used for the CFP

Food items	Iron (mg)	β-Carotene (mg)
Anchovies	6.20	-
Turkey berry	0.80	0.026
Carrot	0.20	23
Soybean	1.20	-

4.2 Proximate composition, iron and carotene content of CFP.

Table 4.2 shows the proximate composition of the CFP and rodent feed with some micronutrients of interest (iron and β-carotene). 100g of the CFP contains 31.96 ± 0.00 g carbohydrate followed by 22.30 ±0.00 g protein, 14.01 ± 0.86 g fat, 12.80 ± 0.00 g moisture and 5.00±0.21g ash in decreasing order. It also contains 18.00 ± 0.00 mg/100g β-Carotene and 13.20 ± 0.00 mg/100g iron. The rodent feed contains 17.03 ± 0.01 g/100g moisture, 15.62 ± 0.78 g/100g of fat, 13.18 ± 0.01 g/100g of protein, 33.28 ± 0.00 g/100g of carbohydrates, 20.91 ± 0.15 mg/100g of ash and 11.65 ± 0.71 mg/100g iron.

Table 4.2: Proximate composition, iron and β-carotene of rodent feed and CFP

Parameters	Rodent feed (Mean±SD)	CFP (Mean±SD)
Proximate composition		
Energy	326.95 ± 5.86	343.09 ± 6.97
Moisture	17.03 ± 0.01	12.80 ± 0.00
Crude fat	15.62 ± 0.78	14.01 ± 0.86
Crude fibre	-	6.40 ± 0.99
Protein	13.18 ± 0.01	22.30 ± 0.00
Carbohydrates	33.28 ± 0.00	31.96 ± 0.40
Ash	20.91 ± 0.15	12.54 ± 0.53
Micronutrients		
β-Carotene	-	18.00 ± 0.00
Iron	11.65 ± 0.71	13.20 ± 0.00

Proximate composition on dry matter basis (g/100g), β-Carotene (mg/100g) and iron (mg/100g)

4.3 Mean feed and water consumed by SD rats.

Table 4.3 shows the mean water and feed consumed after four weeks of intervention feeding. The mean feed consumed for the control group was 12.16±0.33 g, while that of the experimental group was 12.25±0.25 g. There was no significant difference between the control and experimental groups for feed consumed. The mean water consumed for the control group was 22.29±1.55 g, while that of the experimental group was 20.61±1.79 g. There was no significant difference between the two groups for water consumed.

Table 4.3: Mean feed and water consumed by the rats according to groups

Parameter	Mean±SD	P-Value
Feed		
Control	12.16 ± 0.33	0.29
Experimental	12.25 ± 0.25	
Water		
Control	22.29 ± 1.55	0.92
Experimental	20.61 ± 1.79	

Values are means ± standard deviations. Statistical significance was set at p=0.05.

4.4 Growth performance of the SD rats

Table 4.4 shows the mean length and weight after four weeks of intervention feeding. The mean length for the control group was 19.03 ± 1.28 g, while that of the experimental group was 19.13 ± 1.00 g. There was no significant difference between the control and experimental groups for the length of the rats. The mean weight of the control group was 161.37 ± 8.83 g, while that of the experimental group was 166.77 ± 7.91 g. There was no significant difference between the two groups in the weight of the rats.

Table 4.4 Mean weight and length of rats

Parameters	Mean±SD	P-Value
Length		
Control	19.03 ± 1.28	0.78
Experimental	19.13 ± 1.00	
Weight		
Control	161.37 ± 8.83	0.74
Experimental	166.77 ± 7.91	

Values are means ± standard deviations. Statistical significance was set at p=0.05.

The growth pattern of the rats is presented in figures 3 and 4. Figure 3 shows the length of the control and experimental group. It can be observed from the plot that both groups increased in length throughout the study period. Although the experimental group had a higher increase in length, it was not significant. Figure 4 shows the weight of the rats fed for the control and the experimental group. It can be observed from the study that the experimental group decreased in weight; however, a sharp increase was observed from day 21 to 28.

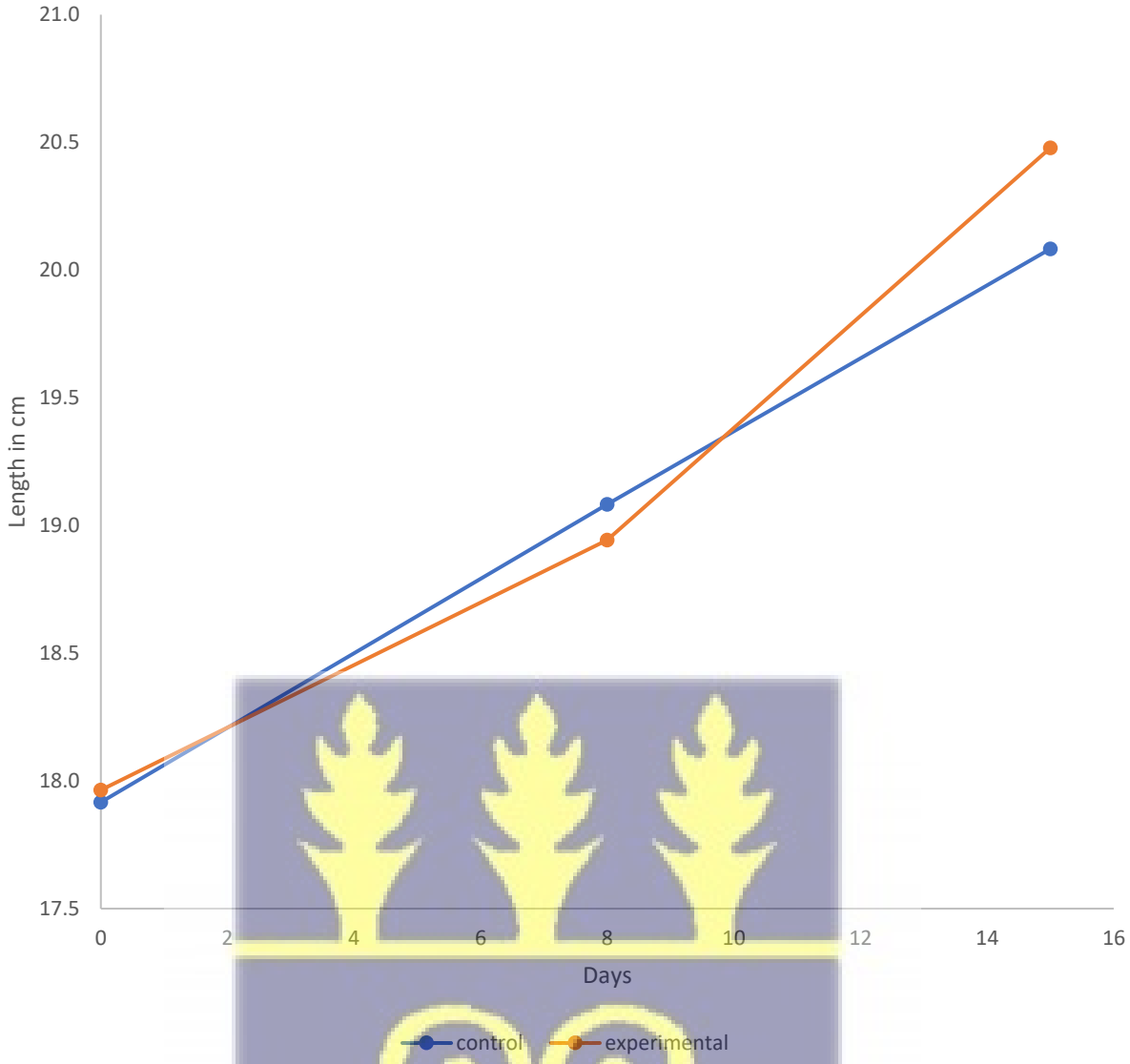

Figure 2: A scatter plot showing the length of rats throughout intervention feeding

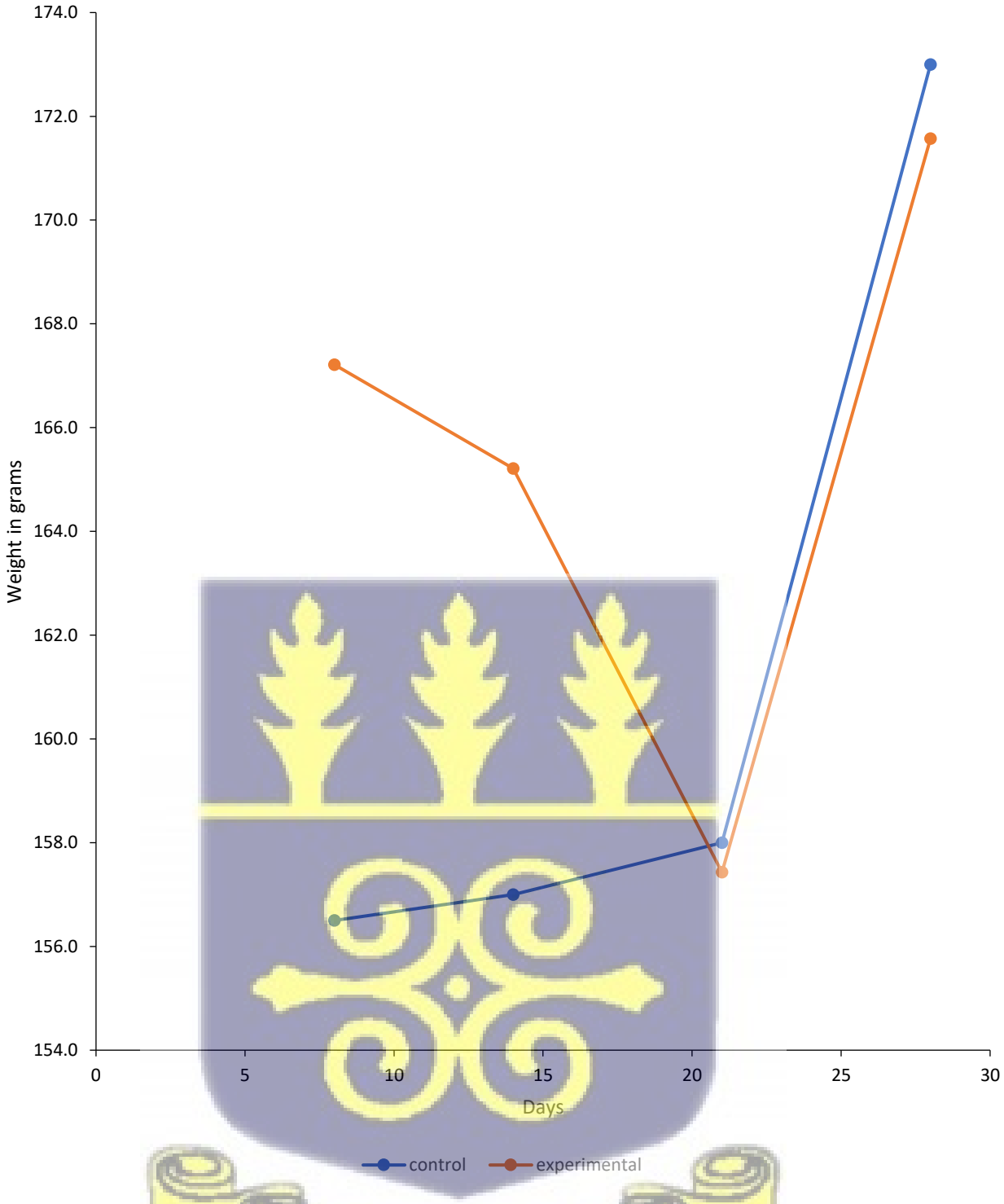

Figure 3: A scatter plot showing the weight of rats throughout intervention feeding

4.5 Haematological profile of the rats

Table 4.5 shows the red blood cell and platelet indices after four weeks of intervention feeding. The Red blood cells count for the control group was 8.20 ± 0.65 10^{12} cells/L, while that of the experimental group was 7.76 ± 1.17 10^{12} cells/L. The haemoglobin for the control group was 13.97 ± 0.76 g/dl, while that of the experimental group was 13.26 ± 1.74 g/dl. The haematocrit for the control group was 0.45 ± 0.02, while that of the experimental group was 0.43 ± 0.06. The mean cell volume for the control group was 55.07 ± 1.95 Fl, while that of the experimental group was 54.80 ± 1.38 Fl. The mean cell haemoglobin for the control group was 17.08 ± 0.66 Pg, while that of the experimental group was 17.15 ± 0.50 Pg.

There was no significant difference between the control and experimental groups for red blood cells count, haemoglobin, haematocrit, mean cell volume and mean cell haemoglobin values. The mean platelet volume for the control group was 7.98 ± 0.30 fL, while that of the experimental group was 7.80 ± 0.22 fL. There was also no significant difference between the control and experimental groups for mean platelet volume and count.

Table 4.5 Mean haematological indicators by the rat groups

Parameters	Mean± SD	P-Value
Red blood cells count (10^{12} cells/L)		
Control	8.20 ± 0.65	0.33
Experimental	7.76 ± 1.17	
Haemoglobin (g/dL)		
Control	13.97 ± 0.76	0.20
Experimental	13.26 ± 1.74	
Haematocrit		
Control	0.45 ± 0.02	0.17
Experimental	0.43 ± 0.06	
Mean cell volume (Fl)		
Control	55.07 ± 1.95	0.38
Experimental	54.80 ± 1.38	
Mean cell haemoglobin (Pg)		
Control	17.08 ± 0.66	0.37
Experimental	17.15 ± 0.50	
Mean cell haemoglobin concentration (g/dL)		
Control	31.0 ± 0.50	0.44
Experimental	31.28 ± 0.67	
Mean platelet volume (fL)		
Control	7.98 ± 0.30	0.45
Experimental	7.80 ± 0.22	
Platelet count (10^9 cells/L)		
Control	584.67 ± 397.94	0.67
Experimental	642.50 ± 360.03	

Values are means ± standard deviations. An Independent t-test was used to determine the statistical significance.

4.6 Haematological profile of the rats continuation

Table 4.6 shows the white blood cell indices after four weeks of intervention feeding. The Lymphocyte per cent for the control group was 84.00 ± 6.36 %, while that of the experimental group was 75.17 ± 7.37 %. The number of lymphocytes for the control group was 7.05 ± 3.77 10^9/L, while that of the experimental group was 6.04 ± 2.42 10^9/L.

Neutrophil per cent for the control group was 12.32 ± 5.54 %, while that of the experimental group was 19.70 ± 8.58 %. The neutrophil number for the control group was 0.85 ± 0.47 10^9/L, while that of the experimental group was 1.64 ± 0.86 10^9/L. Monocyte per cent for the control group was 1.80 ± 0.93 %, while that of the experimental group was 2.29 ± 1.75 %. The number of monocytes for the control group was 0.14 ± 0.10 10^9/L, while that of the experimental group was 0.19±0.15 10^9/L. Eosinophil per cent for the control group was 1.37 ± 0.65 %, while that of the experimental group was 2.16 ± 0.62 %. The number of eosinophils for the control group was 0.10 ± 0.04 10^9/L, while that of the experimental group was 0.32±0.54 10^9/L. Basophil per cent for the control group was 0.52 ± 0.21 %, while that of the experimental group was 0.49±0.40%. The number of basophils for the control group was 0.04 ± 0.01 10^9/L, while that of the experimental group was 0.03±0.02 10^9/L. No significant difference was observed across the control and experimental groups for Lymphocyte per cent and number, Eosinophil per cent and number, monocyte number and per cent, neutrophil per cent and number and basophil number and basophil per cent.

Table 4.6 Mean haematological indicators by the rat groups continuation

Parameters	Mean± SD	P-Value
White blood cell count		
Control	8.20 ± 3.83	0.48
Experimental	7.51 ± 3.12	
Lymphocyte per cent		
Control	84.00 ± 6.36	0.88
Experimental	75.17 ± 7.37	
Lymphocyte number (10^9/L)		
Control	7.05 ± 3.77	0.113
Experimental	6.04 ± 2.42	
Neutrophil per cent		
Control	12.32 ± 5.54	0.36
Experimental	19.70 ± 8.58	
Neutrophil number (10^9/L)		
Control	0.85 ± 0.47	0.25
Experimental	1.64 ± 0.86	
Monocyte per cent		
Control	1.80 ± 0.93	0.37
Experimental	2.29 ± 1.75	
Monocyte number (10^9/L)		
Control	0.14 ± 0.10	0.47
Experimental	0.19 ± 0.15	
Eosinophil per cent		
Control	1.37 ± 0.65	0.91
Experimental	2.16 ± 0.62	
Eosinophil number (10^9/L)		
Control	0.10 ± 0.04	0.21
Experimental	0.32 ± 0.54	
Basophil per cent		
Control	0.52 ± 0.21	0.38
Experimental	0.49 ± 0.40	
Basophil number (10^9/L)		
Control	0.04 ± 0.01	0.57
Experimental	0.03 ± 0.02	

Values are means ± standard deviations. An Independent t-test was used to determine the statistical significance.

4.7 Serum retinol concentration of the rats

The serum retinol concentration of the rats after four weeks of intervention feeding was generally low among the experimental and control groups. The control group had 0.002 ± 0.001 10^3 µg, and the experimental group had 0.002±0.001 10^3 ug. There was no significant difference (p-value = 0.07) between the control and experimental groups.

4.8 Serum biochemistry of the rats

Table 4.7 shows the serum biochemistry after four weeks of intervention feeding. The urea value for the control group was 7.24±1.02 mmol/L, while that of the experimental group was 7.96±1.16 mmol/L. The creatinine for the control group was 71.40±10.90 µmol/L, while that of the experimental group was 82.82±5.74 µmol/L. The total bilirubin for the control group was 10.17±2.56 µmol/L, while that of the experimental group was 18.69±7.61 µmol/L. The direct bilirubin for the control group was 0.77±0.55, while that of the experimental group was 0.96±1.51. The alanine aminotransferase for the control group was 43.00±15.57 µ/L, while that of the experimental group was 39.46±26.03 µ/L. The aspartate aminotransferase for the control group was 218.00±67.99 U/L, while the experimental group was 217.83±187.41 U/L. The alkaline phosphatase for the control group was 335.17±124.13 U/L, while the experimental group was 313.17±69.71 U/L. The total protein for the control group was 63.17±5.60 g/L, while that of the experimental group was 69.75±6.18 g/L.

The albumin for the control group was 28.33±1.86 g/L, while that of the experimental group was 29.50±2.28 g/L. No significant difference was observed across the control and experimental groups for urea, creatinine, direct bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin except total bilirubin. There was a significant difference between the control and experimental groups for total bilirubin.

The total bilirubin for the control group was 10.17±2.56 µmol/L, while that of the experimental group was 18.69±7.61 µmol/L.

Table 4.7: Serum biochemistry of the rats

Parameters	Mean±SD	P-Value
Urea (mmol/L)		
Control	7.24±1.02	0.608
Experimental	7.96±1.16	
Creatinine (µmol/L)		
Control	71.40±10.90	0.34
Experimental	82.82±5.74	
Total bilirubin (µmol/L)		
Control	10.17±2.56	0.001
Experimental	18.69±7.61	
Direct bilirubin (µmol/L)		
Control	0.77±0.55	0.543
Experimental	0.96 ± 1.51	
Alanine aminotransferase (µ/L)		
Control	43.00 ± 15.57	0.56
Experimental	39.46 ± 26.03	
Aspartate amino transferase (U/L)		
Control	218.00 ± 67.99	0.15
Experimental	217.83 ± 187.41	
Alkaline phosphatase (U/L)		
Control	335.17 ± 124.13	0.06
Experimental	313.17 ± 69.71	
Total protein (g/L)		
Control	63.17 ± 5.60	0.45
Experimental	69.75 ± 6.18	
Albumin (g/L)		
Control	28.33 ± 1.86	0.53
Experimental	29.50 ± 2.28	

Values are means ± standard deviations. An Independent t-test was used to determine the statistical significance.

4.9 Histopathological examination of kidney and liver

The histopathological examination of the kidneys and liver did not reveal any abnormalities (Figures 1 and 2). Figure 1 shows the kidney photomicrographs of the control (A) and experimental group (B). The renal tubules, Bowman's capsule and glomeruli were normal. The glomerulus and bowman space were well defined. No pathological features were observed in both the control and experimental groups. However, there was increased cell activity in the bowman capsule, increased vacuolization and overall increased cell activity in the experimental group. In figure 2, photomicrographs A and B show the liver sections of the rats fed with the control and experimental feeds. In both images, there was typical lobular architecture with no inflammation. Even distribution of the hepatocytes. No necrosis or lymphocytes was found in both the control and experimental group. The overall architecture was preserved with no fatty changes. However, increased cellular activity was observed in the experimental groups. Although the hepatocytes were evenly distributed, they were more in the experimental group than the control group.

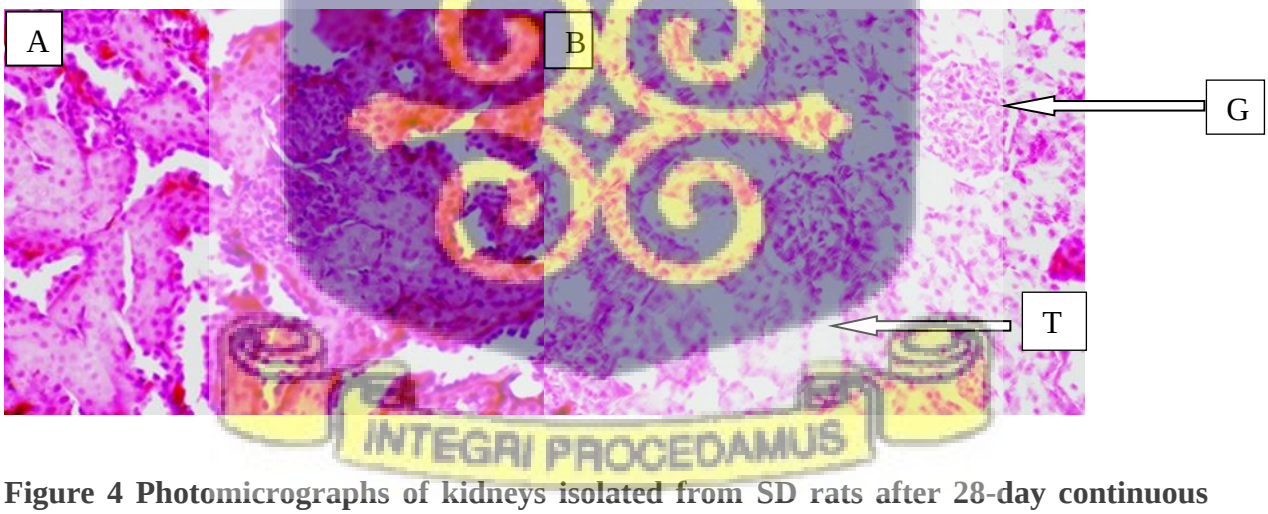

Figure 4 Photomicrographs of kidneys isolated from SD rats after 28-day continuous administration of (A) control feed only, (B) 0.4g CFP. The arrow (T) shows normal renal tubule, and arrow (G) shows normal Glomerulus but increased cell activity (H&E staining, 100×).

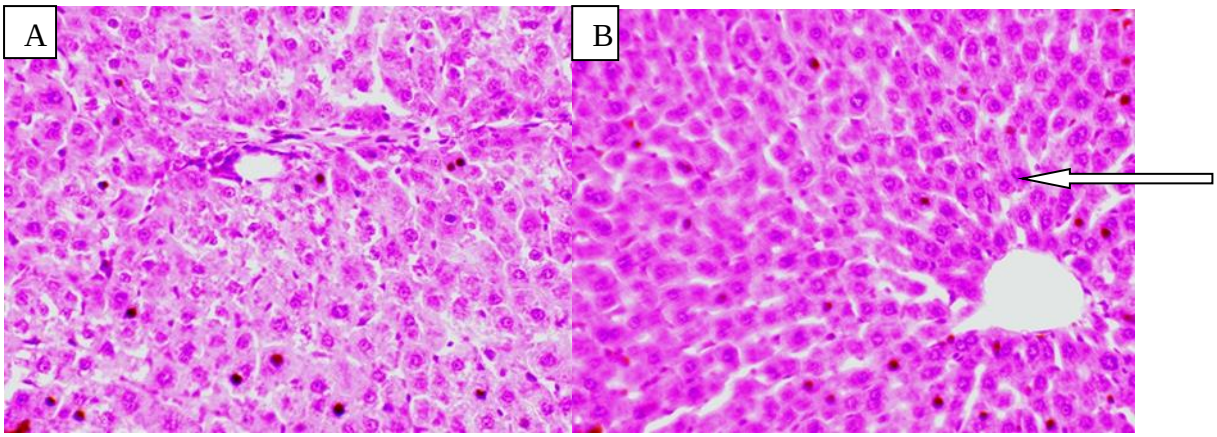

Figure 5 Photomicrographs of livers isolated from SD rats after 28-day continuous administration of (A) control, (B) 0.4g CFP. The arrow shows the hepatocyte, which is increased and evenly distributed (H&E staining, 100×).

CHAPTER FIVE

5.0 DISCUSSION

The study aimed to assess the effect of the composite foods powder on anaemia, vitamin A levels, growth status and toxicological effect in Sprague-Dawley rats. This was a formative study to extend it to human studies. This chapter discusses the findings from the data obtained. The implications of the results are also mentioned.

5.1 Iron and vitamin A content of the composite food powder

Iron is part of the haemoglobin and myoglobin structure needed to transport oxygen into the body. It is also required to form enzymes to transfer the body's electrons, oxidation and reduction processes (Cerami, 2017). In this present study, the iron content of the CFP was 13.20 ± 0.00 mg/100g. This was lower than the iron content (18.81 mg/100g) of an earlier study in Nigeria (Verem et al., 2021). In that study, the proximate, functional and chemical properties of a composite flour made from wheat (60%), soy (20%), and moringa leaf (20%) were used to improve cereal-based products (Verem et al., 2021). Another study conducted in Uganda developed a composite flour from millet grains, pumpkin seeds, carrots, cowpea leaves and skimmed milk powder. The study showed an iron content of 3.6mg/100g, which was not lower than the present study. A possible reason for the observed difference could be the different components of the food products. β-Carotene is an antioxidant, and its anti-inflammatory effects help to protect cells from damage. The bioavailability of β-carotene in food and formulations varies (Kawata et al., 2018). The present study's β-Carotene content was 18.00 ± 0.00 mg/100g. This study was not consistent with a study conducted in Uganda. That study developed a composite flour from millet grains, pumpkin seeds, carrots, cowpea leaves and skimmed milk powder.

The study showed an iron content of 3.6mg/100g, which was not lower than the present study. A possible reason for the observed difference could be the different components of the food products.

5.2 Growth and growth determinants of SD rats

In this study, growth was determined by weight and length. Weight and length increased during the experimental period. Growth was operationalized in this study by finding the growth rate (%) of the length and weight of the animals (see Appendix B). The control and experimental group had moderate growth rates. However, there was no significant difference between the experimental and control groups for weight and length. This result was consistent with a study conducted for 6,279 SD rats to observe the weight of the animals and organs at different ages. It was observed that the animals increased in weight from 13 weeks to 104 weeks (Piao et al., 2013). Another study conducted with protein-deficient diets supplemented with *moringa oleifera* leaf meal showed a decrease in the weight of Wistar rats after experimental feeding (Mhlomi et al., 2022). A possible reason for the observed difference could be attributed to the number of weeks of experimentation; a more prolonged investigation and the composition of the diet given time may influence the study's outcome.

5.3 Serum retinol concentration of SD rats

The amount of carotenoids in the diet plays a vital role in determining the quantity of absorbed carotenoids. Thus, although all carotenoids are derived from the diet, the amount and type absorbed in serum and other tissues are species-dependent characteristics (Institute of Medicine (US) Panel on Micronutrients, 2001). The serum retinol concentration was 0.002 ± 0.001 10^3 ug for the control group, and the experimental group had 0.002±0.001 10^3 ug.

There were no significant differences between the control and experimental groups. This result was consistent with a study conducted to determine the effect of citric pectin on β-carotene bioavailability in rats. That study used 30 weaned rats fed with a composition of citric pectin, soy oil, and 24 ug/g β-carotene ration, while the control group received the same feed but without the citric pectin. After feeding, that study showed no significant difference between the control and experimental groups (Zanutto et al., 2002). A possible reason could be the internal homeostatic control of serum retinol concentration or the diet composition used in experimental feeding.

5.4 Effect of CFP on haematological profile of the rats

Red blood cell indices such as red blood cell counts (RBC), haematocrit and haemoglobin concentration (Hb) are crucial indications of a red blood cell's functioning status. Hb concentration shows the oxygen-carrying capacity of the blood, and haematocrit shows the proportion of blood that comprises cellular constituents and the proportion that is plasma. Red blood cell counts represent the number of red blood cells per unit volume of circulating blood. The RBC, Hb and haematocrit were within the reference values reported for these parameters in the literature (He et al., 2017; Delwatta et al., 2018). These were the findings in this study for RBC (8.20 ± 0.65 and 7.76 ± 1.17 10^{12} cells/L), Hb (13.97 ± 0.76 and 13.26 ± 1.74 g/dL) and haematocrit (0.45 ± 0.02 and 0.43 ± 0.06). The values for RBC were 6.39 – 8.01, Hb was 12.9 – 15.9, and haematocrit was 0.40 – 0.49. It was also observed for haematocrit, RBC and Hb that there were no significant differences between the control and experimental groups. A study conducted in South Africa with protein-deficient diets supplemented with moringa oleifera leaf meal showed no significant difference between the control and experimental groups for haematocrit and RBC. That study observed a significant

difference between the control and experimental groups for Hb (Mhlomi et al., 2022). A possible reason could be the composition of diets used in feeding.

The mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) calculated from the RBC, haematocrit, and Hb are useful in morphologically elucidating and classifying anaemia. They represent an estimate of the changes in size and haemoglobin content of individual red blood cells. In this study, MCH (17.08 ± 0.66 pg and 17.15 ± 0.50 pg), MCV (55.07 ± 1.95 and 54.80 ± 1.38), and MCHC (31.0 ± 0.50 and 31.28 ± 0.67) were similar to a study conducted to determine reference values for haematological parameters for SD rats by Delwatta et al., (2018). MCH for Delwatta et al. (2018) was 13.07 – 41.57 pg, MCV was 15.15 – 123.07 fl, and MCHC was 21.16 – 95.0 fl. There was no significant difference between the experimental and control groups for MCV, MCH and MCHC. This shows that both control and experimental groups had normal red blood indices. This could be attributed to the nutrient content of rodent feed and CFP, especially the high ash content.

White blood cell count (WBC) represents an animal's health status in connection to its response and adjustment to harmful chemicals, stress, and deprivation; the indices are useful for verifying or ruling out a provisional diagnosis, determining prognosis, and guiding therapy. The WBC could also offer data about the severity of an agent, the virulence of an infectious organism, a host's sensitivity, and the kind, severity, and length of a disease process. In this present study white blood cell count (8.20 ± 3.83 and 7.51 ± 3.12), lymphocyte per cent (84.00 ± 6.36 and 75.17 ± 7.37) and number (7.05 ± 3.77 and 6.04 ± 2.42), basophil per cent (0.52 ± 0.21 and 0.49 ± 0.40) and number (0.04 ± 0.01 and 0.03 ± 0.02), eosinophil per cent (1.37 ± 0.65 and 2.16 ± 0.62), monocyte number (0.14 ± 0.10 and 0.19 ± 0.15) and per cent (1.80 ± 0.93 and 2.29 ± 1.75), neutrophil per cent (12.32 ± 5.54 and 19.70 ± 8.58) and number (0.85 ± 0.47 and 1.64 ± 0.86) were similar to studies conducted to

determine reference values for haematological parameters for SD rats. (He et al., 2017; Delwatta et al., 2018). The reference values for white blood cell count were 2.58 - 9.22 10^9/L, lymphocyte per cent was 69.68 – 89.94 % and number was 2.09 – 7.66 10^9/L, basophil per cent was 0 – 2 % and number was 0 – 0.22 10^9/L, eosinophil per cent was 0.54 – 4.29 %, monocyte number was 0.08 – 0.76 10^9/L and per cent was 2.10 – 10.82 %, neutrophil per cent (4.27 – 22.95 %) and number (0.19 – 1.43 10^9/L). However, eosinophil number (0.10 ± 0.04 and 0.32 ± 0.54) was not consistent with those studies (0 – 0.02 10^9/L). This could indicate parasitic infections, an animal's inflammatory disorder, or a different study environment. The histological study did not show inflammation or infection. The difference can be attributed to the micro-and macro-living environments of the animals, the water source and supply, bedding in the cages and cleaning of the cages number of samples and the method of determination of the parameters.

Platelets, also known as thrombocytes, are a haematological indicator frequently used to identify agglutination and foreign substance attacks. Platelets have a role in innate immunity in a variety of ways. For example, platelets have an antibacterial function and interact with bacteria, viruses, and parasites. Platelet activation and secretion of antimicrobial peptides are induced by bacterial contacts with platelets, which contain many proinflammatory cytokines that control the inflammatory response. In this study, there was no significant difference between the groups for both platelet count and mean platelet volume. The results for platelet were not similar to those by He et al. (2017) but similar to Delwatta et al., 2018. This could be due to changes in the animals' macro and micro living habitats, the water source and supply, cage bedding and cleaning, sample size, and the parameter determination method.

5.6 Effect of CFP on biochemical profile of the rats

The serum biochemistry test offers information about the body's organs and tissues and the animal's metabolic state. For example, the primary indicator of renal function is creatinine, a blood metabolic product of muscle creatine phosphate. It's a product that has to do with muscles. Individuals' hydration and nutritional condition are assessed using total serum proteins. Albumin also serves as a binding and transport protein for various substances, such as bilirubin and hormones. The result in this present study the results for urea (7.24 ± 1.02 mmol/L and 7.96 ± 1.16 mmol/L), albumin (28.33 ± 1.86 g/L and 29.50 ± 2.28 g/L) and total protein (63.17 ± 5.60 g/L and 69.75 ± 6.18 g/L) were similar to a study conducted to determine reference values for biochemical parameters of SD rats (urea is 4.32 – 12.67 g/L, albumin is 26.85 – 34.55 g/L and total protein is 51.10 – 64.55 g/L) (He et al., 2017). However, the creatinine value (32.36 – 59.67 µmol/L) for He et al. (2017) was not similar to this present study (71.40 ± 10.90 - 82.82 ± 5.74 µmol/L). In this study, creatinine was higher than He et al. (2017). This could result from declining renal function or high animal protein intake (Walker et al., 1990). However, results from the kidney histology show a normal function. Therefore, creatinine values can be attributed to the protein of the CFP and rodent feed. The result for alanine aminotransferase (43.00 ± 15.57 - 39.46 ± 26.03 µ/L), alkaline phosphatase (335.17 ± 124.13 - 313.17 ± 69.71 U/L), and aspartate aminotransferase (218.00 ± 67.99 - 217.83 ± 187.41 U/L) was similar to a study conducted to determine reference values for biochemical parameters of SD rats (alkaline phosphatase is 160.8 – 838.3 U/L, aspartate aminotransferase is 0.2 – 838.3 U/L and alanine aminotransferase is 1 - 223.3 U/L) (Delwatta et al., 2018). There was no significant difference between the control and experimental groups for urea, albumin, total protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. A study was conducted with fortified vegetarian milk to prevent metabolic syndrome in rats and impact hepatic and vascular complications.

Three (3) different fortified beverages were made from almond milk, carrot juice, honey, stevia, quinoa seeds and banana juice. It was observed from the study that there was no significant difference between the control and experimental groups for creatinine, ALT, AST and albumin (Hussein et al., 2020). This was consistent with the present study. However, there was a significant difference between the control (10.17 ± 2.56 µmol/L) and experimental (18.69±7.61 µmol/L) groups for total bilirubin. Increased total bilirubin levels could indicate decreased kidney function or a diet high in protein; the histopathology results showed no abnormalities. Hence, this can be attributed to the CFP, which has a higher protein content (22.30 ± 0.00 g/100g) than the rodent feed (13.18 ± 0.01 g/100g).

5.7 Effect of CFP on Histopathology

The present study was conducted to assess the toxicity of CFP following a 28-day continuous feeding in SD rats. The histopathological examination of the kidneys and liver did not reveal any abnormalities. The renal tubules, Bowman's capsule and glomeruli were normal. The glomerulus and bowman space were well defined. However, there was increased cell activity in the bowman capsule, increased vacuolization and overall increased cell activity in the experimental group. The liver had typical lobular architecture with no inflammation. Even distribution of the hepatocytes. No necrosis or lymphocytes were found in both the control and experimental group. The results show that CFP consumption produces no adverse effects on the liver and kidney structure and function. However, increased cellular activity was observed in the experimental groups for both the kidney and liver. This could be a result of the protein content of the CFP, which was higher than the rodent feed. An increase in protein content can increase cellular activity. For the liver, one condition necessary for cell proliferation is an increment in protein synthesis (Liu et al., 2017).

5.7 Limitations of the study

Although the study was conducted with caution and within the bounds of both animal and experimental mistakes, it is crucial to emphasize its inherent limitations, which may affect the results. The results show no significant difference for most of the parameters tested. The number of days spent on the experiment could have influenced the results. This study did not exclude differences in metabolic parameters between the groups studied. There may be variations in data on rat nutrient requirements since the study was conducted under different environmental circumstances or with varying stocks of rats or strains. Rats of the same species, strain, age, and sexes were used to address this issue.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study revealed that CFP formulated from locally available food commodities contains recommended nutrients for growth and could meet the micro nutritional needs of infants and children. There was no significant difference between the experimental and control groups for growth indices (weight and length). There was no significant difference between the weight and length of the animals in both the control and experimental groups. The CFP was found to have no significant effect on the SD rats' growth indices and serum retinol. The CFP was found to possess low toxicity on haematological and biochemical parameters and minimal histopathological changes in the isolated organs. Nutrient requirements for laboratory rats are similar to those for other mammalian species. Because of their small body size and higher metabolic rate, rats' quantitative requirements for each nutrient may differ from those of other species. However, more research, including human trials, is required before inferences can be reached about the bioavailability of nutrients, particularly iron, in the CFP or its usage.

6.2 Recommendations

1. The consumption of CFP should be further investigated to ascertain whether it will be established as a dietary strategy for minimizing anaemia and vitamin A deficiency. Digestibility and bio-availability of the macronutrients in the CFP will need further investigation. The CFP can be given to the animals at varying amounts to determine the concentration that provides the maximum effect.
2. Further study should be conducted among anaemia-induced rats to determine the effectiveness of the CFP in improving anaemia status. Anaemia can be induced in animals by using purified diets.
3. Further research ought to be carried out among rats with moderate vitamin A deficiency to determine the effectiveness of the CFP in controlling vitamin A deficiency.
4. Further microbiological and sensory studies should be carried out of the CFP to determine consumer safety and sensory appeal.

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APPENDICES

Appendix A

The derivation of CFP (100g) based on the iron content of the food items.

Ingredient	Percentage (%)	Mass (g)	% Iron	iron (g)
ANCHOVIES	15.00%	15.00	0.0620%	0.0093
CARROTS	10.00%	10.00	0.0400%	0.0040
TURKEY B.	50.00%	50.00	0.0004%	0.0002
SOYA BEAN	25.00%	25.00	0.012%	0.0030
Total	100.00%	100	0.02%	0.0165

0.0165g = 16.5mg

Based on this information 40g of the CFP can meet at least half of the iron requirements for infants.

The derivation of CFP (100g) based on the β-carotene content of the food items

Ingredient	Percentage (%)	Mass (g)	% β-carotene	iron (g)
ANCHIOVES	15.00%	15.00	0.0000%	0.00000
CARROTS	10.00%	10.00	0.0013%	0.00013
TURKEY B.	50.00%	50.00	0.0046%	0.00230
SOYA BEAN	25.00%	25.00	0.000%	0.00000
Total	100.00%	100	0.01%	0.00243

0.00243g = 2.43mg

Based on this information 40g of the CFP can meet at least half of the β-carotene requirements for infants.

Appendix B

List of equipment used in the study

Equipment	Model/Part no./origin
Electronic balance	Mettler AC100
Centrifuge	J-20 XP
Kjeldahl digestor	425
Kjeldahl distillation unit	K-350
Vortex	G560E
Air oven	Genlab™, Model-MINO175/F
Impulse heat sealer machine	
Gallenkamp muffle furnace	REX-C900
Perkin Elmer Atomic Absorption Flame Emission Spectrophotometer	Model 3110
SYSMEX haematology auto-analyzer	Kobe, Japan

Proximate composition of the rodent feed and CFP

Parameters	Rodent feed (%)	CFP (%)
Proximate composition		
Moisture	8.49 ± 0.01	6.38 ± 0.01
Crude fat	7.79 ± 0.39	9.00 ± 0.57
Crude fibre	-	6.58 ± 0.02
Protein	13.18 ± 0.00	22.34 ± 0.00
Carbohydrates	60.29 ± 0.46	49.46 ± 0.82
Ash	10.26 ± 0.07	6.25 ± 0.26

Operationalization of growth rate using weight and length of the animal

Groups	Growth rate (%)	Score
Control		
Weight	12.06	2
Length	11.90	2
Total		4
Experimental		
Weight	3.02	1
Length	14.02	2
Total		3

The growth rate of 15% and above =3
Growth rate of 10% and above =2
Growth rate of 9% and below =1

Score between 5 – 6 is high growth
Score between 3 – 4 is moderate growth
Score 2 and below is low growth

Average weight and length of rats

Groups	Weight (g)		Length(cm)	
	Initial	Final	Initial	Final
male control	164.67±3.06$^{a\,b}$	181.33±1.15^{a}	18.50±0.50^{a}	21.17±3.40^{a}
female control	150.67±1.15^{b}	174±9.59^{b}	17.33±0.29^{a}	19.00±0.50^{a}
male experimental	183.4±14.03^{a}	170.67±8.33$^{a\,b}$	18.21±0.64^{a}	21.24±1.19^{a}
female experimental	165.42±5.38$^{a\,b}$	166.57±2.76$^{a\,b}$	17.71±0.64^{a}	19.71±1.19^{a}

Values are means ± standard deviations of duplicate determinations. Values in the same column sharing the same letters are not significantly different at the 5% level. Post hoc analysis using the turkey HSD test was used to determine the statistical significance.

Appendix C

Mean haematological indicators by the rat groups

Groups	Hb (g/dL)	RDW %	RBC (10^{12} cells/L)	MCV (Fl)	Haematocrit	MCH (pg)	MCHC (g/dL)
1	14.4±0.8$^{a\,b\,c}$	18.5±0.6$^{a\,c}$	8.7±0.6^{a}	53.6±1.2^{a}	0.46±0.03$^{a\,b\,c}$	16.7±0.6^{a}	31.1±0.4^{a}
2	13.5±0.3$^{a\,b\,c}$	14.5±2.1$^{a\,b\,c}$	7.7±0.1$^{a\,b\,c}$	56.5±1.4^{a}	0.44±0.01$^{a\,b\,c}$	17.5±0.5^{a}	30.9±0.6^{a}
3	14.4±0.8^{b}	17.7±2.1$^{a\,c}$	8.5±0.6$^{a\,b}$	54.1±1.4^{a}	0.46±0.03^{b}	16.9±0.5^{a}	31.2±0.5^{a}
4	12.1±1.7^{c}	13.9±1.8^{b}	7.0±1.1^{c}	55.5±1.0^{a}	0.39±0.06^{c}	17.4±0.4^{a}	31.3±0.9^{a}

Group 1 = male control group, Group 2 = female control group, Group 3 = male experimental group, Group 4 = female experimental group.
Values are means ± standard deviations of duplicate determinations. Values in the same column sharing the same letters are not significantly different at the 5% level. Post hoc analysis using the turkey HSD test was used to determine the statistical significance.

Mean haematological indicators by the rat groups continuation

Groups	WBC (10^9 cells/µL)	Lymph# (10^9/L)	Lymph%	Eos%	Eos# (10^9/L)	Mono%	Mono# (10^9/L)	Neut# (10^9/L)	Neut%	Baso %	Baso# (10^9/L)
1	5.6±1.8^{a}	4.43±1.40^{a}	79.1±1.0^{ab}	1.8±0.6^{ac}	0.10±0.05^{a}	2.1±1.1^{a}	0.12±0.07^{a}	0.97±0.38^{bc}	16.3±2.2^{a}	0.67±0.15^{a}	0.04±0.12^{a}
2	10.8±3.7^{a}	9.67±3.61^{a}	88.9±5.3^{a}	0.9±0.3^{c}	0.10±0.03^{a}	1.5±0.9^{a}	0.16±0.13^{a}	0.73± 0.61^{bc}	8.3± 4.8^{a}	0.37±0.15^{a}	0.03±0.15^{a}
3	8.6±3.2^{a}	7.07±2.06^{a}	72.4±2.9^{b}	2.2±0.7^{a}	0.22±0.12^{a}	2.3±1.6^{a}	0.21±0.14^{a}	2.21±0.73^{a}	22.8±3.3^{a}	0.34±0.15^{a}	0.03±0.11^{a}
4	6.4±2.8^{a}	5.01±2.44^{a}	77.9±9.6^{ab}	2.1±0.6^{ab}	0.43±0.77^{a}	2.2±2.0^{a}	0.16±0.16^{a}	1.06±0.55^{bc}	16.6±11.2^{a}	0.63±0.53^{a}	0.03±0.02^{a}

Group 1 = male control group, Group 2 = female control group, Group 3 = male experimental group, Group 4 = female experimental group.
Values are means ± standard deviations of duplicate determinations. Values in the same column sharing the same letters are not significantly different at the 5% level. Post hoc analysis using the turkey HSD test was used to determine the statistical significance.

Platelet indices in the complete blood test for SD rats

Groups	Platelet Count (10^9 cells/L)	Mean Platelet Volume (fL)
male control	373. 1±215.4[a]	7.87±0.12[a]
female control	506.6±292.5[a]	8.15±0.50[a]
male experimental	177.2±67.0[a]	7.77±0.13[a]
female experimental	443.6±167.7[a]	7.84±0.32[a]

Values are means ± standard deviations of duplicate determinations. Values in the same column sharing the same letters are not significantly different at the 5% level. Post hoc analysis using the turkey HSD test was used to determine the statistical significance.

Serum retinol concentration of the rats

Groups	Serum retinol concentration (10^3ug)
male control	2.45±0.30[ab]
female control	1.95±0.62[ab]
male experimental	2.63±0.89[a]
female experimental	1.32±0.09[b]

Values are means ± standard deviations of duplicate determinations. Values in the same column sharing the same letters are not significantly different at the 5% level. Post hoc analysis using the turkey HSD test was used to determine the statistical significance.