UTRITIONAL, PHYSICO-CHEMICAL AND MICROBIOLOGICAL EVALUATION OF CEREAL-BASED COMPLEMENTARY FOODS FORTIFIED WITH PIGEON PEA (*Cajanus cajan*) FLOUR

BY

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CERTIFICATION

This is to certify that this study was undertaken by Mrs ANAEMENE, Doris Ifeoma under my supervision in the Department of Human Nutrition, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

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DEDICATION

This work is dedicated to God Almighty, the most Merciful and Faithful Father.

ABSTRACT

Undernutrition during complementary feeding period remains a significant public health problem and contributes to growth failure, increased morbidity and mortality in children. Pigeon pea is an under-utilised legume that is rich in protein and minerals; however, report on the nutritional quality of cereal-based complementary food (CF) formulated with Pigeon Pea (PP) is scanty. This study was designed to evaluate the nutritional, physico-chemical and microbiological characteristics of two cereal-based complementary foods fortified with PP.

Two diets, fermented PP (CompifO) and roasted PP (CompifR), were formulated from maize, PP, fish, carrot, pumpkin leaves, oil and sucrose in the ratio of 50:20:10:6:4:5:5. The formulated diets were evaluated for physico-chemical and microbial properties using standard methods and compared with Commercial CF (CCF). Thirty-six weanling rats divided in six groups of six were acclimatised on 4% casein diet for five days. One group was sacrificed at zero day for baseline data while five groups were fed the formulated diets, CCF, Casein (protein isolate) and protein-free diets for 21 days. Feed intake measured daily and weight twice weekly were monitored for Protein Efficiency Ratios (PER) and weight change. Urine and faeces were collected for evaluation of protein quality parameters (Biological Value (BV), net protein utilization (NPU)). Blood samples were collected for haematology and serum biochemistry (albumin, globulin, cholesterol,aspartate amino transferase). Data were analysed using one-way ANOVA and Duncan's multiple range test at $\alpha_{0.05}$.

The protein contents of CompifO (17.2%) and CompifR (16.8%) were comparable but calcium (83, 118 mg/100g), iron (3.60, 1.95 mg/100g), zinc (3.35, 1.15 mg/100g), vitamin A (104.21RE, 90.61RE) and phytate (179.0, 146.0 mg/100g) levels of CompifO and CompifR respectively were significantly different ($p<0.05$). CCF had lower protein (15.5g) but significantly higher calcium (410mg), iron (8.52mg), and vitamin A (370RE) contents than CompifO and CompifR. The viscosity values of CompifO (320cps) and

CompifR (322cps) were similar but significantly different from that of CCF (268cps). The microbial counts of the diets ranged from 0.00 to 0.20 (x 10^4cfu/g) and were within safe limits. Casein diet (2.64) had the highest PER followed by CCF (2.22), CompifO (1.69) and CompifR (0.48). Rats fed CCF (+33.1±9.8g), Casein (+32.7±7.5g), and CompifO (+24.1±4.9g) diets had similar weight changes (p=0.09), which varied significantly with those fed CompifR (+5.8±3.6g) and protein-free diets (-14.2± 6.6g) ($p<0.05$). The BV and NPU values of CompifR (47.1%, 43.8%) were significantly low compared to those of Casein (99.5%; 91.0%), CCF (85.9%; 79.2%) and CompifO (78.0%; 71.5%). Haemoglobin level (g/100ml) was highest in the Casein group (15.43), followed by CCF (11.85), CompifR (11.55), CompifO (9.65) and protein-free (7.83) groups. Serum iron and zinc were significantly lower in CompifR (1.88±0.30;0.75±0.24mg/dl) compared to CompifO (3.33±0.44;1.58±0.50mg/dl), Casein (3.25±0.72;2.50±0.53mg/dl) and CCF (4.05±0.70;3.24±0.19mg/dl).

The fermented pigeon pea complementary food compared favourably with commercial complementary food in terms of macronutrient content and growth support. It is therefore recommended for use in formulating quality complementary food rather than roasted pigeon pea.

Keywords: *Complementary foods, Fermented pigeon pea, Roastedpigeon pea, Nutritional quality of infant foods*

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Amino Acid Score
ACUREC	Animal Care and Use in Research Ethical Committee
AG RATIO	Albumin/Globulin Ratio
ALA	Alpha-linolenic Acid
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ARA	Arachidonic acid
ASF	Animal Source Foods
AST	Aspartate Amino transferase
BUN	Blood Urea Nitrogen
BV	Biological Value
CAC	Codex Alimentarius Commission
Carb	Carbohydrate
CCF	Commercial Complementary Food
CFU	Colony Forming Units
COMPIF	**CO**mplementary **M**aize **PI**geon pea with **F**ish
Cp	Centipiose
CP	Crude Protein
C-PER	Corrected Protein Efficiency Ratio
DHA	Docoxahexanoic Acid
DFP	Dried Fish Powder
EAA	Essential Amino Acid
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
$FeCl_3$	Iron Chloride
FER	Feed Efficiency Ratio

FERBP	Fermented Boiled Pigeon pea
GAIN	Global Alliance for Improved Nutrition
HB	Haemoglobin
IAR&T	Institute For Agricultural Research and Training
IITA	International Institute for Tropical Agriculture
LEAA	Limiting Essential Amino Acid
MCH	Mean Cell Haemoglobin
MCHCMean Cell Haemoglobin Concentration	
MCV	Mean Cell Volume
MICS	Multiple Indicator Cluster Survey
NBS	National Bureau of Statistics
NDHS	Nigeria Demographic Health Survey
NNHS	National Nutrition Health Survey
NPC	National Population Commission
NPR	Net Protein Ratio
RAE	Retinol Activity Equivalent
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
RE	Retinol Equivalent
R-NPR	Relative Net Protein Ratio
PAHO	Pan American Health Organisation
PCV	Packed Cell Volume
PDCAAS Protein Digestibility Corrected Amino Acid Score	
PER	Protein Efficiency Ratio
QPM	Quality Protein Maize
Rpm	Rotations per minute
SDG	Sustainable Development Goals
TBW	Total Body Water
TD	True Digestibility
UNICEF	United Nations Children's Education Fund
WBC	White Blood Cell

WHO World Health Organisation

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CHAPTER ONE
INTRODUCTION

1.1 Backgroundinformation

Under-nutrition, particularly micronutrient deficiency among children below five years continues to be a key health concern in Nigeria. Under-nutrition is linked toa greater risk of morbidity, mortality, reduced learning capacity anddeveloping non-communicable diseasesif it occurs before a child turns two years (Dewey and Begum, 2011; Black *et al*., 2013). This is a consequence of poor complementary feeding practices (Bhutta *et al*., 2013). Complementary foods in Nigeria are predominantly staples which make them phytate dense (Roos *et al*., 2013). A high level of phytate in meals offered children has a negative influence on the nutritional status of children (Thacher *et al*., 2009; Bisinwa *et al.,* 2012).

Complementary feeding is the feeding of infants from six months,with other varied, safe and nutritious foods in addition to breast milk. It is a period when older infants and young children are gradually introduced to other foods and liquids while breastfeeding continues. Breast milk cannotsustain the increased energy and nutrients required for the rapid growth from six months (Pan American Health Organisation (PAHO)/World Health Organisation (WHO), 2003). Therefore, the provision of adequate energy and nutrients from other sources is very crucial during this period. The nutrition of young children remains the major focus of nutrition and health researches worldwide because of its proven contribution to human and national development. Feeding infants and older children adequately ensure that they achieve optimal growth and development (WHO, 2009; UNICEF 2012). In the developing countries, growth faltering is highest during the complementary feeding stage because it coincides with

decreased breast milk intake (Pelto *et al*., 2003), increased micronutrient deficiency and diarrhoeal diseases (Dewey and Adu-Afarwuah, 2008). According to the Nigeria

Demographic Health Survey (NDHS), 2018, about 32%, 24% and 13% of children aged 6 to 23 months were stunted, underweight and wasted, respectively (National Population Commission (NPC) and ICF 2019). Stunting prevalence among under-five children increased from 27% at 6 months to 50% at 23 months.Under-nutrition during the complementary feeding period contributes substantially to under-fives deaths in Nigeria.Stewart *et al.* (2013) emphasizes the role of different aspects of complementary feeding in childhood stunting. Factors that contribute to poor quality complementary foods includea high level of anti-nutrients, poor consumption of micronutrient-rich foods and insufficient intake of animal source foods. Given these problems, extensive work has been undertakenon the formulation and evaluation of complementary foodsfrom various blends of cereals/tubers, legumes, animal source foods, vegetable and/or fruit sources(Onabanjo *et al*., 2009a;Wakil and Kazeem, 2012; Oyarekua, 2013;Kinyua *et al*., 2016;Adepoju and Ajayi, 2016; Fasuan *et al.,* 2017).Complementary foods have also been formulated from blends of fruit and legumes (Ijarotimi and Olopade, 2009; Martin *et al*., 2010).

The major constituents of most of these complementary foods are carbohydrate-rich sources which are starchy staples. Starchy granules gelatinize when heated with water or mixed with hot water. The process produces thick (viscous)porridge which requires dilution with water before feeding young children. This affects the energy and nutrient density of complementary foods as well as the digestibility (Michaelsen *et al.,* 2003). The viscosity and other physical properties of complementary foods are important factors that determine their quality. Severalmethods of processing have been employed to improve the functional characteristics of cereals and legume. This positively influences the digestibility, energy/nutrient densities as well as bioavailability of protein and micronutrients of complementary foods. These methods include germination, roasting(Kebebu *et al.,* 2013; Addis *et al*., 2013;Ikujenlola and Adurotoye, 2014; Fikiru *et al*., 2017) and controlled or uncontrolled fermentation (Wakil and Kazeem, 2012; Oyerakua,2013).Also, a combination ofprocessing methods such as germination and then

fermentation have been employed to improvethe quality of complementary foods(Inyang and Zakari 2008; Hemen *et al*., 2012;Okoronkwo *et al.,* 2016).

Food-based strategies remain the most realistic and sustainable approach to tackling undernutrition among children (Thompson and Amoroso, 2011). Although care is fundamental to achieving desired results in complementary feeding, the quest for complementary foods of good nutritional quality continues to occupy the centre stage in nutrition research. Complementary foods of good nutritional quality could be formulated from locally available ingredients using traditional processing methods. The provision of quality complementary foods to older infants and young children would immensely reduce the burden of undernutrition during the complementary feeding period.

1.2Statement of Problem

Under-nutrition during the complementary feeding period is a major public health problem and contributes to growth failure, increased morbidity, and mortality in children. Despite intensiveresearch and enlightenment programmes on adequate nutrition in the early years of life, underweight, stunting and wasting prevalence rates increased from 24.2 percent to 31.5 percent, 34.8 percent to 43.6 percent and10.2 percent to10.8 percent respectively (National Bureau of Statistics (NBS) and United Nations Children's Fund (UNICEF)(2017)).The rising level of under-nutrition coincides with the time complementary foods are started.Poor complementary feeding, particularly, low bioavailability of protein and micronutrients coupled with poor intake has been strongly linked to under-nutrition among under-five children.Diets consumed by young children are of low dietary diversity (Dewey, 2016) as also observed in Ethiopia (Mesfin *et al*., 2015), and Kenya (Macharia-Mutie *et al*., 2010). Most of these complementary foods are usually low in energy and micronutrients, particularly in vitamin A, calcium, iron, and zinc. Furthermore, the dilution of these cereal-based complementary foods with water to reduce the viscosity also negatively affects the quality. The fact that breastfeeding and child-caring practices decline during this period aggravates the problem.World Health Organisation (WHO) recommendsthe inclusion of at least four out of seven food groups in complementary foods to improve dietary diversity (WHO, 2010). Legumes are nutritious and complement cereals in terms of proteins but

contain antinutrients. Apart from some anti-nutritional compounds such as oligosaccharides and protease inhibitors that are easily removed by heat, legumes contain other substances such as phytates. Phytates are heat stable and may form complexes with micronutrients (Gibson *et al.,* 2010), making them unavailable.

1.3Justification of the Study

Under-nutrition during the complementary stage and up to two yearsresults inirreversible and devastating complications. The global targets of achievinga 40% reduction in stunting among under-five children will not be feasible with the present marginal decrease in nutritional indices. Bioavailability, not only adequacy of nutrients in complementary foods is very vital in curbing the menace of under-nutrition. Therefore, more research on effective and sustainable strategies to improve nutrition during the complementary feeding stage is necessary. Enormous work has been undertaken on improving complementary feeding (Adenuga, 2010; Oyarekua, 2013; Ige 2017). However, there is limited data on the nutritional quality of cereal-based complementary foods fortified with pigeon pea, fish and vegetables. Thus, the need to undertake a study on the nutritional quality of maize-pigeon pea-based complementary food enriched with fish, carrot and pumpkin leaf flour.

Pigeon pea (*Cajanus cajan)*is an underutilized legume with an excellent nutrient profile (Onu and Okongwu 2006). Its oligosaccharide content offers a prebiotic property that is beneficial to gut health when fermented and improves better utilisation of nutrients for growth.The addition of pigeon pea to complementary food will improve its utilization, promote diet diversity and strengthen the traditional food system.Pigeon pea is largely grown by women. Hence, increased utilisation of pigeon pea would not only contribute tothe improved nutritional status of children but also women empowerment. It is also eaten as part of the traditional family diet in communities where it is grown; therefore its use goes beyond the complementary stage and ensures sustainability.Maize(*Zea mays*) gruel is very common as the first line of complementary food in most developed countries. It is locally available and affordable when compared to sorghum and millet. Normal maize has low levels of lysine and tryptophan (Serna-Saldivar *et al*., 2008)but Quality Protein

Maize(QPM) possesses reasonable quantities of these amino acids (Iken and Amusa, 2004).Fish is an animal source food(ASF) that provides high-quality protein and minerals. Blue whiting(*Micromesistius poutassou)* specie is preferred because of its low-fat content, availability, and affordability. Carrot and pumpkin leaves are sources of vitamins and minerals particularly, beta-carotene.

Fermentation and germination improve protein/mineral digestibility and bioavailability of plant-based meals(Tufa *et al.,* 2016).Cereal and legume contain oligosaccharideswhich are prebiotics and when fermented produce beneficial microbes stimulating a probioticenvironment. This aids digestion and supports growth in children.Including pigeon pea in a cereal-based complementary food with fish, carrot, and pumpkin leaves as well as employing appropriate processing methods to reduce anti-nutrients would provide a multi-mix complementary food with improved nutritional value in terms of bioavailability of nutrients. Quality complementary food remains the key to reducing older infants' under-nutrition and mortality rates. This is fundamental to achieving sustainable development goals.

Enriching maize-pigeon pea-fish blend with pumpkin leaves and carrot powder would give a quadruple mix of complementary food and provide a new variety of complementary food that will be locally available.This study was designed to evaluate the nutritional quality of enriched maize-pigeon pea-based complementary food using animal model.

1.4 Objectives of the study

1.4.1 Main objective was to:

- To evaluate the nutritional, physico-chemical and microbiological characteristics of maize-pigeon pea-based complementary food enriched with fish, carrot, and pumpkin leave powder.

1.4.2 Specific Objectives were to:

1. Determine the effects of fermentation, germination, and germination-fermentation on nutrient retention and phytate reduction in maize and pigeon pea seeds.
2. Assess the nutrient and anti-nutrient composition as well as adequacy of formulated complementary foods
3. Examine the functional properties, microbial safety and shelf life of formulated complementary foods.
4. Assess the nutritional quality (growth performance characteristics, protein quality parameters, haematological and histological properties) of the formulated complementary foods using an animal model.

1.5Research hypothesis

Ho: There would not be significant differences in the phytate levels of maize and pigeon pea seeds processed using fermentation, germination or combined germination-fermentation methods.

Ho: Chemical composition of formulated complementary foods would not satisfy > 70% ofthe recommendation.

Ho: The microbial load of the selected formulated complementary food would be within recommended safe limit.

Ho: There would not be significant differences in the nutritional quality of the formulated complementary foods and control diet.

Ho: There would not be any significant difference in the nutritional quality of the two formulated complementary foods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Undernutrition during the complementary feeding stage

A global report by UNICEF states that in 2017, an estimated 22.2% of children under-five years were stunted while the rate of wasting among these children was 7.5%. According to Multiple Indicator Cluster Survey (MICS) 2017, more than 30% and 25% of the world's children, who are stunted and wasted, respectively, live in Africa (NBS/UNICEF, 2017). The report reveals that it is only in Africa that the rate of stunting among children increased by 16% while Asia recorded a 51% decrease. Around 11 million children below five years are stunted in Nigeria, with the highestburden in the north-east, north-west and among the poorest group (NBS/UNICEF, 2017). These statistics reveal insufficient progress to reach the World Health Assembly and Sustainable Development Goals (SDG) targets set for 2025 and 2030 respectively.

There is widespread malnutrition among children in Nigeria, particularly, in the rural areas (Awogbenja and Ugwuona,2010; Amosu *et al*., 2011; Ibeanu *et al*., 2012; Quadiri and Ojuore 2013; Samuel, 2014;NPC/ICF, 2019). The National Nutrition Health Survey (NNHS), (2017) report revealed that about 43.6%, 31.5% and 10.8% of children less than five years were stunted, underweight and wasted respectively. This represents about 36.3% and 57.5% increase in the rates of stunting and underweight among under-five children when compared to the report of 2014. According to the Nigeria Demographic Health Survey (NDHS), (2018), about 37%, 23% and 7% of children under-five years were stunted, underweight and wasted, respectively (NPC/ICF, 2019). Health survey reports show a wide range of stunting rates in the southeastern and northwestern/northeastern states of Nigeria. Statistics of stunting rate has remained worrisome despite efforts towards its reduction due to gross protein and micronutrient

deficiencies arising from inappropriate infant feeding practices, inadequate complementary foods,and repeated infections during the critical period of growth (Bhutta *et al*., 2013; Stewart *et al*., 2013; Ochonorgo, 2013; WHO 2015a). The NNHS 2017 report indicates that the under-nutrition rate rose from age 6-8months and reached a maximum between 18-35 months (Fig 2). The high level of under-nutrition period coincides with the period when infants are introduced to complementary feeding foods.

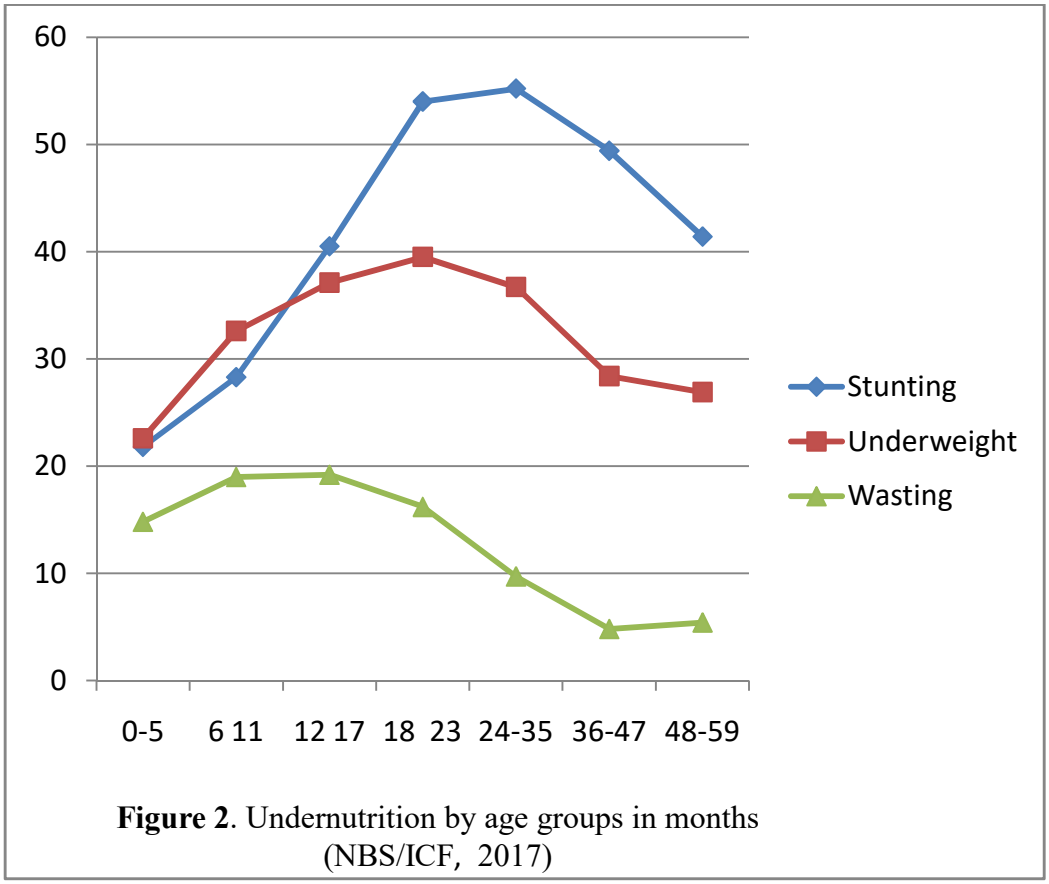


Figure 2. Undernutrition by age groups in months (NBS/ICF, 2017)

2.2 Recommendations for complementary feeding and formulated complementary foods.

The Global Strategy for Infant and Young Child Feeding is one of the global initiatives aimed at improving infant and young child feeding practices. It is recommended that as from six months of age, an infant is gradually introduced to other adequate, nutritious, safe and varied foods including liquids (PAHO/WHO 2003). The mother is expected to continue breastfeeding the child till the second birthday to ensure that increased nutrient needs are met. These foods should be chosen from not less thanfour food groups (WHO 2010). These food groups include dairy products; grains, roots, and tubers; flesh foods; eggs; legumes and nuts; vitamin A-rich fruits and vegetables; other fruits and vegetables. It is also advised that breastfed children aged 6 – 8 months and 9 – 23 months be fed 2 and 2-3 times daily respectively while non-breastfed children should be fed about 4 times daily (Table 2) (PAHO/WHO 2003). Emphasis is placed on the consumption of iron-rich or iron-fortified foods to address anaemia. Complementary foods are formulated based on the nutritional requirements of older infants and young children. It is recommended that these foods should be specifically formulated to complement the family diet and breast milk in providing additional energy and protein needs required for rapid growth. They should never replace breast milk or family diet. Precooked or instant formulated complementary foods are desirable because of ease of preparation, low fuel and time consumption (Global Alliance for Improved Nutrition (GAIN), 2017). Processed complementary foods are usually reconstituted with water to semi-solid gruel to assist the transition from breast milk which is a liquid diet to a normal family diet. Other recommendations include:

2.2.1 Energy needs and energy density of processed complementary foods

The energy needs of infants are very high per kilogram body weight compared to any other stage of life (Dewey 2013). The average energy need for infants aged 6 to 12 months is about 98 kcal/kg body weight (Wong.,2016). Individual infant energy need ranges from 80-120kcal/kg bodyweight depending on some factors such as weight, growth rate, breast milk intake, physical activity and health status among others. For

breastfed infants, energy needs from complementary foods are obtained from the difference between average breast milk energy intake and total daily energy requirement for each age group (Dewey 2003).

Infantsaged 6-8, 9-11 and 12-23months with average breast milk intake residing in developing countries,require about 200 kcal, 300kcal and 550 kcal per day,respectively, from complementary foods(Table 2.1). non-breastfed infants require a greater amount of energy from complementary foods (600-700kcal).The energy density of complementary food as served should be at least 0.8kcal/g (4kcal/g dry weight). Low energy-dense complementary foods (<0.8 kcal/g) would need to be fed more or above the recommended frequency to meet daily energy needs(Dewey 2003).Increased meal frequency may excessively displace breast milk intake. It is recommended that about 10 to 50g of formulated complementary food when prepared should be served in one or more feeding based on the age of the child (Codex Alimentarus Commission (CAC),1981 rev 2006). The quantity of the formulated complementary food offered to a child depends on its energy density. Lower quantity of high energy density complementary food such as lipid-based products would be required per feed. The energy density of formulated complementary food is influenced by the carbohydrates (starchy food and sugars), protein and fatcontents.Low energy-dense complementary food can be improved by adding fat (oily seeds, oil) and/or digestible carbohydrate (simple sugar)(Michaelson *et al.,* 2003).

2.2.2 Protein Needs

Adequate protein intake is required for the rapid growth and development in the first 1000 days of life (Black *et al.,* 2013). From birth to 6 months, infants with average breast milk or consume the right amount of infant formula will meet their protein requirements. However, from seven (7) months of age, quality protein food sources must be added as part of complementary food to meet the protein requirement of 1.2 g/kg body weight. Cereals, legumes, nuts and some oily seeds contribute to the protein content of complementary food. It is recommended that formulated complementary food should contain animal source foodfor improved protein quality. Animal source foods are also rich in micronutrients. Codex Alimentarus Commission (2006) recommended that the

protein efficiency ratio of protein in the complementary food should not be less than 70% of the reference protein,casein. Protein digestibility corrected amino acid score (PDCAAS) was introduced in 1991 as the idealmeasure of protein quality of foods (FAO/WHO, 1991). This is based on comparing the true digestibility of test foods with the amino acid reference pattern of related age group. The PDCAAS is a comparison of the concentrations of the first limiting amino acid in the processed complementary diet and the amino acid in a reference protein (casein) considering digestibility. According to the standard, PDCAAS should be at least 70% of the World Health Organisation amino acid reference pattern for children aged 2-5 years (CAC, 1991 revised 2013). The contribution of energy from protein to the total energy content of the complementary food should range between 6-15% (PAHO/WHO 2003).

2.2.3 Fat needs

Fat is a concentrated form of energy. It is very essential in an infant's diet because it supplies essential fatty acids (linoleic acid (LA), alpha-linolenic (ALA), docoxahexanoic acid (DHA) and arachidonic acid (ARA)) and enables absorption of fat-soluble vitamins. Docoxahexanoic acid (DHA) is very vital in infancy and early childhood because of its role in brain and neurological development. Fats provide energy to the liver, muscles, heart, and brain.The recommended intakes of fat for infants aged 0-6 months and 7-12 months are 31 and 30 grammes, respectively (Wong, 2016). It is estimated that fat contributes about half of the energy in breast milk and infant formular. Consequently, as breast milk or formula intake decreased with age, total fat intake declined and should, therefore,be provided in complementary foods. The proportion of fat in formulated complementary food depends on the level of breast milk intake and its content. According to the guidelines for complementary food for the breastfed infant (Table 2), 6-8, 9-11 and 12-23 months infants who consume an average level of breast milk with about 38g/l fat content would require 0-34%, 5-38%, and 17-42%, respectively, of energy as fat in complementary foods. It is also recommended that the level of linoleic acid in formulated complementary food should not be less than 1.6g in 100g dry product (333mg per 100kcal) (CAC, 2013).Fat increases energy density but may negatively affect

overall nutrient density (decrease protein and iron density), increase susceptibility to rancidity and reduce the shelf life of formulated complementary food (Michaelsen *et al.,* 2003).

2.2.4 Vitamin and mineral needs

Iron, zinc, calcium, and vitamin A recommended nutrient intakes for 7-12 months old infants are 11.6mg, 8.3mg, 500mg, and 400 mcg/RE, respectively (GAIN, 2017). Breast milk contains a substantial amount of many vitamins but relatively low in minerals particularly iron and zinc. Iron stores of infants deplete by 6 months(PAHO/WHO 2003). It is recommended that complementary foods fill this gap. Complementary foods should offer about 97% of iron, 72% of calcium, 81% of phosphorus, 86% of zinc and 76% of magnesium (PAHO/WHO 2003). According to the Codex Alimentarius Commission (1991 rev 2013), the suggested level of micronutrients in a daily serving of the formulated complementary food should be 50% of reference nutrient intakes. The remaining proportion ideally should come from family meals and breast milk. Complementary foods processed from naturally occurring foods including animal-sourced foods may not require fortification depending on the level of losses of micronutrients during processing. It is very challenging to meet the micronutrient requirements of young childrenparticularly, iron, even with dietary diversification and nutrient-rich complementary foods (Dewey, 2016). Specialised infants and young children's products such as fortified blended foods and micronutrient powders have been developed to curb micronutrient deficiency(Dewey, 2016).

Table 2. Energy/nutrient needs from complementary foods (developing countries)

Age of child	Recommended daily feeding frequency		Energy needs from Complementary Food (0.8kcal/g)		Gastric capacity (ml), (30g/kg body weight)	Protein (% of total energy)	Fat(% of total energy)	Fibre g/100g dry weight
	Breastfed	Not breastfed	Breastfed (kcal/d)	Not breastfed (kcal/d)	Average child ml/meal			
6-8	2-3	4-5	200	600	249	6-15% (PDCAAS ≥ 70%)	0-34%	≤5g
9-11	3-4	4-5	300	700	285		5-38%	
12-23	3-4	4-5	550	900	345		17-42%	

Source: PAHO/WHO (2003), Codex Alimentarius Commission, (1991 revised 2013). Protein Digestibility Corrected Amino Acid Score (PDCAAS).

2.3Complementary feeding practices

Inadequate maternal, infant and child nutrition, caring practices, poor hygienic and sanitary environment have been implicated in a high rate of malnutrition in children (WHO, 2009; Ochornogor, 2013). About 17% and 29% of infants are given only breast milk till six months (NPC/ICF, 2014 and 2019) compared to 79.6% in Pakistan, 49% in Tanzania, 42.5% in Bangladesh, 31.5% in India (Hassain *et al.,* 2013), and 46% in Ghana(Gyampoh *et al.,* 2014).In Lagos, 14.7% of mothers breastfed their infants exclusively for six months (Akeredolu *et al.,* 2014).According to some British studies, very few Danish mothers practiced exclusive breastfeeding until six months (Kronborg *et al*., 2015). Infants across Europe are introduced to complementary foods safely between four and six months (Kronborg, 2015). In Nigeria, despite the recommendations, an increasing number of infants are not exclusively breastfed until the sixth month and are introduced to poor complementary foods very early in life (Ochornogor, 2013).It was reported that in a northern community, almost 50% of the mothers started complementary foods much earlier than three months and about 28% washed their hands before giving food to their wards (Anigo *et al*., 2010). In Nasarawa state, children were offered foods before six months (Awogbenja and Ugwuona, 2010). A study in the satellite town of Lagos reported that about 48.4% of mothers introduced their children to other foods at about six months of age and 91.9% continued breastfeeding but 57.1% discontinued breastfeeding before twelve months of age. However, 70.3% and 75.6% practiced responsive feeding and proper hygienic food preparation respectively(Olatona *et al*., 2014).

In Cross River, it was observed that about 85.4% of infants aged 6-8 months were introduced to complementary foods timely but only 7.3% met the minimum acceptable diet (Udoh and Amodu, 2016). Seventy-nine percent of childrenaged 6-8 months given breastmilk in Nigeria consume complementary foods, while 40.2 % of breastfed infants receive foods from at least 4 food groups daily (NBS/UNICEF 2017). In Nigeria, about 11% of 6-23 months old children are fed properly with respect to IYCF recommendations (NPC/ICF, 2019). Timely introduction of complementary foods (47.9%), dietary diversity (16.0%) and minimum acceptable diet (16.0%) for 6 to 9-month-old children in Lagos were low (Olatona., et al, 2017). In Ghana, it was noted that 42%, 64% and 32% of

6-23 months children received dietary diverse meals, the required number of feeds and minimumacceptable diet respectively (Gyampoh *et al*., 2014).The majority of the mothers in resource-poor settings prefer home-based complementary foods to commercial complementary foods and use locally available food sources to prepare these complementary foods for their children (Onabanjo *et al*., 2009a; Agbon *et al*., 2011).These locally prepared complementary foods are oftenlow in key nutrients and mineral availability not guaranteed because they are mainly unsupplemented staple cereals or tubers (Dewey, 2016).

'Pap' prepared from fermented maize, millet or sorghum is the most common and usually the first complementary food is given to infants across the developing countries (Amagloh, 2012; Ogunba, 2012).The majority of mothers offer gruel made from these cereals with very little quantity of milk (adult small satchet milk). Sucrose is seldom added because it is perceived to cause stomach problems commonly called 'Jedi Jedi'. Other new meals introduced include *fura da nunu*, yam vegetable (Awogbenja andUgwuona, 2010), mashed beans and unripe banana porridge (Adepoju and Etukumoh, 2014), soyabean porridge, noodles, amala and soup, mashed rice (Agbon *et al*., 2011)and sweetened cookies, bread, tea (Hassain *et al*., 2013). Awareness of supplementation of cereal/tuber based complementary food with legume or animal source foods termed double mix complementary foods has tremendously increased. This is as a result of health and nutrition information given to mothers at child welfare clinics. However,there is the poor practice of other recommendations despite the high level of adequate knowledge of good complementary feeding practices (Sanusi *et al*., 2016). Agbon *et al*. (2011) also noted that the iron, calcium and zinc intakes from mashed beans given as complementary food were low while protein and energy intakes were met. Complementary foods available in the developing countries have been reported to be of low dietary diversityandmainly plant-based with high phytate content (Gibson *et al*., 2010; Roos *et al*., 2013;Abeshu *et al*., 2016; Makori *et al*., 2017) and lack variety (Kimiywe and Chege, 2015). The bioavailability of proteins and minerals particularly iron, calcium,and zinc was hampered in plant-based diets due to the presence of anti-nutrients unless properly processed or enhancers added (Gibson *et al*., 2010). Cereals and legumes used for preparing semi-solid foods for children are usually fermented or roasted.

2.4Anti-nutritional compounds in foods

Plants particularly, cereals and legumes, in addition to nutrients possess anti-nutritional properties which negatively affect the bioavailability of protein and important minerals. Anti-nutritional factors in cereals are lower than those in legumes. Cereals contain digestive enzyme inhibitors which do not pose serious health issue because they can be easily removed. Legumes are rich in oligosaccharides (raffinose and stachyose). These factors cause flatulence and discomfort because of the lack of enzymes to digest them but can be reduced during germination (Bora, 2014). Other anti-nutrients include phytate, tannin, trypsin and chymotrypsin inhibitors, cyanide, saponin, polyphenols, oxalates among others. The level of anti-nutrients in foods largely depends on the ingredients used in the preparation and methods of processing.Although some anti-nutritional compounds such as isoflavones, phenolic compounds, and lectinsoffer potential anti-cancer and anti-oxidant benefits (Oboh, 2006; Young, 2011;Bora, 2014), the immediate negative influence on the nutritional status of humans particularly, children, is of utmost importance and demands prompt and sustained action.The effectiveness of methods of reduction or elimination of these anti-nutritional factors is influenced by their location, chemical and structural characteristics. Heat labile anti-nutrients include hemagglutinnin, avidin, alpha-amylase, and trypsin inhibitors while phytate, saponins, and alkaloids are among the heat-stable compounds (Bora, 2014).

2.4.1Phytic acid in foods

Phytic acid is referred to as phytate as a salt or inositol hexaphosphate (IP6). Phytate occurs naturally and mainly produced during the ripening of plant seeds and grains. Phosphorus is storedas phytic acid in plants and content varies widely (Abdoulaye*et al*., 2011). Phytic acid is abundant in the seed portion of maize while in cereals such as wheat and rice it is more in the aleurone layer (Abdoulaye *et al.,* 2011). Phytatehas also located hulls of beans, nuts, peas, and grains. Moderate quantities are found in tubers while fruits and vegetables contain minimal quantities.Monogastric animals and humans lack phytase required to metabolize phytate (Singh *et al*., 2011). Phytic acid bindssome minerals and inhibits the digestive enzymes necessary for protein starch degradation(Gupta *et al*.,

2015).The phytic acid content of plants varies. Ghavidel and Prakash (2006) reported 610mg, 480mg, and 600mg per 100g dry green gram, chick pea and cowpea respectively. Roos *et al*. (2013) disclosed that the phytate content of plant-based complementary foods offered to children in the developing countries ranged from 68 to 1536mg/100g. They also observed that phytate:iron molar ratio exceeded the recommended level in 32 of the 36 complementary foods analysed. A study reported that magnesium and zinc absorption reduced from 30% to 13% and 23%,respectively; when participants were fed low phytate and then high phytate bread (Egli *et al*., 2002). Thacher *et al*. (2009) found that consumption of maize mealsresulted in reduced zinc absorption in rachitic and non-rachitic children. The effect was significant and linked to the high phytate content of the meal. Bisinwa *et al.* (2012) attributed the poor result of intervention with fortified ready-to-use complementary food to the high phytic acid content of the diets which might have led to inadequate nutrient absorption.Phytate effect on content and bioavailability of minerals is dose-dependent, but even at low levels; it may still reduce iron absorption (Hurrel, 2004). Mamiro*et al.* (2004) reported thatprocessed complementary foods fed to a group of infants did not have a significant effect on iron availability becauseof phytate,which only reduced by 34%. They argued that despite the reduction, phytate:iron molar ratio of processed complementary foods (11.8) was still high in relation to that of the unprocessed complementary food (16.5) and affected absorption of iron in the infants. To ameliorate this problem, limits and ratios of phytate content to iron, zinc, and calcium were established (Roos *et al.,* 2013).Phytate: iron/zinc/calcium ratios in complementary foods should be less than 1.0,15 and 0.17, respectively, for efficient mineral absorption based on the recommendation(Roos *et al.,* 2013).These phytate:mineral ratios are assumed to facilitate increased mineral absorption from plant-based foods (Thompson andAmoroso 2011). Despite the negative effect of anti-nutrients particularly phytate because others such as trypsin,lectins and chymotrypsin inhibitors are heat sensitive (Bora, 2014), their levels are rarely assessed in processed plant-based complementary foods.

2.5Effect of processing methods on the nutrient and anti-nutritional content of foods

Several works have been carried out on the methods of removal or reductions of anti-nutrients (Egli *et al*., 2004; Abebe *et al*., 2007; Ghavidel and Prakash 2006; Helmatha *et al.,* 2007; Adebowale and Maliki 2009; Thacher *et al*., 2009;Fasoyiro *et al*., 2010).However, essential nutrients may be reduced and undesirable elements such as peroxides introduced during the processing (Bora, 2014).The influence of processing methods on nutrient, functional and other characteristics of foods have been extensively studied (Mbaeyi and Obetta, 2016; Tufa *et al.,* 2016; Fadupin *et al.,* 2017).Harris (2010) reviewed extensively the different traditional and conventional methods of reducing phytates in foods. Among the methods were sprouting; sprouting and fermentation; soaking; soaking and dehulling; soaking, dehulling and sprouting; addition of sprouted flour to non-sprouted flour; co-fermentation of low phytase food with high phytase containing food; dehulling; natural and cultured fermentation among others. All these methods had varying reducing effects on the phytate levels of cereals. Generally, it can be deduced that hydrolysis methods tend to reduce phytates more than other methods (Liang *et al.,* 2008).

2.5.1 Fermentation

Effect of fermentation depends on numerous factors which comprise the pH of the medium, temperature, kind of container, type of plant/variety (cereal, legume), surface area (flour or seed), nature of fermenting medium (natural or cultured) and whether samples are fermented separately or together(Oyerakua, 2013). Reports on the effects of fermentation on nutrients and anti-nutrients vary. Mugula *et al.*(2003)detected reductions in the content of soluble sugars and starch during fermentation. This was associated with hydrolysis of starch to form acids. When boiled pigeon pea was milled into a paste, wrapped in flame-blanched plantain leaves and naturally fermented for five days, ash and crude protein content of the flour increased from 4.61 to 5.52% and 21.88 to 23.90%,respectively (Adebowale and Maliki, 2009). In the same study, it was reported that fat, carbohydrate, bulk density, and water absorption capacity of pigeon pea flour

decreased. During fermentation, the acids (ethanol and organic acids) producedto inhibit the proliferation of pathogenic organisms (Bora, 2014). Tufa *et al*. (2016), in their work on maize/soybean blend, reported that fermentation significantly ($p<0.05$) lowered tannin, phytate, iron, calcium, and zinc but increased fat and carbohydrate levels. Fermentation increased the fat level in millet but decreased it in pigeon pea(Mbaeyi and Obetta, 2016). The increased activity of the lipolytic enzymes during fermentation was implicated in the reduction of fat. However, these effects were time-dependent as also noted by Onweluzo and Nwabugwu (2009) who reported that twenty-four (24) hours fermentation reduced crude protein levels in both pigeon pea andmilletflours but the significant increase occurred after seventy-two (72) hours. They also observed increased content of fat and energy while ash, tannin, phytate and cyanide contents decreased. Both Onweluzo and Nwabugwu (2009)and Tufa *et al.*(2016)observed a decrease in bulk density, water absorption capacity and increased least gelation concentration of the cereals and legumes with fermentation. Another study also recorded increased protein, carbohydrate, fat, iron and zinc levels in sorghum while fibre, ash, calcium, tannin, and phytate composition decreased during fermentation (Kinyua *et al.,* 2016). Wakil and Kazeem(2012)assessed the effect of starter or controlled fermentation on the nutritive and sensory properties of sorghum-cowpea complementary food. It was observed thatcrude protein content increased asthe fermentation period increasedwhile moisture, crude fibre, total ash,ether extract, total carbohydrate, and anti-nutrients decreased. Natural lactic acid fermentation had very poor results when compared to fermentation with lactobacillus and yeast controlled fermentation.These researchers suggested the use of cowpea to fortify sorghum-based complementary food. They also proposed the use of fermentation with mixed cultures of *Lactobacillus Plantarum* and yeast to improve protein and organoleptic properties and reduce anti-nutrients. Lactic acid fermentation reduced tannin content and improved protein quality resulting in increased iron absorption (Onyango *et al*., 2005). In another study, natural co-fermentation of maize/pigeon pea and sorghum/mucuna particularly after seventy-two (72) hours increased the crude protein, crude ether and reduced carbohydrate, ash and anti-nutrients content of the blend(Oyerakua, 2013). This study revealed thatthe co-fermentation of maize and pigeon pea resulted in greater

reductions in anti-nutrients than that of sorghum and mucuna but not to the recommended levels.

2.5.2 Germination

Germination is an age-long household processing method as fermentation. It involves soaking of grains or legumes for a specified period and allowing the moistened product to sprout or malt using its endogenous carbon source/starch in the presence of oxygen, light and favourable temperature. During germination of cereals, alpha-amylase hydrolyse starch (amylose and amylopectin) to simple sugars,dextrins and maltose (Sade, 2009). This reduced the viscosity of thick cereal porridges, thereby producing thin gruels with higher energy and nutrient density (Nnam 2000). Sprouted seeds were susceptible to enterobacteria, fungi and bacillus contamination (Thompson and Amoroso 2011). Therefore, it was recommendedthat cereal grains should be treated with chemicals such as 70% ethanol, formaldehye (0.2%) or sodium hypochlorite (1%). The grains may also be periodically rinsed during germination and the germinated flour decontaminated by heating before use. Germination reduced the oligosaccharides (stachyose and raffinose) and anti-nutrients present in legumes (Bora, 2014). Increased period of germination (Agostini *et a*l., 2010) and temperature (Liang *et al.,* 2008) resulted in a greater decrease in phytic acid. Germination has been shown to improve the functional properties and nutrient quality of complementary foods (Tufa *et al.,* 2016). Soaking (12 hours) and germination (24 hours) of green gram, cowpea, lentil, and chick pea resulted in reductions in levels of ash, fat, and anti-nutrients while protein, total dietary fibre, and thiamin levels increased significantly (Ghavidel and Prakash, 2006). They argued that the significant decrease in the mineral content could be a result of leaching duringthe soaking processbefore germination. Ghavidel and Prakash (2006) also observed that the percentage of bioavailable iron increased significantly in germinated samples and correlated negatively with phytic acid, tannin, and total dietary fibre contents.Addis *et al.*(2013) formulated and evaluated the physicochemical properties and acceptability of a reduced bulk nutrient-dense complementary food. It was observed thatthe addition of 5% malted finger millets to the blend of sorghum, malted pigeon pea, soybean, and skimmed milk reduced the high viscosity of the formulated complementary food from 23733 to

450 mPas.Malting increased the fat and moisture but reduced protein, carbohydrate, energy, ash and fibre contents of complementary foods formulated from malted quality maize and steamed cowpea (Ikujenlola and Adurotoye, 2014).In this study, quality protein maize seeds were steeped for eighthours, spread to germinate for seventy-two (72)hours,watered twice daily, oven-dried,sprout separated,milled, sieved and packaged.The viscosity, bulk density, and water absorption capacity were also found to be lowered in the malted samples. When sorghum and pigeon pea were processed (fermentation and germination) into an instant complementary food, it was observed that malting increased carbohydrate, fat, and zinc while fibre, total ash, calcium, iron, phytate and tannin contents were reduced (Kinyua *et al*., 2016). However, reductions in ash and phytate were observed to be greater in malted samples while Makokha *et al.* (2002) reported that fermentation and not malting removed more phytate in sorghum and finger millet.

2.5.3 Combined Strategies

A combination of processing methods is suggested to be more effective in eliminating anti-nutritional factors in foods (Larsson and Sandberg, 1992). A combination of methods such as germinating and then fermenting grains and legumes (Inyang and Zakari, 2008; Hemen *et al*., 2012; Okoronkwo *et al.,* 2016) among others have been employed. Thacher *et al.* (2009) indicated that enzymatic methods of dephytinization reduced phytate concentration of typical maize meal while fermentation had no significant effect. Germination and roasting processes were reported to reduce the phytate composition of maize and broad beans by 33% (Kebebu *et al.,* 2013).In a research carried out by Inyang and Zakari,it was reported that combined germination and fermentation of millet increased the protein content of seeds ofpearl millet.These seeds were soaked at room temperaturefor twelve hours, sprouted for 48 hours, washed, then fermented for 48 hours, dried and milled to flour (Inyang and Zakari, 2008). The flour was then used to produce germinated-fermented '*Fura'*which was compared with germinated '*Fura',*and traditional '*Fura'.* Result of the analysis showed significant ($p<0.05$) rise in the protein level of

germinated-fermented'*Fura'* (10.67%) compared to the control (8.82%) which was also higher than the protein levels of germinated '*Fura'*(10.39%) and fermented '*Fura'* (9.18%). It was also reported that fat, carbohydrate including phytic acid contents reduced while calcium and iron levels increased. Incorporation of animal source foods reduced the effect of phytate in complementary foods. Robust probiotic digestive health produces more phytase that can help to neutralize some phytic acid.Assessment of anti-nutritional factors and reducing the level to the recommended levels through simple technology should be one of the priorities included in the formulation of complementary foods to ensure nutrient bioavailability.

2.6 Complementary food formulation and evaluation

2.6.1 Cereal/tuber-legume based complementary food formulations

High phytate content, low dietary diversity, and animal source foods have been related to poor quality complementary food (Stewart *et al*., 2013).This has generated a lot of research interest in complementary feeding. Consequently, several strategies have been suggested to improve complementary feeding practices.The literature is replete with works done on complementary food formulations and evaluations. These complementary foods have been produced with a range of locally available ingredients in ways to conform to recommendations on infant and young child feeding. Most of the formulations are cereal-based, enriched with legume with or without animal source foods and/or micronutrient fortification. These are processed into flourrequiring reconstitution with water and cooking to form porridge before consumption. Predominant methods of processing grains and legumes include dehulling, soaking or fermentation with or without agents and germination.

Various blends of cereal or tuber (maize, sorghum, millet, plantain, sweet potatoes, cassava among others) based complementary food supplemented with one or more legumes- soyabean, groundnut, cowpea among others have been formulated (Wakil and Kazeem, 2012; Oyarekua, 2013; Aderonke *et al*., 2014; Ikujenlola and Adurotoye,

2014; Ewuola *et al*.,2015; Kinyua *et al.,* 2016; Okoronkwo *et al.,* 2016; Tufa *et al.,* 2016; Adeola *et al.,* 2017; Ige 2017; Oludumila and Enujiugha 2017; Okudu *et al.,*2017).Cereal-legume blend or double mix is widely used in the processingof complementary foods because their amino acid contents complement each other in relation to lysine and sulphur amino acids.corn-soy-blend plus (CSB+) and corn-soy-blend plus plus (CSB++) food supplements havebeen promoted for utilization in the management of moderate acute malnutrition (WFP, 2010).

In a study mentioned earlier(Oyerakua, 2013), sorghum was co-fermented with mucuna (S/M) while maize was co-fermented with pigeon pea (M/P). The result of the proximate analysis revealed that the crude protein contentsof S/M and M/P blendswere10.38and10.32 g/100g, respectively. The zinc and iron contents were 0.68, 1.23 mg/100g and 0.75, 0.93 mg/100g, respectively. Ikujenlola and Adurotoye (2014) observed that 30% cowpea substitution in malted quality protein maize increased protein, ash, moisture, energy but reduced carbohydrate content of the blend. The moisture, protein and fibre contents of the blends ranged from 5.68-10.32%, 7.80-15.21%, and 3.67-5.08% while ash and fat content were 0.66-1.53% and 1.80-2.51%, respectively. However, calcium (22.67-33.34 mg/100g), iron (0.26-0.39mg/100g) and magnesium(24.15-31.05mg/100g) levels did not meet the stipulated levels for complementary food.Some researchers studied the proximate and mineral contents of composites from fermented maize (M), soybean (S) and pigeon pea (P) in the ratio of 70:15:15 for the three ingredients, and 70:30 for maize and either soybean or pigeon pea (Aderonke *et al.,* 2014). Results recorded for calcium, iron and zinc, ranged from 17.4mg/100g (fermented maize flour) to 48.7mg/100g (maize:soybean:pigeon pea); 4.82mg/100g(fermented maize flour) to 10.12mg/100g (maize:soybean:pigeon pea) and 2.75mg/100g (fermented maize flour) to 6.55mg/100g (maize:soybean:pigeon pea). The crude protein levels were 20.75% in MSP and 19.6%;16.7% in MS and MP blend, respectively.

A lot of work has been done on improving the quality of double mix complementary foods to meet the minimum dietary diversity as recommended by the World Health Organisation. This ensures thatat least one animal source food and either fruit or

vegetable are consumed in combination with the staple foods in one meal.In some works, cereal/tuber:legume blends have been enriched with vegetable and/or fruit sources (Onabanjo *et al.,* 2008a; Onabanjo *et al*., 2009a; Yusuf*et al*., 2013; Shiriki*et al*., 2015; Achidi *et al*., 2016; Mbaeyi and Obetta, 2016; Fikiru *et al*., 2017; Gbadamosi *et al*., 2017).Mbaeyi and Obetta(2016) examined the nutrient, functional and microbial properties of complementary foods formulated from millet, pigeon pea andseedless breadfruit (*Artocarpus altillis*) leaf powder. On analysis, the ranges of crude protein, ash, fat and crude fibre contents of the composites were 14.59-23.45%, 3.01-3.48%, 2.77-4.85%, and 4.76-10-75%, respectively. In a study, the effect of combination ratio of maize, malted barley, roasted pea and carrot on total carotenoids, micro-nutrients and anti-nutritional factors using D-optimal mixture designwas evaluated. The calcium, phosphorus,zinc, ironof the 14 formulations ranged from 70.0-90.1 mg/100g, 42.0-68.1mg/100g, 2.0-3.4mg/100g, and 4.3-5.5 mg/100g, respectively, while phytic acid, condensed tannin, and total carotenoids ranged from2.41-2.71g/100g,2.0-4.52g/100g and1238-2464 µg/100g, respectively (Fikiru *et al.,* 2017).They observed that the mineral contentsand anti-nutritional factors increased with more proportion of pea but that the least phytic acid content was observed in the blend with the highest content of malted barley which they attributed to increased phytase activity. This is a typical triple mix (cereal:legume:vegetable) with mineral levels lower than recommended and high phytate content.

Another work on cereal:legume:vegetable-based complementary foods compared complementary foods formulated from germinated wheat (W), soybeans (S) and carrot (C)in the ratio of 60:30:10 (WSC) and 50:30:20 (WSD) with wheat and soybeans blend (WS) (60:40). The results of the analysis of WSC, WSD and WS showed24.7, 25.0, 22.4%; 1.3,1.3,2.6%;16.3, 16.3, 1.73% for crude protein, crude fat, crude fibrecontents respectivelyand 10.1, 11.9, 12.0; 5.5, 6.7, 6.6 mg/100giron and zinc levelsrespectively (Gbadamosi *et al*., 2017).These blends had a better mineral profile and lower anti-nutrient contents than the maize-malted barley-roasted pea-carrot blend formulated by Fikiru *et al.*(2017). This difference may be attributed to the inclusion of soybean and wheat (high phytase). Blends of banana and Bambara groundnut (Ijarotimi and Olopade, 2009) or

banana and soybean or cowpea (Martin *et al*., 2010)were also formulated. Blending banana with 10-20% cowpea improved protein level from 9.58 to 13.74%, while 10% soybean addition increased it to 17.87%(Martin *et al.,* 2010).Onabanjo *et al*., (2008a) formulated complementary foods from cassava roots, soybean, groundnut, cassava leaves, and carrot-based on WHO/FAO codex standard using Owl Tech Wizard software. It was reported that iron, zinc,and calcium ranged from 7.38-7.54mg/100g, 6.2-6.3mg/100g, and 238.0-270.0 mg/100g dry flour respectively.The four diets provided adequate amounts of most minerals except for iron and zinc. It was concluded that the inclusion of animal source foods improves the protein quality of complementary foods.

Several blends of cereal, legume, and animal source foods have also been formulated (Addis *et al*., 2013; Ijarotimi and Keshinro, 2013; Adepoju and Ajayi, 2016; Fasuan *et al.,*2017).After processing of complementary foods, the nutritional constituents of complementary food produced from white sorghum(*Sorghum bicolor*), sesame seeds (*Sesamum indicum*), carrot(*Daucus carota*), crayfish (*Euastacus spp*) was assessed (Onabanjo *et al.,* 2009a).According to the report, the moisture, ash,crude protein, crude fibre, crude fats, iron, zinc, total carotenoids, and vitamin C levels were significantly higher in the composites than in the control (Cerelac), but the control had higher total carbohydrate and calcium than the composites. This report supported the fact that quadruple complementary mix (contains foods from at least 4 food groups) was superior, compared to either cereal-legume only mix or cereal-legume-animal source food/vegetable/fruit mix.The work of Addis *et al.* (2013)also supported that quadruple mix complementary food was better in nutritional content than double or triple mix blends. In their study, complementary food was formulatedfrom popped sorghum, germinated pigeon pea, toasted soybean (200^{o}C,20 minutes), skimmed milk and sucrose in the ratio of 65:15:10:5:5, then combined with 5% malted finger millet (95:5). The formulated complementary food contained protein-16%, fat-4%, and dietary fibre-12.8%. It was concluded that although the complementary food met the recommended levels for macronutrients, fortification may be required for some micronutrients. The energy content of processed complementary foods could be increased by including oil or oilseeds such as groundnut and sesame seedsin the formulation. The addition of oil seeds does not

only increase fat and energy content but also improves protein content. Whenmalted maize, roasted sesame seeds, and crayfish were combined to formulate complementary foods, the blends had 13.26-25.59% and 20.78-28.09% ranges of fat and protein content respectively (Fasuan *et al.,* 2017). They also observed that both protein and ash content increased as the quantity of crayfish increased.

2.6.2Functional and microbiological characteristics of cereal-based complementary foods

Apart from nutrient and anti-nutrient contents of complementary foods, the physical characteristics and safety are very important. The common functional properties of food include viscosity, bulk, and loose density, water absorption capacity, swelling capacity, least gelation concentration, gelling temperature, solubility, dispersability among others. Pasting properties that include peak and final viscosities, trough, breakthrough, set back, peak time and pasting temperature are part of functional characteristics investigated. Processed complementary foods are usually in dry flour form and would require reconstitution with water and sometimes cooking before consumption. The effect of such treatments on the functional properties influences the energy and nutrient density of complementary foods and therefore the nutritional quality. Moisture content of the food also affected the microbial load with time (Laroche *et al.,* 2005).Low viscosity, bulk density, and water absorption capacity are desirable qualities of complementary foods. The methods of processing the ingredients used for the formulation of complementary foods greatly influence functional and microbiological characteristics. Thus, many researchers investigate these properties in addition to the chemical composition.

According to the report of a work done on the functional properties of cereal-legume blend, the bulk densities of unmalted quality protein maize (QPM), malted QPM, 70% unmalted QPM-30% cowpea and 70% malted QPM-30% cowpea were 0.73g/ml, 0.68g/ml, 0.70g/ml and 0.65 g/ml, respectively (Ikujenlola and Adurotoye, 2014). The bulk density of the unmalted QPM significantly reduced from 0.73 to 0.70 g/ml when

30% of the maize was substituted with cowpea. Similarly,the substitution of malted QPM with 30% cowpea reduced the bulk density from 0.68 to 0.65 g/ml while it increased the water absorption capacity (WAC) from 310 to 361 % at room temperature.Aderonke *et al.*(2014) also observed that the WAC, least gelation capacity and swelling power increased as legumes were added to fermented maize. The binding property of protein bodies is one major factor that determines the water absorption capacity of foods. A greaterquantity of legume in formulated complementary food results in higherwater absorption capacity (Gbadamosi *et al*., 2017). A report revealed that fermentation of millet for forty-eight (48) hours increased the swelling power from 1.58% to 1.89%, water absorption capacity from 1.52g/g to 2.17g/g, least gelation concentration from 8.00% to 19.25% and viscosity from 220 cPa to 310 cPawhile it reduced the bulk density from 0.67g/ml to 0.61g/ml (Mbaeyi and Obetta, 2016).

The moisture content of food affects the microbial load with time (Martorell 2007). The higher the moisture levels of formulated foods, the greater the risk of contamination. In a study to assess the microbial load of maize-based complementary foods, it was reported that the total viable count of the toasted and oven-driedcrayfish enriched ogi ranged from 1.2 to 2.5 x 10^3cfu/g(Ajibola *et al.,* 2016). Molds (1 x 10^3cfu/g)were detected in both blends while yeast was found only in the oven-dried crayfish enriched complementary food. The researchers concluded that the total viable counts recorded for all the formulated diets were below the recommended maximum level of 1.0 x 10^5.Similarly, microbiological analysis of complementary foods formulated from fermentedpigeon pea and milletflour combined with breadfruit leaf powder revealed 1.0 x 10^3 cfu/g of the mold but higher total viable count (2.0-2.9 x 10^4) (Mbaeyi and Obetta, 2016).

2.6.3Evaluation of nutritional quality of cereal-based complementary foods using animal models

The adequacy of formulated complementary foods to support optimal growth and micronutrient status of young children is evaluated using experimental animals (Onabanjo *et al*., 2009b;Adepeju *et al*., 2014; Abiose *et al*., 2015;Amagloh *et al*., 2015;Okoronkwo *et al.,*2016).There is a need to evaluate the adequacy of formulated complementary foods in terms of bioavailability of vital nutrients using animals before giving to children. The assessment with animals would also provide the researcher with information on any allergic, toxic or negative effect of the blend for necessary adjustment. Many of the animal studies utilized albino Wistar rats (*Rattus novergicus*) in controlled feeding trials to evaluate the nutritional quality of processed complementary foods (Onabanjo *et al*., 2008b; Onabanjo *et al*., 2009b; Adepeju *et al*., 2014; Abiose *et al*., 2014).Rats are the smallest monogastric animals which share almost the same gastric morphology with humans and have been confirmedgood alternative to humans (Ochei and Kolhatkar 2009).Rats are quite accessible, affordable and easier to manage.

Quality Protein Maize (QPM) is better than normal maize (NM)and this has been confirmed through several controlled trials. The QPM has more lysine and tryptophan and as such expected to enhance more growth than common maize. In arandomized controlled trial, the nutritional quality of complementary foods formulated from malted and fermented QPM or NMfortified with soybean was assessed using 160 white rats aged 3-4 weeks (Abiose *et al*., 2015). Formulated diets prepared at 10% protein level were given to the rats for twenty-eight (28) days while one group was fed a non-protein diet. Dietary intake and growth changes were monitored regularly. At the end of the feeding period, protein efficiency ratio(PER) and feed efficiency ratio (FER) of the complementary food from malted QPM fortified with soybean were the highest(2.44 and 0.24, respectively). These were comparable to those of casein(2.5) and a commercial diet(2.3). The packed cell volume increased from 23% (basal) to 46%(soy fermented NM). The QPM-based diets particularly the soy malted QPM had better biological value, protein digestibility and supported the fast recovery of protein depleted rats than normal maize-based diets. In another study, Onabanjo *et al*.(2009b)evaluated the protein quality

and heamatological parameters of rats fed complementary diets processed from cassava roots,soybean, groundnut, cassava leaves, and carrots.The diets were formulated by mixing the quantity of complementary foods that gives protein levels of 10% with 10% corn oil, 1% vitamin and mineral mix, 1% oyster shell, 2% bone meal, 0.25% salt, 5% non-nutritive cellulose and corn starch. Each dietwas made up to 100%. In a randomized controlled trial, 21 day old male Wistar rats weighingabout 30g were divided into six groups of eight rats each in a randomized block design and given the formulated diets for twenty-one (21) days while a group was fed a non-protein diet. The residual food was collected daily, dried and weighed. Dietary intake and growth changes were assessed constantly. In the end, all were sacrificed and the blood sample collected for haematological analyses. Faecal and urinary discharge were also collected and utilised for nitrogen analysis. The weight changes, protein and feed efficiency ratios, true digestibility and net protein utilisation were evaluated.It was observed that the control and the other test diets gave similar results forfeed efficiency ratio (FER), protein efficiency ratio(PER), net protein utilization (NPU),biological value, true digestibility. The rats on the casein diet had the highest mean weight gain while total mean weight gain ranged from 28.50g to 68.75g.The protein efficiency ratio of all the diets ranged from 2.37-2.54 and all the cassava-based complementary diets met the recommendation (> 2.3). Haemoglobin and packed cell volume (PCV) levels of the rats fed the test diets were significantly higher than those fed casein and basal diets. They concluded that the test diets are suitable for maintaining healthy nutritional status in weanling rats.

In another study, the effect of complementary food formulated from mature but unripe breadfruit (B) (*Artocarpus altilis*), soybean (S) (*Glycine max*), groundnut (G)(*Arachis hypogeal*) on the weight and haematological parameters of albino ratswas investigated (Adepeju *et al*., 2014). The blends were formulated as Diet 1- 100:0:0, Diet 2- 80:10:10, Diet 3-80:5:15, Diet 4- 80:15:5 (B:S: G) ; Diet 5- 80:20(B:S),Diet 6- 80:20(B:G) and Diet 7--Commercial complementary food.Forty-five Wistar rats of both sexes were weighed and allocated into nine groups of five rats in well ventilated metabolic cages (randomized controlled trial). A group of rats served as zero-day animals and blood samples collected through cardiac puncture. They were fed the formulated

diets 1-6 and control diet for twenty-eight (28) days. Blood samples were collected 14th and 28th days. Dietary intake and growth changes were measured regularly. At the end of the trial, it was found that the weight of the animals fed the basal diet decreased with time. Diet 6(breadfruit and groundnut) supported the highest weight gain among the formulated diets and compared well with the control while the mixed diet at a 10% level supported the least weight gain. The PCV ranged from 22%(basal) to 50.67%(diet 3). The white blood cells counts (WBC) of the rats that were fed the basal diet were higher than those that were fed the other formulated diets but all decreased as the study progressed. It was concluded that breadfruit-based complementary foods can support growth in rats and thus infants.

In a similar animal study (Shiriki *et al*., 2015), feeding of rats with diets based on maize, soybean, peanut at a ratio of 60:30:10 and fortified with 5%, 10% and 15% moringer leaf powder, resulted to PER values less than the recommended value of 2.1. PER values increased significantly ($p<0.05$) up to 10% leaf substitution (1.90) from 1.77 in the unfortified blend but reduced at 15% substitution level (1.69) while the net protein ratio (NPR) and Feed efficiency ratio (FER) ranged from 1.89 to 2.78 and 3.10 to 3.39, respectively.

Ajibola *et al.* (2016) investigated the nutritional quality of maize-crayfish based complementary foods formulated using combined processing methods. The maize grains were germinated for seventy-two (72) hours and then fermented for twenty-four (24) hours. The milled grains were divided into two portions and separately toasted at 80^{o}C and oven-dried at 50^{o}C and then enriched with crayfish powder. The weight gain of the rats fed with Cerelac and casein were higher than those fed with the maize-crayfish based diets. The two formulated diets had similar growth effects on the rats which were significantly better than those fed with ordinary ogi. The formulated diets and control diets did not influence the haematological variables ($p>0.05$). The feed efficiency ratio varied from 0.22g (toasted crayfish enriched ogi) to 0.26g (casein-based diet) and the net protein ratio varied from 2.60 (toasted crayfish enriched ogi) to 3.25 (casein-based diet). True digestibility (76.0-89.5%), biological value (66.5-87.6%) and net protein utilization (50.5-77.4%) values were lowest in the toasted diet.

The PCV of male Wistar rats fed feeds with 100% raw pigeon pea was significantly lower ($p<0.05$) (35%) than values for other groups including the control group (43.8%) (Soetan *et al*., 2017). The WBC counts differed significantly ($p<0.05$) among treatment groups. The blood urea nitrogen (BUN), Aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity recorded in the study ranged from 10.66-15.90mg/dl, 37.40-42.00u/l, 20.40-31.40u/l, and 108.40-114.60u/l, respectively. There were some distortions in the histology of liver cells but no lesion was detected in the histology of the kidneys.

2.6.4Evaluation of Complementary foods using human feeding interventions

Also, numerouscommunity based complementary feeding trials have been undertaken to evaluate the effect of processed complementary food on nutritional status ofinfants and young children (Adu-Afarwuah *et al*., 2008; Lin *et al*., 2008; Bisinwa *et al*., 2012; Phu *et al*., 2012). Most of these complementary feeding interventions were preceded by acceptability studies to assess acceptability and pitfalls before large scale studies (Bisinwa *et al*., 2012; Owino *et al*., 2014). A longer period of acceptability study gives an in-depth assessment of a child's or mother's reaction to formulated complementary foods than a cross-sectional or one-day sensory test (Paul *et al.,* 2008).

2.7Pigeon pea nutrition

Pigeon pea (*Cajanus cajan [L]. Millsp*) is known by several names which include red gram, congo pea, tuar, Angola pea, dahl among others (Mula and Saxena 2010). The cultivation of pigeon pea dates back to about 3500 years in India. India still contributes over 90% of the production in the world (Odeny, 2007; Saxena *et al.,* 2010). It came to East Africa and West Africa from India, where it was first encountered by Europeans and obtained the name Congo pea. Studies on pigeon pea over the years have recorded varying chemical constituents and nutritive values particularly, protein profile. The variations were attributed to genetic differences, differences in environmental conditions

where it is grown, methods of sampling, storage, and analysis among other variables (Saxena *et al.,* 2010). Pigeon pea contains about 20%-26% protein, 2.2%-7.5% crude fibre, 1.62%-4.43% crude fat and 58%-62% carbohydrate (Onu and Okongwu, 2006;Adebowale and Maliki, 2009;Onweluzo and Nwabugwu, 2009;Ajayi *et al*., 2010). Although some anti-nutritional constituents such as trypsin inhibitors, hemaglutinin, phytic acid, saponin, and oxalates are present (Onweluzo and Nwabugwu, 2009), they can be eliminated by boiling, soaking and other processes (Fasoyiro *et al.,* 2005;Onu and Okongwu, 2006; Ajayi *et al*., 2010). Moreover, the cream or white seeded variety largely grown in Africa contains relatively less anti-nutritional factors (Odeny, 2007).

There are contrasting reports on the effect of fermentation and sprouting alone or in combination with the chemical composition and functional properties of pigeonpea particularly protein quality (Adebowale and Maliki, 2009; Onweluzo and Nwabugwu, 2009; Fasoyiro *et al*., 2010; Mbaeyi and Onweluzo, 2010). Pigeon pea has been reported to possess antioxidant activities due to the high content of phenol compounds (Oboh, 2006). Pigeon pea seeds in form of vegetable, dry seeds, and dehulled seeds are only consumed after cooking. This makes cooking time and other related factors very relevant issues in pigeon pea nutrition. Many studies have investigated the effects of cooking time, different cooking methods, various processing and handling methods on the nutritional and organoleptic properties of pigeon pea (Onu and Okongwu, 2006;Fasoyiro *et al.,* 2010). In summary, it has been inferred that cooking pigeon pea enhanced the bioavailability of nutrients and at the same time destroyed some anti-nutritional factors in it. However, the lines which take a longer time to cook generally face the danger of losing important nutrients from food. Long cooking time is the major challenge to pigeon pea consumption in Nigeria. As an alternative protein source for livestock feed, pigeon pea has undergone various nutritional studieswith several livestock species. The majority of the researchers concluded that the inclusion of boiled pigeon pea seeds in animal feed at not more than about 30% by weight improved the growth of the experimental animals (Onu and Okongwu, 2006;Ahamefula *et al*., 2007; Ani and Okeke, 2011).

2. 8Maize (*Zea mays*)

Maize (*Zea mays*), also called corn, is a very common cereal which belongs to the grass family. Maize is one of the major food crops and widely distributed throughout the world. The highest producer of maize in the United States of America (42%).The introduction of maize to Africa dates back to the 1500s (International Institution for Training in Agriculture (IITA)).Nigeria is the largest African producer of maize with about 20 million tonnes (Udegbunem, 2019). Maize exists in so many grain colours, shapes, sizes, and textures. The most common varieties are the white, yellow and red types. Maize is used as human food, animal feed, biofuel, and industrial raw material. Nutrient composition varies across geographic regions. The protein content of maize is about 9g/100g dry weight, but it is low in calcium, vitamin B12, ascorbic acid, lysine, and tryptophan unless fortified (FAO 1992).

2.9Fish

Fish is an animal source food that provides high-quality protein, vitamins, and minerals.Protein content ranges from 16.24-68.4mg/100g in fresh and dried catfish respectively (Olayemi *et al.,* 2011). It also possesses omega 3 and omega 6 fatty acids which are very important for brain development. Fish is readily available and affordable compared to meat (Ikutegbe and Sikoki 2014). It can be consumed fresh, frozen, canned, smoked or dried and fried. The majority of the frozen fishes consumed in Nigeria are imported. Common frozen fishes in Nigeria include mackerel (titus), herring (sawa), horse mackerel (kote), blue whiting (panla), Argentina silus (ojuyobo) and croaker fish among others.Fish is highly perishable, and prone to contamination with microorganisms if not hygienically handled. Amusan *et al.,* (2019) reported that about 16.9% of fishes found marketed in Lagos were contaminated with *Listeria spp*. Therefore appropriate processing is very crucial when fish is used in food preparation, particularly complementary food formulation. Its nutritional quality in complementary feeding has been widely studied with a positive influence on some growth parameters (Lin *et al*., 2008).

2.9.1*Micromesistius poutassou* (blue whiting)

Blue whiting, commonly referred to as *'panla*' is a specie of *Micromesistius* in the cod family. It is not grown in Nigeria. It is found in the northeast Atlantic Ocean, across Iceland and northern parts of the Mediterranean.Blue whiting is a small lean fish measuring about 22-30cm in length (Amusan *et al*., 2019). It is a relatively abundant and nutritious marine fishconsumed widely in Lagos and other Southwestern states (Kolade, 2015). It is available in fresh, smoked and fried forms. Its moisture, protein, fat and carbohydrate contents are approximately 75.0%, 18.1%, 2.6%, and 2.4% respectively (Kolade, 2016). According to Kolade (2016), *Micromesistius poutassou*also possesses appreciable quantities of minerals.

2.10*Telfairia occidentalis* (Fluted pumpkin)

The fluted pumpkin fruit is quite large and harbours the seeds which produce the dark green leaves when planted. Fluted pumpkin is common among the southerners where it is referred to as 'ugu'. The fruit, oilseeds, shoots, and leaves are edible.and nutritious. Ugu leaves are nutritious and belong to the green leafy vegetable group. Fresh *Telfairia occidentalis* leaves possess about 81-83% moisture, 0.03-0.53% protein, 0.30-0.90% crude fat, 2.09-4.76% crude fibre, 0.37-0.47% ash and 12.76% carbohydrate (Okonwu *et al*., 2018).*Ugu* leaves have in addition to nutrients, some antinutritional factors (Aletor and Adeogu, 1995). These include oxalate (68 mg/100g), tannin (35 mg/100g), phytic acid (85 mg/100g) (Ekpenyong *et al*., 2012). *Ugu* leavesare used mainly for soup preparation. It can also be steamed and served as a vegetable with staples.

2.11*Daucus carota* (carrot)

Carrot is a root vegetable rich in carotenoids, dietary fibre and other minerals (Krishan 2012). The largest producer of carrot is China (FAO 2008). Carrots roots are consumed raw or used in the preparation of salads. It can also be eaten with staples, juiced, diced, mashed and dried into a powder. Carrot is considered as a functional food with health benefits because of its high content of beta-carotene, ascorbic acid, and tocopherol

(Hager and Howard, 2006). Carrot roots have about 86-88% moisture, 0.9% protein, 0.2-0.5% crude fat, 1.2%crude fibre, 6.0-10.6% carbohydrate, 1.1% ash, 4.0 mg/100g vitamin C, 5.3 carotenoids,0.4-2.2 mg/100g iron, 80 mg/100g calcium and 0.2-1.0 mg/100g zinc (Gopalan 1991, Holland 1991). Controlled thermal processing frees the available carotene but increased temperature and time reduces carotenoid contents (Krishan 2012). A higher quantity of beta-carotene is retained when carrots are blanched for short periods and dehydrated (Negi and Roy 2001).

CHAPTER THREE
MATERIALS AND METHODS

3.1Study design

The study was experimentalin design and conducted in two phases: formulation/analysis of diets and animal experimentation.In the first phase, maize and pigeon pea seeds were fermented, germinated and germinated-fermented separately. The processed seeds were analysed for nutrients and antinutrients contents to select maize and pigeon pea seeds that had lowest phytate contents (>80% reduction) and retained more iron, calcium, zinc and protein contents after processing. The selected processed maize (one) and pigeon pea (one) seeds were combined with fish,carrot, pumpkin leaf powder to formulate CompifO. Another batch of raw maize and pigeon pea seeds was soaked separately for four hours and roasted at 110^0C for about 60 minutes to produce roasted maize and roasted pigeon pea seeds. The roasted seeds were combined with the same ingredients in the same ratio to formulate CompifR. The nutrient, anti-nutrient, functional and microbiological properties of the formulated foods were investigated.

In the second phase, nutritional quality(growth performance characteristics, protein quality parameters, haematological and histological characteristics) of the formulated foods were evaluated using Wistar rats.

3.2Study location

Processing of samples was carried out at the Department of Human Nutrition, University of Ibadan Laboratory as well as Department of Chemistry and Food Sciences, Bell's University of Technology laboratory. Analyses of samples were carried out at the laboratory units of the Departments of Human Nutrition and Animal Science, University of Ibadan. Anti-nutrient contents of the formulated food were evaluated at SMO Laboratory, Joyce B, Ibadan. The animal study was undertaken at the animal house, Department of Animal Science, University of Ibadan

3.3Ingredient identification, collection, processing, and analysis.

3.3.1Ingredient identification and collection

Quality Protein Maize (ART 98/SW6-OB) is a white maize (*Zea mays*) variety with improved lysine and tryptophan content. Pigeon pea (*Cajanus cajan*) varieties (NWSCC 34 and 34A, and 7D) were collected from the Institute of Agricultural Research and Training (IAR&T), MOOR Plantation, Ibadan. Variety 7D had lower phytate content and related nutritional qualities to the market variety (Iseyin) and was selected. Blue whiting (*Micromesistius poutassou)* was obtained from a cold roomopposite Grait International College, Ota, Ogun State. Carrots (*Daucus carota*)were purchased from a fruit and vegetable shop beside Justrite supermarket Ota, Ogun State while pumpkin leaves were purchased from a farm in Ota. Grand soya oil (UAC) and granulated sugar (Dangote) were purchased from a minishop opposite Bells University of Technology, Ota, Ogun State.

3.3.2Ingredient preparation and processing

Production of fermented pigeonpea and white maize seeds flour: One kilogram of pigeon pea seeds and 1kg of white maize seeds were cleaned, washed and soaked separately in excess tepid water (1:3w/v) and allowed to ferment naturally for 72 hours at 28^{o}C (Onweluzo and Nwabugwu, 2009). The fermented seeds were washed and oven-dried at 60^{o}C for 19-22 hours, partially milled, dehulled, milled and sieved with 450 μm pore sieve into maize and pigeon pea fermented flours.

Production of sprouted pigeonpea and white maize seeds flour: About 2kg of pigeon pea and 2kg of white maize seeds were cleaned, soaked separately in excess tepid water for 24 hours (rinsed at 12th hour and 24th hour), washed and spread on moist jute bags, then washed, drained and spread again at 24th hour and 48th hour of germinationand allowed to continue to sprout at room temperature till 72 hours, a modified method of Onweluzo and Nwabugwu(2009). The seeds were turned and water sprinkled once a day to encourage aeration. Half of the sprouted seeds were oven-dried at 60^{o}C for 18-21

hours. The rootlets and hulls were removed and seeds weremilled, sieved with a 450µm pore sieveand tagged asmaize and pigeon pea sprouted flour (2 products).

Production of sprouted fermented pigeonpea and white maize seeds flour: The remaining (half) of the sprouted seeds weredivided into two and fermented separately in water for 24 and 48hours. The sprouted fermented seeds were oven-dried at 60^{o}C for 20-22 hours, dehulled, milled and sieved with a 450µm pore sieve into maize and pigeon sprouted fermented flours (4 products).

Roasted pigeon pea and maize seeds flour: Whole maize and pigeon pea seeds weresoaked in water for 4 hours (Addis *et al*., 2013**)**, drained and roasted at about 110^{o}c in the oven for about 75 minutes with intermittent stirring. The toasted seeds were partially milled, dehulled and milled into flour. The flour was sieved with 450µm pore sieve.

The flours obtained were stored in Zip-lock bags (26.5cm x 27.3cm, BJohnson) at 4^{o}C for analysis and diet formulation.

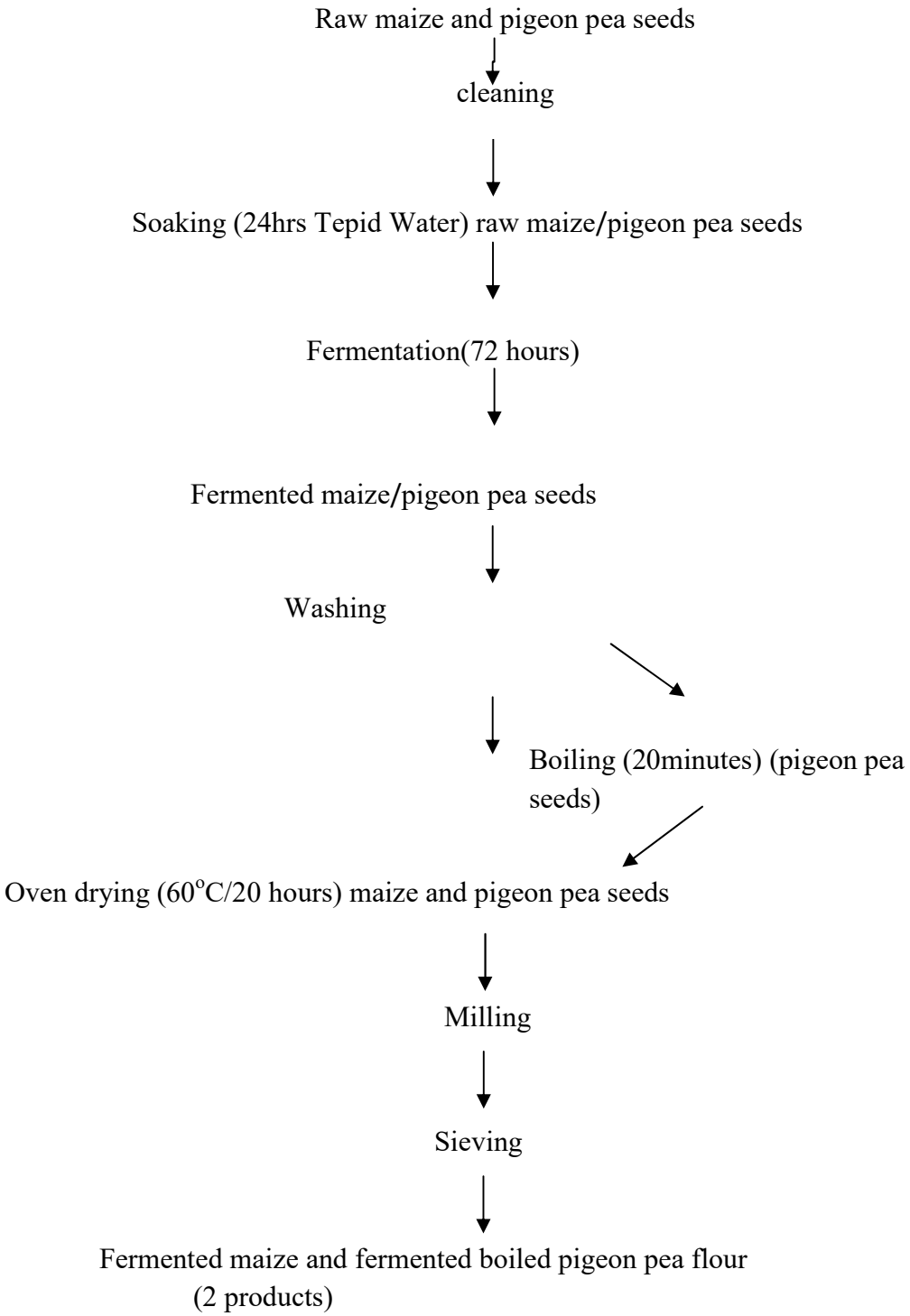


Figure 3.1 Production of fermented, maize and pigeon pea flour (Onweluzo and Nwabugwu, 2009)

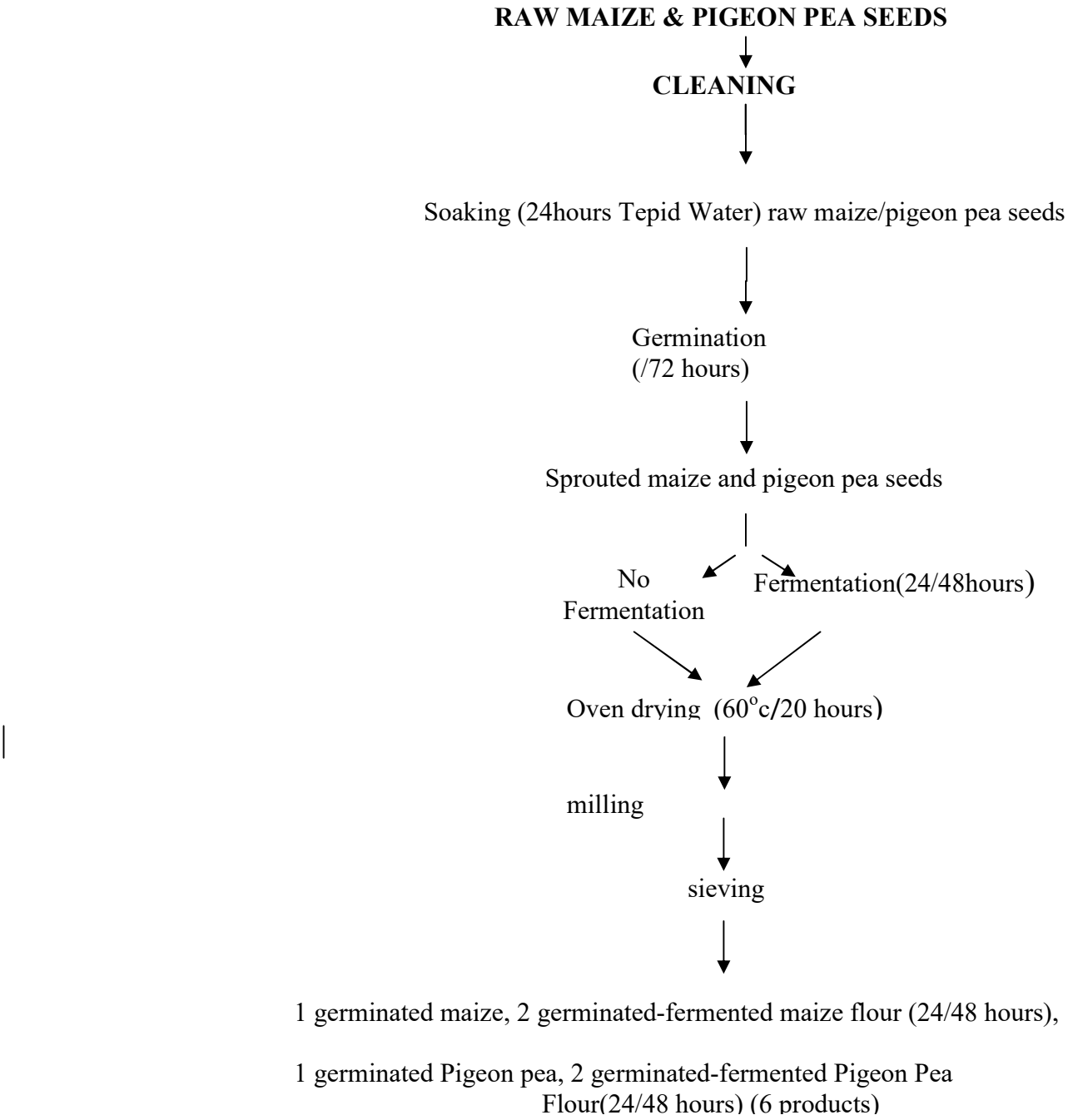


Figure 3.2 Production of germinated and germinated-fermented maize and pigeon pea flour (Onweluzo and Nwabugwu, 2009)

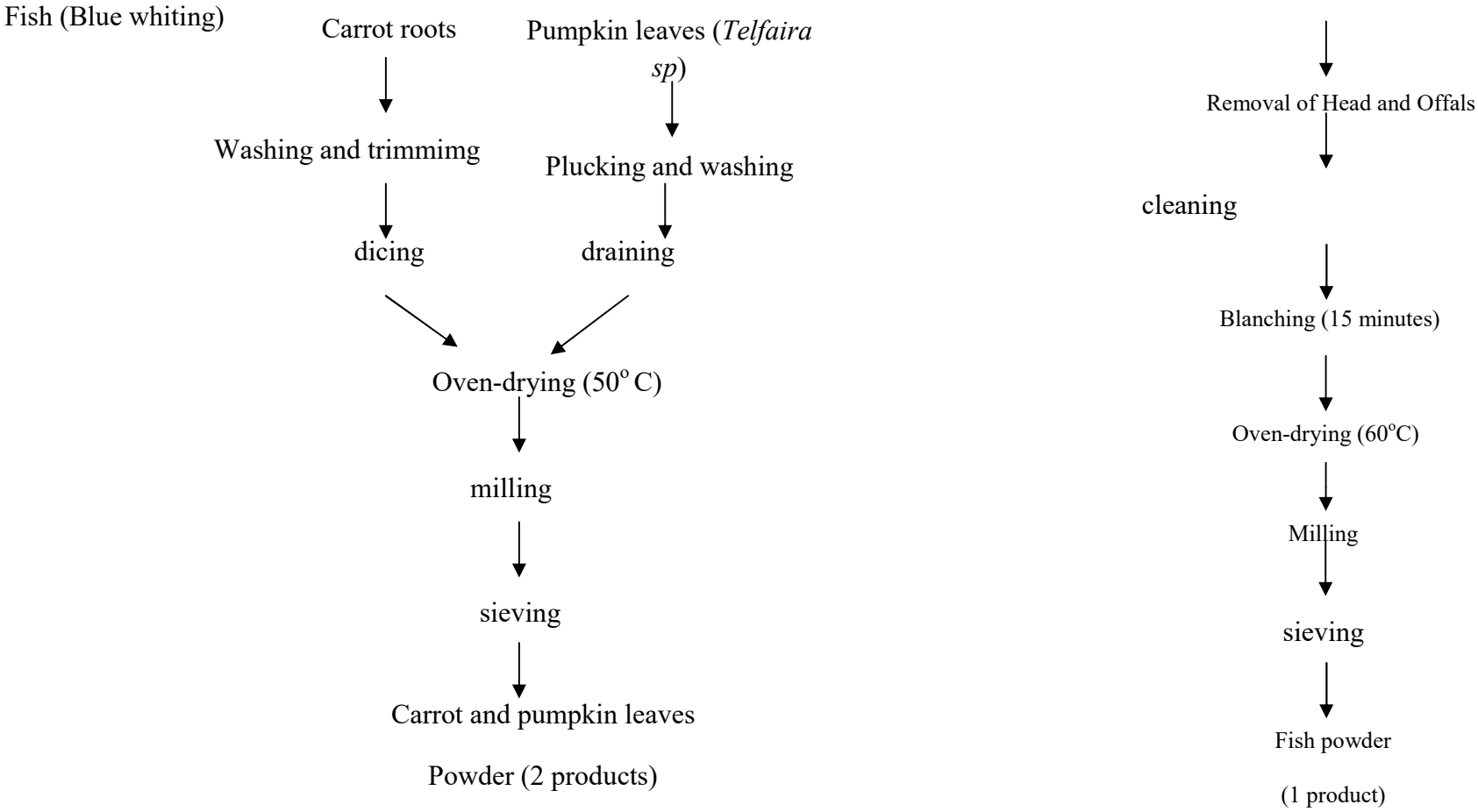


Figure 3.3 Flow chart for production of carrot, *Telfaria* leaves and fish powder

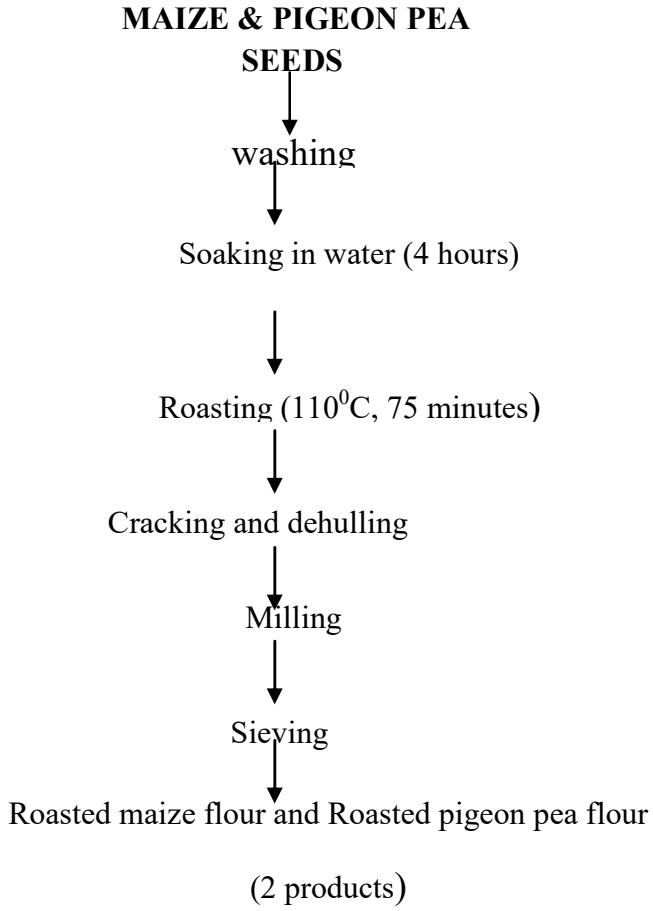


Figure 3.4 Flow chart for roasting of maize and pigeon pea , and milling into flour (Addis *et al*., 2013)

3.4Formulation of complementary foods (Compif O and Compif R)

Five processed maize products and five processed pigeon pea products, one fish product, one carrot product and onepumpkin (ugu) leaves product was obtained at the end of the processing.Fermented pigeon pea seeds were boiled for about 20 minutes, dried, milled and sieved with 450 μm pore sieve into fermented pigeon pea flour.Proximate, mineral and anti-nutrient analyses of the ingredients were performed. After chemical analysis, selected maize and pigeon pea processed flour were used to formulate complementary food which was referred to as COMPIFO (Complementary Maize, pigeon pea, carrot, pumpkin leaf, and fish powder). The selection was based on the phytate contents of processed maize and pigeon pea to ensure low phytate: zinc/iron molar ratios. Another diet COMPIFR was also formulated using roasted maize and pigeon pea flour since roasting was employed in the production of the common ready-to-eat therapeutic food, Tombrown. Also, mothers prefer roasting to save energy and time.

Nutri Survey packagewas used to adjust combination ratio of the processed maize, pigeon pea, carrot, pumpkin leaves and fish powder needed for energy, protein and fat contents of 400kcal, 15g and 10-25g per 100g, respectively,which was based on Codex Alimentarius Commission Recommendations for older infants and young children. This was done based on the nutrient composition of individual ingredients added to the database. The combination ratio50:20:10:6:4:5:5 for maize, pigeon pea, fish, carrot,pumpkin leaves powder, sucrose, and oilwas adopted. The formulated diets werechemically analysed and compared to CODEX recommendation and commercial complementary food (CCF) (Codex Alimentarius Commission, 2006).

3.4.1Formulation of Compif O flour (1000 g)and Compif O grits

Table 3.1 Composition of Compif O flour(1000 g)

Ingredients	Proportion (%)	Quantity (g)
Germinated-fermented maize (*Zea mays*)	50	500
Fermented boiled pigeon pea (*Cajanus cajan*)	20	200
Dried fish powder	10	100
Ugu leaf powder	4	40
Carrot powder	6	60
Sugar	5	50
Oil	5	50

1000g **Compif O flour**+ 1500 mls tepid water⇨Compif O gruel

1500 mls hot water (boiling) was added and the mixture stirred properly. The mixture was cooked for about thirty (35) minutes to form **Compif O gruel** (3600 mls). About 450 mls gruel was poured in a tray (< 0.3 cm thick) and dried in the oven (L-501535, UNISCOPE, United Kingdom) at 50^0C for about five to six hours. The Compif O flakes obtained were ground in a blender into **Compif O grits**(870 g).

50 g of Compif O grits + 150 mls boiling water ⇨Compif O meal (200 mls)

3.4.2 Formulation of Compif Rflour (1000 g) andCompif R grits.

Table 3.2 Composition of Compif R flour(1000 g)

Ingredients	Proportion (%)	Quantity (g)
Roasted maize (*Zea mays*)	50	500
Roastedpigeon pea (*Cajanus cajan*)	20	200
Dried fish powder	10	100
Ugu leaf powder	4	40
Carrot powder	6	60
Sugar	5	50
Oil	5	50

1000 g **Compif R flour**+ 1750 mls tepid water ⟹ Compif R paste

1750 mls hot water (boiling) was added and the mixture stirred properly. The mixture was cooked for about thirty (35) minutes to form **Compif R gruel** (5000 mls). About 450 mls gruel was poured in a tray (< 0.3 cm thick) and dried in the oven (L-501535, UNISCOPE, United Kingdom) at 50^0C for about five hours. The Compif R flakes obtained were ground in a blender into **Compif R grits**(975 g).

40 g of Compif R grits + 150 mls boiling water ⟹ Compif R meal (200 mls)

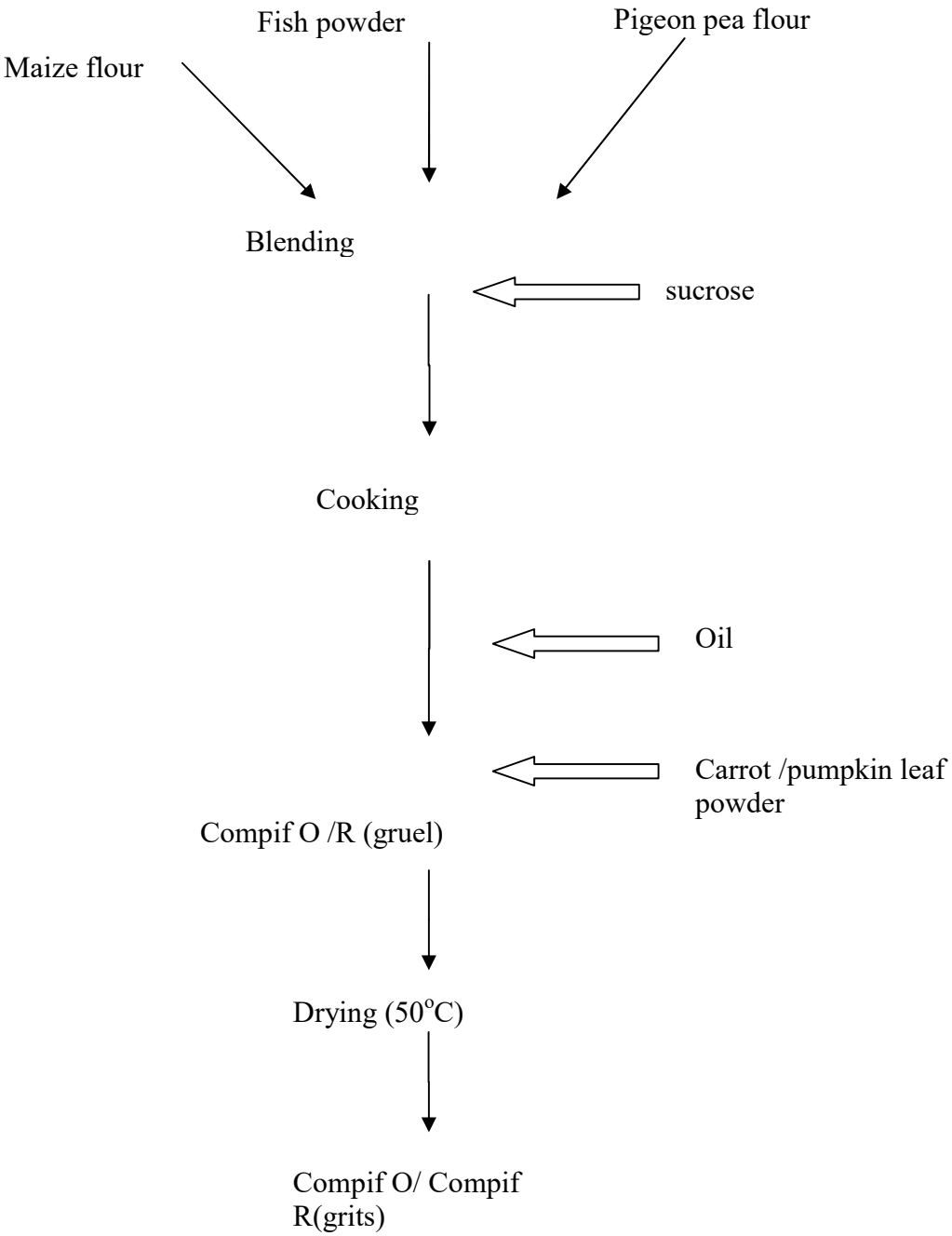


Figure 3.5 Flowchart for production of CompifO and CompifR

3.5Chemical analysis

3.5.1 Proximate analysis

The moisture, fat, crude protein (N x 6.25), crude fibre and an ash content of the food ingredients, as well as the formulated diets,were determined in triplicates according to the approved procedures of the Association of Official Analytical Chemists (2005).

3.5.1.1 Moisture content determination(AOAC(2005), METHOD 967.08)

Moisture contents of the samples were determined using the oven drying method. Petri dishes were weighedafter drying and cooling in a desiccator. Two (2)grammes of each sampleweremeasured into each of the weighed petri dishes (W). The petri dishes with the samples (W_1)were placed in the UNISCOPE oven(SM9053, Surgifriend Medicals, England) at 105 ± 1°C for 4 hours for samples to dry. After the drying process, the samples were cooled in a desiccator and weighed. The samples were repeatedly dried, cooled and re-weighed until a constant weight was obtained (W_2). Percentage moisture content was calculated as follows:

$$weight\ of\quad dish \qquad = \quad W$$

$$Initial\ weight\ of\ dish + sample \quad = \quad W_1$$

$$Final\ weight\ of\ dish + sample = \quad W_2$$

$$Moisture\ content = W_1 - W_2$$

$$\%\ Moisture\ content = \frac{W_1 - W_2}{W_1 - W} \times \frac{100}{1} \quad \text{(Joslyn, 1970)} \qquad (1)$$

$$\%\ Dry\ matter = \frac{W_2 - W}{W_1 - W} \times \frac{100}{1} \qquad (2)$$

3.5.1.2Ash content determination(AOAC (2005),METHOD 942.05)

Two grammes of each sample was weighed into porcelain crucibles and placed in a UNISCOPE muffle furnace (SM9080, Surgifriend Medicals, England) at 550°C for 4 hours until white or light grey ash resulted.The ashed samples were removed from the

furnace, left for some time for the temperature to decrease and then transferred to a desiccator. The sample was reweighed. The percentage of ash was determined using the stated formula:

$$weight\ of\quad empty\ crucible \qquad = \quad W$$

$$weight\ of\ crucible + sample\ before\ ashing \quad = \quad W_1$$

$$weight\ of\ crucible + sample\ after\ ashing = \quad W_2$$

$$Ash\ content = \quad W_2 - W$$

$$\%\ Ash\ content\ (dry\ basis) \qquad = \frac{W_2 - W}{W_1 - W} \times \frac{100}{1} \qquad (3)$$

3.5.1.3 Fat content determination(AOAC(2005), METHOD 920.39C)

Crude fat content was determined using an automated semi-continuous solvent extraction method (Soxlet method).The extraction cup was dried for 1-2 hours in the oven, cooled in a desiccator and weighed (W_1).Two (2) grammes of each ground sample(W)was wrapped with cotton wool and placed in the thimble. The thimble was fitted to the Soxtec^{Tm}extractor (2055, FOSS, Sweden). Eighty (80 ml) of petroleum ether was poured into the dried and weighedextraction cup. The extraction cup was placed in the cup holder and fixed to the extractor. The temperature of the extractor was set at 135^{0}C using the control unit. The extraction continued its reflux for about 1- 1 hour 20 minutes. When extraction was complete, the extraction cupsweighed dried in the oven (60^{o}C)for about 1 hour to eliminate traces of petroleum ether, cooled and weighed (W_2). The percentage of crude fat present in the sample was calculated.

$$Final\ weight\ of\ extraction\ cup \quad = \quad W_2$$

$$Initial\ weight\ of\ extraction\ cup = \quad W_1$$

$$Weight\ of\ sample \qquad = \quad W$$

$$Weight\ of\ fat = W_2 - W_1$$

$$\%\ Fat = \frac{W2-W1}{W} \times \frac{100}{1} \quad (4)$$

3.5.1.4 Crude fibre determination(AOAC (2005), METHOD 958.06)

About 2 grammes of each sample was weighed and defatted using petroleum ether (Soxlet extraction). The fat-free sample wasput in fiberglass and heated under reflux with 200 mls of 1.25% sulphuric acid (H_2SO_4) for about thirty minutes. The hot solution was filtered through fibre sieve cloth, rinsed with boiling water and the filtratediscarded. The residue was transferred to another fibre glass and heated for another thirty minutes with 200 mls of 1.25% Sodium hydroxide (NaOH). The solution was filtered using fibre sieve cloth with an addition of 10% acetone to dissolve any remaining organic constituent. About 50 mls of hot water was poured on the residue in the sieve cloth to rinse off the chemicals. The residue was transferred to crucibles and dried in the oven overnight at 105^{o}C. The dried residue with the crucible was cooled in a desiccator and weighed (W_1).The dried residue inthe crucible was transferred to a muffle furnace to ash at for 4 hours at 550^{o}C. After ashing, the crucible with the sample was cooled in a desiccator and later weighed again (W_2). The proportion of crude fibre was determined using the formula below:

$$weight\ of\ sample = W$$

$$weight\ of\ crucible + treated, dried\ sample = W_1$$

$$weight\ of\ crucible + sample\ after\ ashing = W_2$$

$$Fibre\ content = W_1 - W_2$$

$$\%\ Fibre\ content = \frac{W_1-W_2}{W} \times \frac{100}{1} \quad (5)$$

3.5.1.5 Crude protein determination (AOAC (2005), METHOD 988.05)

Digestion: About 0.5 grammes of the well ground and homogenized samples were carefully added to the bottom of Kjedahl digestion tubes. A tablet of Kjedahl catalyst and concentrated H_2SO_4 (10 mls) were added to the tubes. The tubes were then placed in the digestion block heaters inside the fume cupboard for about 4 hours. The digest, a clear colourless solution was allowed to cool and transferred to 100 ml volumetric flask. The tubes were thoroughly rinsed with water and poured into the flasks. Water was added carefully to the flasks to make up to the mark on the flasks.

Distillation: About 5 mls of the digest was pipetted into a steam distillator. Five millitres of 40% (w/v) sodium hydroxide (NaOH) was added to the digest using 5 ml pipette. The mixture was steamed for 2 minutes and the distillate collected via a 50 ml conical flask. The ammonia was distilled into a solution of 10 mls of 2% Boric acid and mixed indicator. The change in colour from red to green indicated that all liberated ammonia has been trapped.

Titration: The green coloured solution (Ammonium Borate) was titrated with standardized 0.01N HCl contained in a 50 ml burette till wine colour appeared (Ammonium Chloride). The percentage of nitrogen was calculated as indicated below:

$$\%\ Nitrogen\ content = NHCl\ x \frac{Corrected\ acid\ volume}{g\ of\ sample}\ x\ \frac{14gN}{mol} \times \frac{100}{1} \quad (6)$$

$$\%\ protein\ content = \%\ N\ X\ 6.25 \quad (7)$$

3.5.1.6 Carbohydrate determination

Carbohydrate compositionwas obtained by subtracting the total of the percentage of fat, moisture, ash and protein content from 100 (Nielsen, 2010).

3.5.2Determination of amino acid content

Amino acids were determined through spectorophotometry using ninhydrin chemical reaction (Schroeder *et al*., 1990). One hundred (100) millilitres of 6 Moles hydrochloric acid was added to 1 gram of ground sample in a stoppered 250 ml conical flask. The mixture was incubated for 16 hours to hydrolyse the sample and filtered through a double layered Whatman No 42 Filter paper into another conical flask covered tightly. Two millitres of the hydroxylate was put in a 30 ml test tube using a pipette and 10 mls of buffered ninhydrin reagent was added. The mixture was allowed to heat in a boiling water bath for 15 minutes, cooled to room temperature and 3 mls of 50% Ethanol added. Working standard amino acids (0-5 µg/ml) were prepared from each standard solution of amino acids to obtain the corresponding gradient factor from the calibration curve. Each working standard was heated with the buffered ninhydrin reagent, cooled and Ethanol added as done above. The absorbances of sample and working standard solutions were measured at the wavelength of colour developed by each amino acid.

$$\%Amino\ Acid = \frac{Absorbance\ of\ sample\ x\ Average\ Gradient\ x\ Dilution\ Factor}{10000} \quad (8)$$

3.5.3Mineral and vitaminanalysis

3.5.3.1 Determination of calcium (AOAC (2005), Method 975.11)

About 2 grammes of the samples were degraded to ash according to the method described previously. Five millilitres of 10% perchloric acid (HCI03)was used to digest the ashed sample in the crucible and heat was applied to the mixture using heating mantle till it dried. The process was repeated with additional10% perchloric acid (HCI03)but the mixture was not allowed to dry. The boiled mixture was filtered using a Whatman No. 1 filter paper into a 100ml volumetric flask. Deionised water was added to the filtrate up to the mark. The concentration of calcium in the filterate was read on the Jenway Digital Flame Photometer(PFP7, Bibby Scientific Limited, United Kingdom) using the filter

corresponding to calcium element. Concentration of calcium was determined using the formula:

$$\%\ calcium\ content = \frac{meter\ reading\ x\ slope\ x\ dilution\ factor}{10000} \quad (9)$$

3.5.3.2 Determination of iron and zinc (AOAC(2005), Method 975.23)

The iron and zinc contents were determined using the atomic absorption spectrophotometer (AAS). About 2 grammes of the samples were degraded to ash according to the method described previously. The derived ash samples were digested with five millitres of 10% perchloric acid (HCI03) and washed into 100ml volumetric flask with deionised water upto the mark. The diluent was extracted into theatomic absorption Spectrophotometer (Buck 200, Buck Scientific, USA) usingthe suction tube. The mineral elements were read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination. Percentage values of Iron and Zinc were determined as follows:

$$\%\ Iron/Zinc\ contents = \frac{meter\ reading\ x\ slope\ x\ dilution\ factor}{10000} \quad (10)$$

$$Dilution\ Factor = \frac{Volume\ of\ Solvent}{Sample\ weight.} \quad (11)$$

3.5.3.3 Determination of beta carotene (AOAC (2005), Method941.15)

Beta carotene was determined using the method described by AOAC (2005), and Rodriguez-Amaya, (2004). Two (2) grams of flour sample was weighed into a 250ml volumetric flask and 50mls of petroleum ether: Acetone (2:1v/v) mixture was added. The mixture was properly mixed with a shaker at 5 g (Relative Centrifugal Force (RCF)) for 20 minutes at 27 $\pm$2 ^{0}Cand thereafter centrifuged at 2130g (RCF) for 10minutes. The supernatant obtained was made up to 50ml with the solvent mixture poured through a 250ml funnel to separate the organic layer (upperlayer). The organic layer was put into 50ml volumetric flasks and made up with solvent mixture for reading of ß-carotene while the aqueous layer was thrown away. A standard solution of ß-carotene of range 0-50ppm

was prepared from betacarotene stock solution of 100ppm concentration. The absorbances of samples and the standard solutions were read on a Spectrophotometer (2483, CECIL Instruments, England)at a wavelength of 450nm against blank.

$$B-carotene\ \left(\mu g/_{100g}\right) = \frac{Absorbance\ of\ sample\ x\ Average\ Gradient\ x\ Dilution\ Factor}{10000} \quad (12)$$

3.5.4Evaluation of selected anti-nutritional factors

3.5.4.1 Determination of oxalate

The method of Onwuka (2005) was employed in the determination of oxalate. About 190 mls of distilled water was poured into 250 ml volumetric flasks containing to 2 g of each food sample. Ten mls of 1M HCl was added to the solution. The mixture was heated for one hour to digest the sample. After cooling, distilled water was poured into the flask to the 250 ml mark. The mixture was filtered and about 125 mls of the filterate was measured into three beakers. Three drops of methyl red was added to each of the beakers. There was a change in colour from pink to faint yellow. The beakers were then heated at 90^{o}C and ten millitres of $CaCl_2$solution added gradually with continuous stirring. The solution was left overnight at 5^{o}C to cool. The solution was centrifuged at 832 g (2500 rpm) for 5 minutes. After, the supernatant was carefully filtered through Whatman No. 42 filter paper.The precipitate formed was dissolved in 10 mls of 20% H_2SO_4. Distilled water was poured into the solution to reach the 300 ml mark and ammonium hydroxide (NH_4OH) was added to reprecipitate oxalic acid. The contents were boiled and allowed to settle overnight. The quantity of oxalic acid was determined by titrating the mixture with 0.05N $KMnO_4$ as indicated below:

$$1\ ml\ of\ 0.05N\ KMnO4\ = 0.00225\ anhydrous\ oxalic\ acid$$

$$\%\ oxalic\ acid = \frac{Titre\ value\ x\ 0.00225}{2} x\ 100$$

$$= T.V\ x\ 0.1125 \quad (13)$$

3.5.4.2 Determination of tannin

Tannin was determined by the method described by Joslyn (1970). About 0.2g of the sample was weighed into a 50mls beaker and then 20mls of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80^{o}C for 1 hour with constant agitation. The filtrate was then filtered with a double layer Whatman No 41 filter paper into 100ml volumetric flask. About 20mlsdistilled water, 2.5 mls Folin-Denis reagent and 10mls of 17% Na_2CO3 were added and solution mixed properly. Water was added to make up to the mark and the mixture after thorough mixing was left standing for 20 minutes till bluish-green colour developed. The absorbance of the solution and prepared tannic acid standard solutions were read after colour developmenton a UV-Vis spectrophotometer (V6300, Venway, UK) at a wavelength of 760 nm (AOAC, 2009)

% Tannin was calculated using the formula:

$$\%Tannin = \frac{Absorbance\ of\ sample\ x\ Average\ Gradient\ x\ Dilution\ Factor}{Weight\ of\ Sample\ x\ 10{,}000} \quad (14)$$

3.5.4.3Determination ofPhytate

The method of Maga (1983) was employed. One hundred millitresof 2% Hydrochloric acid was added to2g of each sample in 250ml conical flask to soak each sample for 3 hours. The mixture was filtered through a double layer of hard filter paper. Then 50mls of the filtrate was placed in 250ml conical .flask and 107mls distilled water added and 10mls of 0.3% Ammonium Thiocyanate (NH_4SCM) solution was added into each solution. The resulting solution was titrated with standard iron (III) chloride solution having 0.00195g iron per ml. Slightly brownish-yellow colour which persisted for 5 minutes was obtained. The % phytic acid was determined using the formula:

$$\%\ Phytic\ Acid = \frac{Titre\ value\ x\ 0.00195\ x\ 1.19\ x\ 100\ x\ 3.55}{weight\ of\ sample} \quad (15)$$

The results of the phytic acid analysis were used to calculate phytic acid to iron molar ratio and phytic acid to zinc molar ratio using the formula:

$$Phytic\ acid: iron\ molar\ ratio\ = \quad \frac{phytic\ acid(g/100g)\ /\ 660.04}{Iron\ (g/100g)\ /\ 55.85} \tag{16}$$

$$Phytic\ acid: zinc\ molar\ ratio = \quad \frac{phytic\ acid(g/100g)\ /\ 660.04}{Zinc\ (g/100g)\ /\ 65.4} \tag{17}$$

$$Phytic\ acid: calcium\ molar\ ratio = \quad \frac{phytic\ acid(g/100g)\ /\ 660.04}{Calcium\ (g/100g)\ /\ 40.07} \tag{18}$$

3.5.4.4Determination of Saponin

The spectrophotometric method of Brunner (1984) was used to determine the saponin content of the samples. One hundred millilitres of isobutyl alcohol was added into a 250ml beaker containing 1g flour sample. The mixture was thoroughly mixed with a shaker and filtered into a 100ml beaker using a Whatman No1 filter paper and 20mls of 40% saturated solution of $MgCO_3$ was added. Themixture obtained was refiltered. One millilitre of the filtrate was then pipetted into 50ml volumetric flask into which 2 ml of 5% $FeCl_3$ solution was added and distilled water added to make up to the mark. The mixture was left for about 30minutes till blood red colour appeared. The absorbance of the solution and prepared standard saponin solutions were read after colour development in a spectrophotometer(V6300, Venway, UK) at a wavelength of 380nm.

$$\%\ Saponin \quad = \quad \frac{Absorbance\ of\ sample\ x\ gradient\ factor\ x\ dilution\ factor}{Weight\ of\ sample\ x\ 10000} \tag{19}$$

3.5.4.5 Determination of hemagglutinin

According to the method of Liener (1995), 10 mls of 0.1M phosphate and 10 mls of 0.85% NaCl were added into a screw cap centrifuge tube containing 0.20g of defatted sample. The mixture was thoroughly agitated at $27 \pm 2\ ^0$CusingUDY shaker and allowed

to stand for 18hours. Thereafter, the suspension was centrifuged at 300 g (RCF) for 15minutes. The supernatant was decanted into another clean centrifuge tube. The hemaglutinin inhibitior activity was tested using standard solutions of hemaglutinin prepared from serial dilutions of 0 to 1.0ml of the stock hemaglutinin. The sample and standard solutions were treated with 0.9% satrain. After colour development, the absorbance of sample as well as standard solutions was read on the spectrophotometer (V6300, Venway, UK).The hemaglutinin activity was determined as follows:

$$HU/mg = 98.56\ (Absorbance\ of\ sample - absorb\ blank) \quad (20)$$

3.5.5Determination of functional properties

3.5.5.1Determination of water absorption capacity

One gram of each sample was weighed into two marked centrifuge tubes and 10 ml distilled water was added. The mixture was stirred using a warring mixer for 30 seconds as described by Onwuka (2005). The sample was left to stand for 30 minutes at $27 \pm 2\ ^{0}C$ and centrifuged at 3328 g (RCF) for 30 minutes. The supernatant was read directly from the graduated centrifuge tube and absorbed water calculated by difference. The absorbed water was converted to weight (in grams) by multiplying by the density of water (1 g/ml). The water absorption capacity was expressed in grams of water absorbed per gram of flour sample.

3.5.5.2Determination of swelling capacity

The method of Leach *et al.,* (1959)was employed. About 0.1g of sample was weighed into a test tube 10 ml of distilled water added. The slurry was heated in a water bath at $60^{o}C$ for 30 minutes with continuous shaking.. The heated slurry was cooled and centrifuged at 1000 rpm for 15 minutes and the supernatant cautiouslypoured out. Weight of the paste was calculated and swelling capacity calculated as follows:

$$Swelling\ capacity\ (g/g) = \frac{weight\ of\ paste}{Weight\ of\ dry\ sample}$$

(21)

3.5.5.3Determination of bulk density

The bulk density was determined employing the method described by Ukpabi and Ndimele (1990) slightly modified. About 10 g of the flour sample was put into a 25ml measuring cylinder. The cylinder was gently tapped several times for about 10minutes to eliminate air spaces till a constant volume was obtained. The bulk density was calculated as:

$$Bulk\ density\ (g/ml) = \frac{weight\ of\ sample\ (g)}{volume\ of\ sample\ (ml)}$$

(22)

3.5.5.4Determination of Least Gelation Concentration (LGC)

Percentage proportion ofsample suspensions of the flour blends (2,4, 6, 8, 10, 12, 14,16) were prepared with 5 ml distilled water in test tubes and heated for one hour in a boiling water bath. The heated suspensions were then cooled rapidly under running water and further cooled for 2 hours in a cold water bath at 4^{o}C. The minimum concentration at which the sample from inverted test tube did not slip was determined as the LGC (Onwuka 2005).

3.5.5.5Determination of Viscosity

One gram of flour sample was measured into a 30 ml centrifuge tube and 20 ml of distilled water was added. The mixture was shaken thoroughly to obtain a homogenous slurry. Two millilitres of the slurry was pipetted into aTubular Jaw of the Kiln Viscometer. The maximum time taken by the slurry to move at 30^{0}C at normal range was noted (AOAC 1984).

$$Viscosity\ at\ 30^{o}\mathrm{C} = \frac{volume\ of\ flow\ to\ maximum\ time\ at\ 30^{o}\mathrm{C}}{maximum\ time\ used\ at\ 30^{o}\mathrm{C}}$$

(23)

$$Apparent\ viscosity\ in\ Cp = viscosity\ x\ 10.03$$

(24)

3.6 Microbiological analysis

The presence of microbes in the formulated complementary foods was investigated applying the microbiological procedures specified in the International Commission on Microbiological Specifications for Foods (ICMSF, 1996). Microbiological assays were performed to detect the presence and enumerate bacteria (total viable count), yeast/mould and *Escherichia coli.*Culture media used were nutrient agar, potatoe dextrose agar and Eosin Methylene Blue Agar were. One gram of finely ground sample was weighed aseptically and diluted in distilled water (9 mls) in sterilized McCartney bottles to achieve 1:10 dilution. The mixture was stirred vigorously to obtain a homogenous suspension and up to 10^{-5}serial dilutions prepared.

3.6.1Total viable count (bacteria)

Total viable colony count was determined using the pour plate method. One millitre of the dilutions (10^{-5}) was inoculated on sterile petri dishes and nutrient agar media (Oxoid)was poured on the diluents in duplicate plates. The plates were incubated at 35-37^{o} C for 48 hours and colonies were counted with the aid of magnifying glass as colony forming units per millilitre.

3.6.2 Enumeration of fungi (yeasts/moulds)

Potato dextrose agar (Oxoid) was poured on 1 ml of diluents in duplicate plates and incubated for 72 hours at 22-25^{o}C.After incubation,colonies were counted as fungal forming units per ml (cfu/ml).

3.6.3 Detection of *Escherichia coli*

One millilitre of each of the sample dilutions were measured onto sterile petri dishes and Eosin Methylene Blue Agar (Oxoid) was poured on the diluents. The plates were incubated for 24 hours at 35-37^{o} C.

Total viable counts of the isolates were determined as colony forming units per ml (cfu/ml) using the formular :

$$Total\ viable\ count\ (cfu/g) = total\ number\ of\ colonies\ x\ dilution\ factor\ x\ plating\ factor \quad (25)$$

(ICMSF (International Commision for Microbiological Specification for Foods), 1996].

3.7Nutritional quality evaluation using Wistar rats

3.7.1 Experimental diet formulation

Experimental test diets were formulated by fromCompif O, Compif R, casein, and Commercial Complementary Food (CCF) at 10% protein levels. Quantities of the formulated complementary mixes, casein and CCF that had 10 g protein were calculated and used to prepare iso-nitrogenous.A basal diet which was protein free was formulated from corn starch.The fat content of the experimental diets were also adjusted to 10% levels with varying quantities of soya oil (iso-caloric diets).The fibre contents of the experimental diets were adjusted to not more than 5% with non-nutritional cellulose. Other ingredients such as sucrose, oyster shell and vitamin/mineral mix were added to all the experimental diets including the basal diet. The basal diet served as the negative control. These experimental diets were formulated a day before the feeding of the animals commenced.

Table 3.3 Test Diet Composition (g/100g, dry matter basis)

Ingredient & proportion in test diets (%]	A	B	C	D	E
Corn Starch (to make up to 100%)	13.40	57.00	20.29	20.13	67.00
Soy oil (10%)	5.00	10.00	5.40	4.40	10.00
Sucrose	5.25	8.25	5.25	5.20	8.25
Glucose	3.00	6.00	4.00	4.00	6.00
CCF	66.60	-	-	-	-
Casein	-	10.00	-	-	-
Compif O	-	-	58.31	-	-
Compif R	-	-	-	59.52	-
Non Nut. Cellulose (5% if test food has fibre<5%)	3.00	5.00	3.00	3.00	5.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00	2.00
Tablesalt	0.25	0.25	0.25	0.25	0.25
*Vit.Premix (0.5%)	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

A = CCF based diet, B= casein based diet, C= compif O based diet, D= compif R based diet, E= protein-free diet.

*Vitamin A, 200 000.001U,Vit D_3: 40 000.001U, Vit E (mg) 460,Vit K3 (kg) 40, Vitamin B, (mg) 60, Vitamin B_2 (mg) 120, Niacin (mg) 1000, Calcium pentothenate (mg) 200, Vit B_6 (mg) 100, Vit B_{12} (mg) 05, Folic acid (mg) 20, Biotin (mg) 1,Chlorine chloride (mg) 8000, Manganase (mg) 2400, Iron (mg) 2000, Zinc (mg) 1600, Copper (mg) 170,Iodine (mg) 30, Cobalt (mg) 6, Selenium (mg)24, Anti-oxidant (mg) 2400.

3.7.2Animal feeding experimentation

Forty weanling albino rats (Wistar strain) of both sexes aged 25-28 days and weighing about 30-40g were used for protein quality evaluation of formulated diets. The experimental rats were randomly distributed into 6 groups of 6 rats. Each rat was housed separately in metabolic cages with facilities for separate feeding, faecal and urine collection. They were fed a 4% casein diet for 5 days for acclimatization, after which they were re-weighed, tail and body length measured and re-grouped into six groups in a random block design. One rat escaped while three rats were returned to the farm. A group of six rats were sacrificed on the first day as zero day animals (baseline control). The remaining five groups (30 rats) were fed the formulated diets, commercial complementary food (CCF), casein-based diet (positive control) and a protein free diet (negative control) for 21days (period before puberty age of rats). Ten grams of diet and water were offered daily. Daily dietary intake, residual, and spilled food were calculated. Weight changes were assessed twice weekly. Faecal and urinary discharge were collected and utilised for nitrogen analysis. Collective samples of faeces were dried in an oven at 105^{o} C for 24 hours and ground for nitrogen determination by the Kjedahl method. At the end of the feeding period, the experimental ratswere sacrificed and blood sample collected through occular puncture for haematological and biochemical analyses. The liver, heart and kidney sections were weighed and used for histopathological examination. The carcass and faecal nitrogen, weight gain, feed and protein intake were used to determine the biological value (BV), protein and feed efficiency ratios (PER and FER), net protein utilization (NPU), amino acid score, true protein digestibility, protein digestibility corrected amino-acid score (PDCAAS) (AOAC 2005).

3.7.2.1 Body weight and length measurement of the experimental rats.

Weight of the rats was checkedtwice weekly at the same time (morning) using a chemical weighing balance. Body length was measured from nose to rump and tail length(rump to tail tip) at baseline and endpoint. Weight and length of the rats were also compared to their age and feeding period.

3.7.2.2 Carcass nitrogen analysis

The carcasses were chopped into relatively small pieces and dried at 95°C for 48-72 hours to constant weight to determine the Total Body Water (TBW). The dry carcasses were ground and mixed with 5g aliquot in a pyrex beaker. The slurry was heated with 20mls of 50% sulfuric acid for 3 hours at 100°C. The hydroxylate was allowed to cool and transferred to a 500ml volumetric flask. Distilled water was added into the flask make up to 500ml level. Carcass nitrogen was determined using the Kjeldahl method.

3.7.2.3Determination of protein quality parameters

From the carcass of the animals sacrificed on the first day, the initial body water and body nitrogen of each animal were determined. These values were subtracted from those derived at the end of the experiment to obtain the change in body water and body nitrogen of the test animals.The weight gain, feed intake, protein intake, carcass nitrogen and nitrogen intake values for the twenty-one days feeding period were used to calculate protein quality parameters as indicated below (Pellet and Young 1980):

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Total\ feed\ consumed}{weight\ gain} \quad (26)$$

$$Feed\ Efficiency\ Ratio\ (FER) = \frac{Weight\ gain}{Total\ feed\ consumed} \quad (27)$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{Weight\ gain}{Total\ protein\ intake} \quad (28)$$

$$Net\ Protein\ Retention\ (NPR) = \frac{Weight\ gain\ of\ test\ animals\ +\ Loss\ by\ 0\%\ Protein\ group}{Total\ protein\ intake\ of\ test\ group} \quad (29)$$

$$Relative\ NPR\ (R-NPR) \quad = \quad \frac{NPR\ of\ diet\ \ x\ \ ANCR\ Casein\ (4.2)}{NPR\ of\ Casein\ diet}$$

(30)

$$Net\ Protein\ Utililisation\ (NPU)$$

$$= \frac{Carcass\ N\ of\ test\ group - \ carcass\ N\ of\ protein-free\ group\ +\ nitrogen\ intake\ of\ protein-free}{Nitrogen\ intake\ of\ the\ test\ group}$$

(31)

$$True\ Digestibility\ (TD)$$

$$= \frac{Nitrogen\ intake\ of\ test\ group - \ (faecal\ nitrogen\ of\ test\ group - \ faecal\ nitrogen\ of\ protein-fre}{Nitrogen\ intake\ of\ the\ test\ group}$$

(32)

$$Biological\ value\ (BV) = \quad \frac{Net\ protein\ utilization\ (NPU)}{True\ digestibility\ (TD} x\ 100$$

(33)

$$PDCAAS\ (Protein\ digestibility\ corrected\ amino\ acid\ score)$$

$$= \quad \frac{mg\ of\ first\ limiting\ amino\ acid\ in\ 1g\ of\ test\ protein\ \ x\ \ faecal\ nitrogen\ digestibility}{mg\ of\ same\ amino\ acid\ in\ 1g\ reference\ protein\ \ (6m-3\ years)}$$

(35)

3.7.2.4 Haematologicalevaluation

At the end of the feeding period, about 5mls of blood was collected through the orbital vein and 2mls put into sample bottles containing sodium ethylene diamine tetracitric acid (Na_2EDTA) and utilized for haematological parameters (packed cell volume (PCV), haemoglobin (Hb), red blood cells(RBC), white blood cells (WBC) with differentials and blood platelets.The PCV and Hb were determined using the microhaematocrit and cyanmethalmoglobin methods respectivelyas described byMitruka and Rawnsley (1977). The RBC and WBC were analysed using the Neubauer haemocytometer after appropriate dilutions (Jain 1986). WBC differential (neutrophils, lymphocytes, monocytes and

eosinophils) count was calculated from the respective percentages of each set of cells in the total leucocyte count on blood smear stained with Giemsa stain.

3.7.2.4.1 Calculation of blood indices

Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated from the values of PCV, HB and RBC using the formulae outlined below:

$$MCV = \frac{PCV(\%)}{RBC(/ul)}\ x\ 10\ fl \tag{36}$$

$$MCH = \frac{Hb\ (g/100ml)}{RBC(/ul)}\ x\ 10\ pg \tag{37}$$

$$MCHC = \frac{Hb\ (g/100ml)}{PVC\ (\%)}\ x\ 100\ \% \tag{38}$$

3.7.2.5Serum biochemical analysis

About 2 mls of blood was collected in clean bottles without EDTA. The collected blood sample was allowed to clot and centrifuged for 10 minutes at 532 g (RCF) to isolate the serum. The serum was carefully transferred to other clean bottles and stored in the freezer until required for serum biochemical and mineral analysis. A commercial diagnostic cholesterol reagent kit (Erba Diagnostic Mannasm Gmbh) was used to determine total lipid profile. The total protein was determined using biuret reaction while colourmetric method was used to measure albumin levels using reagent. Globulin was obtained by difference between total protein and albumin. Creatinine and urea levels were estimated using the photoelectric colourimeter(Coles 1989), while Aspartate Amino transferase (AST) and Alanine Amino transferase(ALT) were determined using spectrophotometric method as described by Rej and Hoder (1983), and McComb *et al.,*(1983) respectively. The alkaline phosphatase (ALP) activity was examined using the method described by Talwar *et al.,* (2004).

3.7.2.6Histopathology of kidney and liver

Sections of the liver, kidney and heart from three rats per treatment were harvested, weighed and fixed in 10% formalin and later utilized for histopathologic examinations at the department of Veterinery Pathology of the University of Ibadan, Ibadan followingthe procedure described by Ewuola (2009). Photomicrographs of tissue lesions were observed using Olumpus microscope fitted with camera to check for vacuola degenerations, necrosis, inflammationsand lesions of tissues.

3.8 Quality control measures

- **Varieties of seeds used:**Known varieties of maize and pigeon pea seeds were collected from a research institute.
- **Duplication of samples:** Analysis of samples was done in duplicates and triplicates.
- **Confirmatory analysis:**Samples were sent to another laboratory for comparison. Seed varieties from research institute were included among the samples as blind controls.
- **Use of Experts:**The animals were monitored by Dr (Mrs) Akangbe of the Department of Animal Science, University of Ibadan who is an animal scientist, undertook her doctoral research in rat study and has monitored related studies. A research assistant who is also a product of the department was engaged throughout the study period including weekends.
- **Calibration of scales:**Digital weighing scale was calibrated before each weighing session (every three days). The measurements were done at the same time (in the mornings before feed and water were served).
- **Matching**:Same age range, stock and strain of rat were used for the study**.**

3.9Statistical analysis

Data analysis was undertaken using SPSS version 20.0. Data was expressed as means and standard deviations where applicable. Differences for continuous variableswereevaluated using a one-way analysis of variance and Duncan's Multiple Range Test was used to separate mean differences when the ANOVA was significant ($p< 0.05$).

3.10 Ethical consideration

Ethical approval was obtained from University of Ibadan, Animal Care and Use Research Ethics Committee (UI-ACUREC/18/0089). The animals were handled according to the rules and regulations guiding the use of animals.

3.10.1 Animal Housing and Management (Repeat experiment)

The animals were purchased from a breeding farm and transported in a ventilated container to the animal house of the Department of Animal Science, University of Ibadan. The animal house is located at the top most floor. Each animal was housed individually in steel cages. The cage is a stainless metallic cage, well ventilated and has the provision for urine and faecal collection. The house was cleaned and disinfected before the animals were brought in. The animal house is well ventilated. The room temperature was controlled at 18-22^{o}C and relative humidity of 45-50%. The animals were fed on 4% casein based diet to stabilise them for five days. Experimental diets based on the formulated complementary foods, casein and corn starch were offered on a daily basis. The food was not toxic. The use of the animals was necessary to assess the nutritional quality and safety of the formulated complementary foods before human study. These animals were the best available alternative. All the procedures were undertaken at the animal house of the Department of Animal Science, University of Ibadan, Ibadan.

3.10.2 Animal Monitoring

The animals were monitored by a research assistant (Miss Grace) and the principal investigator. An expert, Dr (Mrs) Akangbe of the Department of Animal Science, University of Ibadan was also available to ensure adequate monitoring of the animals throughout the period of study. Food was given daily and water given liberally. The research assistant of the project was always available to handle any emergency and call the veterinary expert when it could not be handled. Serving containers were cleaned daily and dietary intake noted daily. The weight measurement was taken twice per week to monitor the growth performance. The cages were cleaned every two days to prevent food contamination and infection.The animals were held for the maximum period of twenty-eight days. The project was terminated by euthanasia method. This was done by injecting chemical anesthetics and tissue harvest (Crusan, 2013). The killing of the animals was done in a designated section of the animal house. The service of an experienced veterinary laboratory scientist was utilised. The animal tissues were not shared with other investigators.

The animals were handled with great care and kindness during the period of study. All surgical and other procedures that involved pain or discomfort were done with utmost care and gentleness. At the completion of the project, the remaining animals were returned back to the breeding farm.

These were some of the factors that determined the humane end point of the first experiment:

a. Drastic weight loss of some of the animals.

b. More than 20% death rate in a group.

c. Out-break of infection.

The experiment was repeated with the assistance of the experts.

CHAPTER FOUR

RESULTS

4.1 Results

This section displays the research findings in tables, figures and histographs,preceded by explanatory pages aimed at interpreting clearly the graphics. The findings were presented thematically based on the specific objectives in three major divisions:

- Effect of processing methods on anti-nutrient reduction and nutrient retention in maize and pigeon pea seeds.
- Nutritional adequacy and microbial safety of two complementary foods formulated from selected processed maize/pigeon pea seeds, fish (panla), carrot and ugu leaves.
- Protein quality parameters and growth, hematological, biochemical characteristics of experimental rats fed complementary foods and control diet.

4.2 Effect of processing methods on anti-nutrient reduction and nutrient retention in maize and pigeon pea seeds.

4.2.1 Proximate and mineral composition of fermented, germinated and germinated-fermented white maize seeds

The row labeled Mo displays the proximate and mineral contents of raw white maize while the values after processing are displayed on the rows below it (Table 4.1). The table shows that the crude protein level of white maize (10.04g/100g) increased significantly ($p<0.05$) after72 hours of fermentation (10.44g/100g), while it decreased significantly($p<0.05$) to 9.12g/100g when germinated (72 hours) and fermented for 48 hours continuously. Other treatments did not have significant effect($p>0.05$) on the crude protein content. Crude fat of maize decreased significantly with all the treatments.

The lowest value of 3.20g/100gwas observed when the seeds were germinated for 72 hours. The ash content of maize was lowest (0.99 g/100g) when germinated-fermented for 48 hours. Carbohydrate composition of maize increased significantly ($p<0.05$), while calcium and iron content decreased significantly($p<0.05$) with all the treatments. Zinc contents decreased significantly ($p<0.05$) with all the treatments except combined germination-fermentation for 48 hours where there was a slight insignificant increase.

4.2.2Proximate and mineral composition of fermented, germinated and germinated-fermented pigeon pea seeds (Table 4.2)

Table 4.2 reveals that all the treatments increased the crude protein level of pigeon pea significantly ranging from 21.20g/100g (control) to 24.49g/100g dry weight (fermentation). Ash contents of pigeon pea reduced significantly at $p<0.05$ with fermentation (4.20g/100g to 3.21g/100gwhile crude fat contents increased significantly at $p<0.05$ (1.42g/100g to 2.29 g/100g with the four processing methods. Calcium level increased significantly ($p<0.05$) with fermentation (22.93mg/100g to 35.33mg/100g) while it reduced significantly with the other treatments. Germination and germination-fermentation (72/48 hours) decreased iron levels of pigeon pea seeds significantly while other methods had almost no effect but all the treatments had significant ($p<0.05$)negative effect on zinc and beta-carotene levels with lowest values recorded following germination-fermentation (72/24 hours) and fermentation respectively.

4.2.3 Anti-nutrient composition of fermented, germinated and germinated-fermented white maize seeds (Table 4.3)

The effect of fermentation, germination and germination-fermentation on the anti-nutrient contents of maize is presented in Table 4.3. It shows that the levels of phytate, oxalate, saponin, polyphenol and hemaglutinin reduced significantly ($p<0.05$) with germination and germination-fermentation methods.Germination and combined germination-fermentation methods were more effective in phytate reduction in maize (>90%) compared to fermentation (14%).The tannin contents of raw maize seeds reduced by only 1.8% when the seeds were fermented for 72 hours. Suprisingly, the oxalate, saponin and

hemaglutinin contents of maize increased with fermentation. Phytate levels decreased from 967.50 mg/100g (control) to 828.50 mg/100mg (fermentation), 85.50 mg/100g (germination),74.50mg/100g (germination (72hours)-fermentation (24 hours)) and 61.00 mg/100g (germination (72hours)-fermentation (48 hours)).

4.2.4Anti-nutrient composition of fermented, germinated and germinated-fermented pigeon peaseeds (Table 4.4)

Phytate values ranged from 97mg/100g (fermentation) to 1236 mg/100g in combined germination (72 hours)-fermentation (48 hours). The phytate contents of pigeon pea seeds decreased significantly with fermentation and germination while the combined germination and fermentation process did not have any effect.All the treatments reduced the tannin contents of pigeon pea seeds by more than 80%.The process of fermentation was very effective in decreasing the anti-nutrients contents of pigeon pea.Combined germination-fermentation (24 hours) did not have any effect on the level of oxalate in pigeon pea seeds (967.50 vs 975.50 mg/100g).

4.2.5 Summary of effect of processing methods on nutrient retention and antinutrient (phytate) reduction in maize and pigeon pea seeds (Table 4.5)

Fermentation of maize reduced phytate content by 14%, increased protein content by 4% and retained about 72% of calcium. Combined germination (72 hours)-fermentation (24 hours) of maize decreased phytate content of maize by more than 90% and retained more iron (79% vs 61%) and zinc (80% vs 74%) compared to fermentation. Fermentation and germination of pigeon pea reduced above 90% of phytate but increased protein content by 16% and 5% respectively. Although, fermentation and germination reduced >90% phytate in pigeon pea, more nutrients were retained after fermentation.

Table 4.1 Proximate and mineral composition of fermented, germinated and germinated/fermented white maize flour(dry weight basis)

	Moisture(%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	CHO (%)	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	B-carotene (µg/100g)
M_O	$8.63^{a} \pm 0.02$	$10.04^{b} \pm 0.06$	$4.72^{a} \pm 0.02$	$1.41^{b} \pm 0.03$	$1.79^{a} \pm 0.02$	$74.82^{d} \pm 0.08$	$10.38^{a} \pm 0.07$	$3.70^{a} \pm 0.53$	$2.28^{a} \pm 0.06$	NA
M_1	$7.33^{d} \pm 0.02$	$10.44^{a} \pm 0.13$	$4.30^{b} \pm 0.02$	$1.18^{c} \pm 0.02$	$1.11^{d} \pm 0.01$	$76.81^{c} \pm 0.12$	$7.05^{b} \pm 0.14$	$2.25^{bc} \pm 0.35$	$1.68^{c} \pm 0.04$	NA
M_2	$7.43^{c} \pm 0.03$	$10.04^{b} \pm 0.05$	$3.20^{e} \pm 0.03$	$1.20^{c} \pm 0.01$	$1.49^{b} \pm 0.02$	$77.84^{b} \pm 0.05$	$4.35^{d} \pm 0.07$	$1.90^{c} \pm 0.07$	$1.60^{c} \pm 0.07$	NA
*M_3	$7.47^{c} \pm 0.02$	$9.92^{b} \pm 0.13$	$3.40^{d} \pm 0.02$	$1.59^{a} \pm 0.02$	$1.41^{c} \pm 0.02$	$77.79^{b} \pm 0.14$	$4.63^{c} \pm 0.11$	$2.93^{b} \pm 0.11$	$1.83^{b} \pm 0.03$	NA
M_4	$7.74^{b} \pm 0.02$	$9.12^{c} \pm 0.12$	$3.80^{c} \pm 0.01$	$1.19^{c} \pm 0.02$	$0.99^{e} \pm 0.01$	$78.35^{a} \pm 0.12$	$4.23^{d} \pm 0.07$	$2.83^{b} \pm 0.11$	$2.38^{a} \pm 0.01$	NA

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at $P < 0.05$.M_O– raw maize; M_1- fermented maize (72h); M_2-germinated maize(72h); M_3- germinated/fermented (72h/24h); M_4-germinated/fermented (72h/48h), CHO-carbohydrat

Table 4.2 Proximate and mineral composition of fermented, germinated and germinated/fermented pigeon pea flour(dry weight basis)

	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	CHO (%)	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	B-carotene (µg/100g)
P_O	$8.52^{a} \pm 0.02$	$21.20^{d} \pm 0.05$	$1.42^{d} \pm 0.02$	$2.20^{b} \pm 0.02$	$4.20^{a} \pm 0.01$	$64.66^{b} \pm 0.05$	$22.93^{d} \pm 0.11$	$18.88^{a} \pm 0.18$	$2.34^{a} \pm 0.01$	$1180.0^{a} \pm 1.37$
***P_1**	$7.88^{b} \pm 0.02$	$24.49^{a} \pm 0.05$	$2.29^{a} \pm 0.02$	$1.60^{c} \pm 0.03$	$3.21^{d} \pm 0.01$	$62.13^{d} \pm 0.03$	$35.33^{a} \pm 0.25$	$18.40^{a} \pm 0.14$	$1.99^{c} \pm 0.09$	$979.4^{e} \pm 0.13$
P_2	$6.67^{e} \pm 0.03$	$22.30^{c} \pm 0.05$	$1.90^{c} \pm 0.03$	$1.00^{d} \pm 0.03$	$4.10^{b} \pm 0.02$	$65.02^{a} \pm 0.03$	$25.48^{c} \pm 0.11$	$17.60^{b} \pm 0.07$	$2.13^{b} \pm 0.04$	$1035.6^{d} \pm 0.19$
P_3	$7.00^{d} \pm 0.02$	$23.27^{b} \pm 0.13$	$2.11^{b} \pm 0.02$	$3.19^{a} \pm 0.01$	$3.70^{c} \pm 0.01$	$63.92^{c} \pm 0.13$	$22.28^{e} \pm 0.18$	$18.80^{a} \pm 0.28$	$1.80^{d} \pm 0.01$	$1092.7^{c} \pm 0.31$
P_4	$7.56^{c} \pm 0.03$	$23.27^{b} \pm 0.13$	$2.11^{b} \pm 0.01$	$2.19^{b} \pm 0.02$	$3.21^{d} \pm 0.01$	$63.84^{c} \pm 0.13$	$26.50^{b} \pm 0.35$	$17.88^{b} \pm 0.18$	$2.13^{b} \pm 0.03$	$1124.2^{b} \pm 0.09$

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at $P < 0.05$. P_O– raw pigeon pea; P_1- fermented pigeon pea (72h); P_2-germinated pigeon pea (72h); P_3- germinated/fermented pigeon pea (72h/24h); P_4- germinated/fermented pigeon pea (72h/48h).

Table 4.3 Anti-nutrient composition of fermented, germinated and germinated/fermented maize flour (dry weight basis)

Parameters	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	Polyphenol (mg/100g)	Hemaglutinin (HU/100g)
M_O	$28.00^{a} \pm 1.41$	$967.50^{a} \pm 10.61$	$590.00^{a} \pm 84.86$	$425.00^{b} \pm 49.50$	$1110.00^{a} \pm 56.57$	$17.31^{b} \pm 0.04$
M_1	$27.50^{a} \pm 4.95$	$828.50^{b} \pm 2.12$	$646.00^{a} \pm 5.66$	$545.00^{a} \pm 35.35$	$715.00^{c} \pm 35.36$	$19.53^{a} \pm 0.07$
M_2	$20.00^{ab} \pm 1.41$	$85.50^{c} \pm 2.12$	$42.50^{b} \pm 4.95$	$195.00^{c} \pm 21.21$	$985.00^{b} \pm 35.36$	$13.65^{c} \pm 0.04$
**M_3	$20.50^{ab} \pm 4.95$	$74.50^{cd} \pm 4.95$	$31.00^{b} \pm 2.83$	$140.00^{c} \pm 14.14$	$795.00^{c} \pm 49.50$	$9.18^{d} \pm 0.04$
M_4	$17.00^{b} \pm 2.83$	$61.00^{d} \pm 1.41$	$29.50^{b} \pm 4.95$	$135.00^{c} \pm 35.35$	$745.00^{c} \pm 35.36$	$8.83^{e} \pm 0.05$

Values are expressed as mean ±SD (n=2). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$. M_O – raw maize; M_1- fermented maize (72h); M_2-germinated maize (72h); M_3- germinated/fermented (72h/24h); M_4-germinated/fermented (72h/48h).

Table 4.4 Anti nutrient composition of fermented, germinated and germinated/fermented pigeon pea flour(dry weight basis)

Parameters	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	Polyphenol (mg/100g)	Hemaglutinin (ug/mg)
P_O	303.50^{a}± 9.19	1235.00^{a} ± 35.36	967.50^{a}±3.54	2225.00^{a}±77.79	4635.00^{a} ±77.78	59.89^{a}±0.20
**P_1	49.00^{c} ± 2.83	97.00^{b} ±7.07	83.00^{c}±4.25	995.00^{c}± 49.50	2785.00^{c}±35.36	18.69^{e}±0.10
P_2	58.50^{bc} ±3.54	116.00^{b}±4.24	100.00^{b}±2.83	1130.00^{b}±14.14	2910.00^{bc}± 56.57	21.33^{d}±0.10
P	57.00^{bc} ±4.24	1244.50^{a}±50.21	975.50^{a}±7.78	1185.00^{b}±35.36	2910.00^{bc}±28.28	23.89^{c}±0.05
P_4	64.50^{b} ±2.12	1236.50^{a} ± 4.95	109.50^{b}±7.78	1205.00^{b}± 7.07	3020.00^{b}±56.57	26.26^{b}±0.07

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$. P_O– raw pigeon pea; P_1- fermented pigeon pea (72h); P_2-germinated pigeon pea (72h); P_3-germinated/fermented pigeon pea (72h/24h); P_4- germinated/fermented pigeon pea (72h/48h).

Table 4.5 Summary of effect ofprocessing methods on nutrient retention and antinutrient reduction

	Increase	Decrease	No effect
Fermentation			
Maize	Protein, fibre, Ca, carb	Fat, ash, iron,zinc, **phytate** (14%)	
Pigeon pea	**Protein(more),** fat, Ca,	Fibre, ash,carb,zinc, betacarotene **Iron (less)**, **anti nutrients**(more)	
Germination			
Maize	Fibre, carb	Anti nutrients, ash,iron, zinc	protein
Pigeon pea	Protein,fat, carb,Ca,	Anti nutrients, ash, fibre,iron, zinc, betacarotene	
GerFer (72/24hrs)			
Maize	Carb, fibre	**Phytates (>90%), Protein(less),**fat, ash, **iron (less), Ca (less),**zinc	
Pigeon pea	Protein,fat, fibre	Ash, carb,zinc, Ca, anti nutrients,betacarotene	Iron,phytate
GerFer (72/48hrs)			
Maize	Carb	Ca, Iron, zinc, fat,protein,fibre,antinutrients	
Pigeon pea	Ca,protein	Iron, zinc,betacarotene,carb, antinutrients	Fibre,phytate

4.3 Nutritional evaluation of complementary blends

4.3.1 Macronutrient composition of ingredients for complementary foods (Table 4.6)

This table displays the proximate, calcium, iron, zinc, beta-carotene and phytate contents of ingredients used for formulation of the complementary foods. From the Table, it was observed that dried fish powder (DFP) had the highest protein content (77.93±0.26%) followed by fermented boiled pigeon pea (23.49±0.05%), and pumpkin leaves (19.50±0.14%). The highest fibre content was observed in pumpkin leaves powder (3.30±0.14%) even after sieving, followed by carrot powder (2.60±0.14%). The ash content of germinated fermented maize was the lowest (1.41%) while DFP and carrot powder had appreciable ash contents of 13.36±0.38% and 11.85±0.21%, respectively.

4.3.2 Mineral and phytate composition of ingredients for complementary foods (dry weight basis) (Table 4.7)

DFP had the highest content of calcium (362±21.14 mg/100g), iron (8.15 mg/100g) and zinc (7.85 mg/100g) but lower vitamin A (RE) (39.43±2.81 RE) when compared to carrot powder (576.13±55.44 RE), pumpkin leaves (212.73±21.22 RE) and fermented pigeon pea (163.23±10.99 RE). Fermented boiled pigeon pea and roasted pigeon pea flours had appreciable quantities of iron content of 18.40±2.14 and 17.44±1.98 mg/100g respectively. Pumpkin leaf powder (TSP) had the highestphytate content of 629 mg/100g which was about four times of the value in carrot powder (CP) (156 mg/100g). The processed products which were germinated-fermented maize, fermented boiled pigeon pea, roasted maize and pigeon pea had low phytate levels (72.85-97.00 mg/100g). The highest quantity of beta-carotene (3456.78µg/100g) represented as retinol equivalent (RE) was observed in carrot which had a mean of 576 RE (1728.39µgRE). Vitamin A in form of beta-carotene was not detected in the white maize products, germinated-fermented maize and roasted maize (GFM, RM) as was expected.

4.3.3Energy and Chemical composition of formulated diets

The proximate, energy, mineral, vitamin and anti-nutrient contents of the two formulated complementary foods is shown in Table 4.8. The blends were significantly different at $p<0.05$ in all examined variables except for protein, crude fibre and carbohydrate contents. Compif O had higher moisture content (6.97%) compared to Compif R (5.94%). The energy content of Compif O (402.51 kcal) was lower than that of Compif R (412.76 kcal). The zinc content of Compif O (3.35%) was significantly higher than that of Compif R (1.15%) at $p<0.05$. CompifR had significantly higher ($p<0.05$) calcium content of 118 mg/100g compared to the 83mg/100g of CompifO, but CompifO had significantly higher ($p<0.05$) iron and vitamin A equivalentlevels.

The roasted diet, Compif R had lower levels of phytate, tannin, oxalate, polyphenol and saponin compared to Compif O.

Table 4.6 Macronutrient composition of ingredients for complementary foods per 100g dry weight

Ingredients	Moisture %	Crude P %	Crude Fat %	Crude fibre %	Ash %	Carb[a] %
GFM	7.47 ± 0.02	9.92 ± 0.13	3.40± 0.01	1.59± 0.02	1.41± 0.01	77.79± 0.12
RM	5.54 ± 0.78	9.85 ± 0.14	4.20 ± 0.08	1.35± 0.14	1.30 ± 0.22	79.11 ± 0.17
FERBP	8.88± 1.02	23.49 ±0.05	2.29± 0.02	1.60± 0.03	3.21± 0.01	62.13± 0.03
RP	5.33± 0.02	20.45± 0.04	2.95 ± 0.15	3.09 ± 0.01	3.10 ± 0.71	74.10 ± 0.87
DFP	7.21±0.014	77.93±0.26	1.50±0.14	ND	13.36±0.38	NIL
SUCROSE	-	-	-	-	-	100
OIL	-	-	100	-	-	-
TSP	8.12±0.03	19.50±0.14	2.80±0.14	3.30±0.14	7.05±0.07	59.10±0.14
CP	7.26±0.08	9.20±0.14	2.90±0.11	2.60±0.14	11.85±0.21	66.30±0.28

GFM: Germinated fermented maize flour, RM: Roasted maize, FERBP: Fermented boiled pigeon pea flour, RP: Roasted pigeon pea, DFP: Dried steamed fish powder(codfish), TSP: Dried pumpkin leaves powder, CP: Carrot powder.

[a] by difference.

ND = Not detectable

NA= Not analysed

Table 4.7 Mineral and phytate composition of ingredients for complementary foods (dry weight basis)

Ingredients	Ca (mg/100g)	Fe (mg/100g)	Zn (mg/100g)	Vitamin A (RE)	Phytate (mg/100g)
GFM	4.63 ± 0.11	2.93± 0.11	1.83±0.03	ND	74.50±4.95
RM	9.42± 1.22	2.13 ± 0.57	1.79 ± 0.12	NA	72.85 ± 2.99
FERBP	35.33 ± 4.25	18.40 ±2.14	1.99 ± 0.09	163.23 ±10.99	97.00 ± 7.07
RP	37.57 ± 3.88	17.44 ± 1.98	1.78± 1.14	152.21± 11.24	79.81± 1.22
DFP	362.00 ± 1.14	8.15±0.07	7.85±0.16	39.43 ±0.81	97±0.42
SUCROSE	NA	NA	NA	NA	NA
OIL	NA	NA	NA	NA	NA
TSP	236.50±1.20	3.20±0.15	2.95±0.24	212.73± 2.22 (638.20µgRE)	629±2.52
CP	27.45±0.07	0.45±0.05	0.30±0.05	576.13±5.44 (1728.39µgRE)	156±0.98

GFM: Germinated fermented maize flour, RM: Roasted maize, FERBP: Fermented boiled pigeon pea flour, RP: Roasted pigeon pea, DFP: Dried steamed fish powder(codfish), TSP: Dried pumpkin leaves powder, CP: Carrot powder.
[a] by difference.
ND = Not detectable
NA= Not analysed
RE= Retinol equivalent

Table 4.8 Energy and chemical composition of formulated diets (dry matter basis)

Parameters	Compif O	Compif R
Moisture	6.97[a]± 0.09	5.94[b]±0.05
Crude Protein (%)	17.18[a]± 0.04	16.79[a] ± 0.02
Crude Fat (%)	7.75[b]±0.07	9.30[a] ± 0.28
Crude Fibre (%)	2.00[b]±0.14	1.70[b] ±0.14
Ash (%)	2.21[b]±0.14	2.50[a] ± 0.14
Carbohydrate (%)	66.01[b]±0.08	65.48[b] ±0.50
Energy(kcal/100g)	402.51[b]±0.16	412.76[a]±0.65
Ca (mg/100g)	83.00[b]± 0.14	118[a]± 0.42
Fe (mg/100g)	3.60[a]± 0.25	1.95[b]± 0.17
Zn (mg/100g)	3.35[a]±0.07	1.15[b]± 0.14
Vitamin A(µg RE)	312.89[b]	271.82[b]
Phytate (mg/100g)	179.00[a]	146[b]
Tannin(mg/100g)	4.60[a]	3.10[b]
Oxalate(mg/100g)	215.0[a]	184[b]
Polyphenol(mg/100g)	136[a]	109[b]
Saponin(mg/100g)	247[a]	212[b]
Hemaglutinin(µg/mg)	6.29[a]	5.48[b]
Alkaloids(mg/100g)	8940[a]	8760[b]

4.3.4Proximate, mineral and vitamin content of complementary foods compared with CCF and WHO recommendation (Table 4.9)

There were significant differences ($p<0.05$) in the moisture, crude protein, crude fat, energy, calcium and iron content of the formulated diets and the control (CCF). The moisture contents of Compif O (6.97%) and Compif R (5.94%) significantly lower than that of CCF (4.50%) at $p<0.05$. The two formulated foods did not meet the <5% limit for moisture content recommended for processed complementary foods. The crude protein contents of Compif O (17.18%) and Compif R (16.79%) were higher than 15% recommended by the Codex Alimentarius Commission. CCF had the least protein content of15.5%. The control was superior in terms of crude fat (10%), energy (425 kcal/100g), calcium (410 mg/100g) and iron (8.52 mg/100g). The two formulated foods met the protein, crude fibre and energy specifications of the Codex Alimentarius Commission Standard for complementary foods for infants and young children. Both formulations had fat contents lower than the specified values, 7.75% for Compif O and 9.30% for Compif R, but Compif R satisfied about 93% of the stipulated value (10-25%). The ash contents of the two formulated diets were not more than 3% as recommended. Compif O had ash content of 2.21% which was significantly lower than those of Compif R (2.50%) and CCF (2.60%). The carbohydrate contents of the complementary foods were comparable and within the recommended range of 60-65%. The control had significantly greater ($p<0.05$) proportions of calcium, iron and vitamin A compared to the two formulated complementary foods. Compif O had higher zinc value compared to that of the CCF by approximately 12% (3.35 vs 3.00mg/100g) and met the recommended zinc value. Compif O satisfied the iron, zinc and vitamin A specification at least 50% of reference nutrient intake for the age group. CompifR met recommended range of vitamin A, iron (lower border) but did not meet that of zinc. The phytate level of control (66.92 mg/100g) was significantly ($p<0.05$) lower than those of Compif R (146mg/100g) and Compif O (179mg/100g).

Table 4.9 Proximate, mineral and vitamin content of complementary foods compared with WHO recommendation

Parameters	Compif O	Compif R	CCF[#]	Codex Standard*
Moisture	6.97[a]± 0.09	5.94[b]±0.05	4.50[c]±0.00	< 5
Crude Protein (%)	17.18[a]± 0.04	16.79[a] ± 0.02	15.50[b]±0.00	15
Crude Fat (%)	7.75[c]±0.07	9.30[b] ± 0.28	10.00[a]±0.00	10-25
Crude Fibre (%)	2.00[b]±0.14	1.70[b] ±0.14	2.20[a]±0.02	<5
Ash (%)	2.21[b]±0.14	2.50[a] ± 0.14	2.60[a]± 0.00	<3
sCarbohydrate (%)	66.01[b]±0.08	65.48[b] ±0.50	67.30[a]±0.00	60-65
Energy(kcal/100g)	402.51[c]±0.16	412.76[b]±0.65	425.00[a]±0.02	400-425
Ca (mg/100g)	83.00[c]± 0.14	118[b]	410[a]	250 (50% of RNIs)
Fe (mg/100g)	3.60[b]± 0.00	1.95[c]	8.52[a]	1.95-5.80[β](50% of RNIs)
Zn (mg/100g)	3.35[a]±0.07	1.15[b]	3.00[a]	1.20-4.15[β](50% of RNIs)
Vitamin A(µg RE)	312.89[b]	271.82[c]	370.00[a]	200 (50% of RNIs)
Phytate (mg/100g)	179.00[a]	146[b]	[$]66.92[c]	

*Codex Alimentarius Commission, 2013

[#]Nutrition label

[$] Amagloh, 2014

[β] Iron and zinc values given for high to low bioavailability.

4.3.5Contribution of energy and some nutrients of the formulated complementary foods compared to recommended energy/nutrient needs from complementary foods of 6 to 24 months children (developing countries)

Table 4.10 displays the proportion of recommended energy and nutrients provided by the formulated complementary foods. The energy density of one feeding (40g dry portion) of Compif O (4.03 kcal/g) and Compif R (4.13 kcal/g) satisfied the recommended energy density of ≥4 kcal/g for the three age groups. Compif O and Compif R (40g dry food) supplied about 14% and 11%, respectively, of 200 kcal and 300 kcal as protein. These values are within the recommended range of 6-15% for ages 6-8 months and 9-11 months. Two feedings of the formulated foods provided about 12% of 550 kcal as protein for 12 to 24 month children. The estimated fat intakes from the formulated foods did not meet the recommended intakes from complementary foods for age groups 9-11 and 12-24 months. CompifO and CompifR satisfied 80 and 83% , 67 and 69%, 73 and 75% respectively, of the energy needs for processed complementary foods for ages 6-8, 9-11 and 12-24 months.

4.3.6Phytate, iron, zinc and calcium molar ratios of formulated complementary foods (Table 4.11)

The two complementary foods did not meet the recommendation for phytate:iron molar ratio (4.17,6.31 vs <1) for plant based complementary foods. The maximum recommended phytate:zinc ratio (<15) was met for Compif O (5.32) and Compif R (12.28). However, there were significant differences between the two formulated complementary foods in phytate:calcium,iron and zinc ratios. Compif O performed better in phytate:iron and phytate:zinc ratios than Compif R while Compif R was better in phytate:calcium ratio.

Table 4.10 Contribution of energy and some nutrients from formulated complementary foods compared to recommended energy/nutrient needs from complementary foods of 6 to 24 months children (developing countries)

	6 -8 months			9-11 months			12-24 months		
Energy/nutrients	Compif O	Compif R	Ref value@,x	Compif O	Compif R	Refvalue	Compif O	Compif R	Ref value
Quantity of food (g)	40	40	40*	50	50	10-50[x]	100	100	10-50[x] per feed
No of feedings	1	1	2-3	1	1	3-4	2	2	3-4
Energy density (kcal/g)	4.03	4.13	≥ 4	4.03	4.13	≥ 4	4.03	4.13	≥ 4
Energy(kcal/day)	161±0.0	165 ± 0.00	200	201.26	206.38	300	402.51	412.76	550
Protein (g/day)	6.87 ±0.04	6.72 ± 0.02	5 (10%)	8.59	8.40	7.5	17.18	16.79	13.75(10%)
Fat (g/day)	3.10±0.04	3.72±0.05	(6-15%) 0	3.88	4.65	(10%) 6.67 (20%)[X]	7.75	9.30	(6-15%) 12.22 (20%)

*Dewey and Brown, 2003
@ PAHO/WHO (2003)
[x] Codex Alimentarius Commission (2013)

Table 4.11 Phytate, iron, zinc and calcium molar ratios of Complementary foods

Complementaryfood	Phytate	Iron	Zinc	Calcium
Compif O (mg/100g)	179	3.60	3.40	83
Compif R (mg/100g)	146	1.95	1.15	118
Compif O (molar ratios)		4.17^{b}	5.32^{b}	0.13^{a}
Compif R (molar ratios)		6.31^{a}	12.28^{a}	0.08^{b}
P value		0.008	0.010	0.002
Maximum recommended Molar ratios*		<1	<15	<0.17

* Gibson et al., (2014).

4.3.7Essential amino acid pattern (mg/g) of formulated foods

The table shows the essential amino acid profile of the two formulated foods (Table 4.12). The total sulfur amino acids (cysteine and methionine) of Compif O and Compif R were 27.0 and 25.8 mg/g, respectively. Compif O met the recommended limit for the total sulfur amino acids while Compif R did not. The two formulated complementary foods had more than the recommended total aromatic amino acids (tyrosine and phenylalanine). Compif O and Compif R recorded leucine and isoleucine contents that were higher than that of the CCF. Isoleucine contents of the two formulated complementary foods were higher than the reference pattern by more than four times. There was no significant difference ($p>0.05$)in the amino acid scores (AAS) of CompifO (75%) and CompifR(74%) but they differed significantly ($p<0.05$) with that of the CCF (89%). The limiting amino acid in the two formulations was lysine.

4.4Functional and microbiological properties of the formulated complementary Foods

4.4.1 Functional properties of formulated complementary foods and CCF

The selected functional properties of complementary foods are displayed inTable 4.13. Water absorption capacity (WAC), bulk density and swelling capacity of the formulated diets were comparable and lower than those of the CCF. Lower WAC, bulk density and swelling capacity are desirable qualities of complementary foods.Water absorption capacity values of the formulated complementary foods and CCF ranged from 123 to 134 g/100ml. Compif R had the least bulk density (0.62 g/ml) and least gelation capacity (6.93%) butthe highest viscosity value of 321.46 cps.The formulated diets had higher least gelation capacity and viscosity compared to those of CCF which are not desirable qualities of complementary foods.

Table 4.12 Essential Amino acid pattern(mg/g dry weight) and protein correctedamino acid score of formulated diets

Amino acid	FAO/WHO EAA pattern [δ]	Compif O	Compif R	Cerelac*
Cystine		6.4	5.6	
Methionine		20.6	20.2	
Total sulfur A.A	27	27	25.8	46.1
Lysine	57	42.8	42.0	50.5
Valine	43	45.5	42.2	59.6
Tryptophan	8.5	72.5	71.8	15.3
Tyrosine		26.1	25.1	
Phenylalanine		57.9	57.6	
Total aromatic A.A	52	84.0	82.7	96.9
Leucine	66	109.0	108.4	87.8
Isoleucine	32	150.1	148.8	47.1
Threonine	31	40.3	39.4	38.5
Histidine	20	62.5	62.8	29.5
AAS[@]		0.75[b]	0.74[b]	0.89[a]
LEAA[R]		lysine	lysine	lysine

[δ] Reference essential amino acid pattern for 6 months to 3 years old children

@Amino acid score (AAS) = Amino acid content of test diet divided by the reference value

[R] Limiting essential amino acid (LEAA) = The amino acid with the lowest amino acid score ratio.

Table 4.13 Functional properties of formulated complementary foods and CCF

Complementary foods	Water Absorption Capacity (g/100ml)	Least Gelation Capacity (%)	Swelling Capacity (g/g)	Viscosity (cps)	Bulk Density (g/ml)
Compif O	125[b]	7.14	15.03	319.76	0.63
Compif R	123[b]	6.93	14.79	321.46	0.62
Cerelac	134[a]	9.86	19.84	268.30	0.67

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$.

4.4.2 Microbiological analysis of formulated foods at weekly intervals (4 weeks)

The total viable count (TVC) and fungal count values obtained for Compif O and Compif R at weekly intervals for four weeks are displayed in Table 4.14. There were significant differences ($p<0.05$) between the microbial counts of Compif O and Compif R at the weekly counts. The microbial counts increased continuously from the first point (first week) to the fourth week.Compif O had higher microbial load than Compif R at all the evaluation points. The TVC ranged from 0.5 to 2.0 x 10^4cfu/g for Compif O and 0.0 to 0.7 x 10^4cfu/g for Compif R. There were significant differences at $p<0.05$ in the microbial count of Compif O from the first week upto the fourth week (Figure 4.3.2b) while for Compif R after the first week, the TVCs were similar ($p>0.05$).No fungal or mould growth was detected till the fourth week when about 0.5 x 10^4cfu/g of fungi was observed in Compif O. *Escherichia coli* was not found in the two formulated complementary foods throughout the four weeks study period. The microbial counts were below the limit of 10^5specified for dry foods.

The trend of the microbial (bacterial) growth at weekly intervals is presented in Figure 4.1. The rate of bacterial growth was higher in the first week compared to the other weeks. The figure clearly shows the difference in the TVCs of Compif O and Compif R.

Table 4.14 Microbiological analysis of formulated complementary foods at weekly intervals (4 weeks)

SAMPLE	Compif O	Compif R	Compif O	Compif R	Compif O	Compif R	Compif O	Compif R
Weeks	Week 1		Week 2		Week 3		Week 4	
Total viable count (cfu/g) Week1	$0.5^{a} \times 10^{4}$	$0.0^{b} \times 10^{4}$	$1.5^{a} \times 10^{4}$	$0.5^{b} \times 10^{4}$	$1.8^{a} \times 10^{4}$	$0.6^{b} \times 10^{4}$	$2.0^{a} \times 10^{4}$	$0.7^{b} \times 10^{4}$
Fungi/mould count point1(cfu/g	No growth	No growth	No growth	No growth	No growth	No growth	0.5×10^{4}	No growth
E.coli count (cfu/g)	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth

Values are expressed as mean ±SD (n=2). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$.

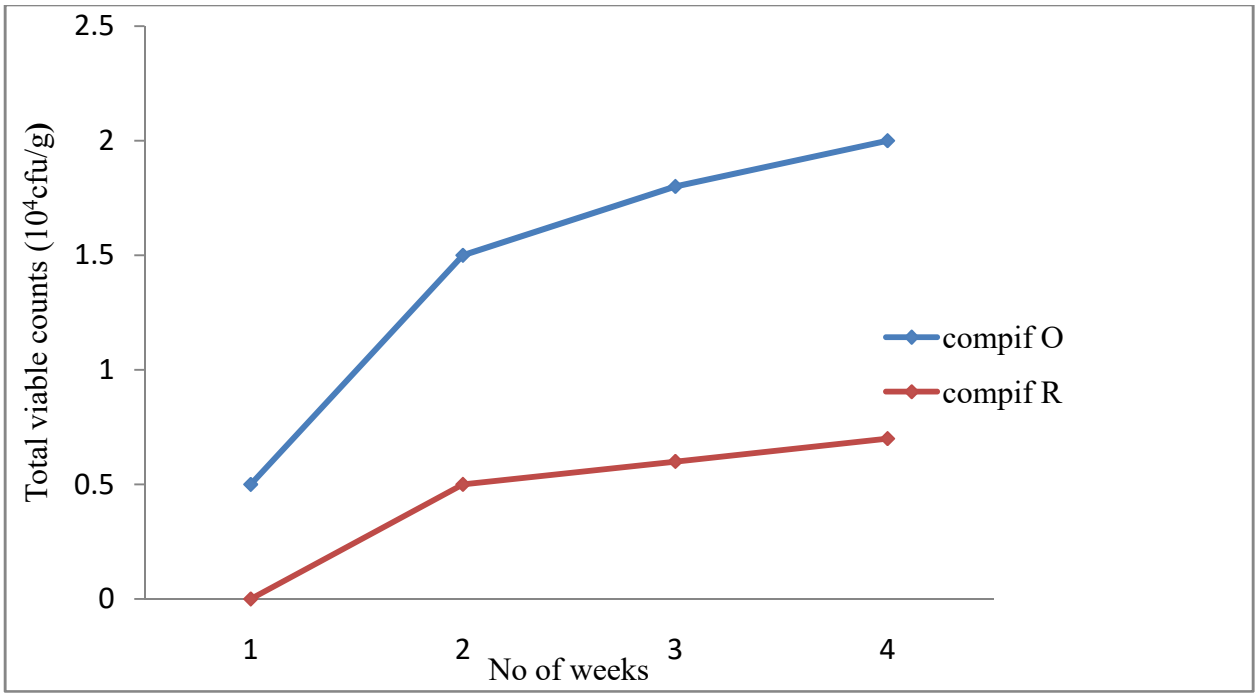


Figure 4.1 Changes in the TVC of the formulated diets at weekly intervals

4.5 Nutritional quality of the formulated and control diets

4.5.1 Initial and final mean weight and length of the experimental rats

The initial mean weight and length of the experimental rats are shown in Table 4.15. The initial mean weight of the rats (after acclamitisation period) ranged from 35 to 40 g while the initial mean length ranged between 17 and 18 cm.

4.5.2 Weight changes of experimental rats during 21 days feeding period

The weight changes of the experimental rats in relation to days of feeding are displayed in Figure 4.2. Weight was measured on Mondays and Thursdays in the morning. Figure shows that for the first four days, the weight of the rats fed Casein-based diet, CCF based diet and Compif O based diet increased almost at the same rate. The weight of the protein-free group decreased continuously while that of the Compif R groupremained static and later increased very slowly. However, after the eighth day, the rate of weight increase of the rats fed Compif Obased diet dropped compared to the weight increase of the Casein and CCF groups but no significant difference existed in the increase in relation to feeding period.

4.5.3 Weight for age of experimental rats fed the experimental diets

Figure 4.3 shows a dramatic change in weight-for-age pattern after five weeks of age between Casein/CCF/Compif O groups of rats and Compif R/ protein free groups. Rats fed Casein based diet and CCF based diet showed better growth pattern which was not significantly different from those of Compif O group but significantly different ($p<0.05$) from those of Compif R and protein free groups.

Table 4.15 Initial and final mean weight and length of the experimental rats

Parameters	CCF	Casein	Compif O	Compif R	Protein free
Initial weight (g)	35.44±5.78	38.31±3.97	39.44±3.70	39.84±3.85	39.68±3.58
Final weight (g)	68.51± 4.02	71.05±3.46	63.62±8.00	45.61±5.19	21.39±2.40
Weight gain/loss(g)	33.07 ±9.81[a]	32.74 ± 7.50[a]	24.05 ±4.94[a]	5.75 ± 3.63[b]	-14.17±6.56[c]
Initial tail length (cm)	8.20 ±0.57	8.88 ± 0.18	8.20 ± 0.76	8.34 ± 0.96	7.60±1.38
Final tail length (cm)	10.64 ±0.05	11.55 ± 0.21	10.66 ± 1.04	10.16 ± 0.42	8.17±1.30
Tail length gain(cm)	2.44 ±0.38[a]	2.68 ± 0.04[a]	2.46 ± 1.58[a]	1.82 ± 0.81[b]	0.53±0.36[b]
Initial body length (cm)	17.16 ±0.77	18.20 ± 0.21	17.16 ± 1.37	17.14 ± 1.22	17.12±1.95
Final body length (cm)	22.34 ±1.15	23.80 ± 0.57	22.51 ± 1.81	21.26 ± 1.10	18.37±1.91
Total body length gain(cm)	5.18 ±1.26[a]	5.60 ± 0.85[a]	5.35 ± 2.02[a]	4.12 ± 1.08[a]	1.25±0.44

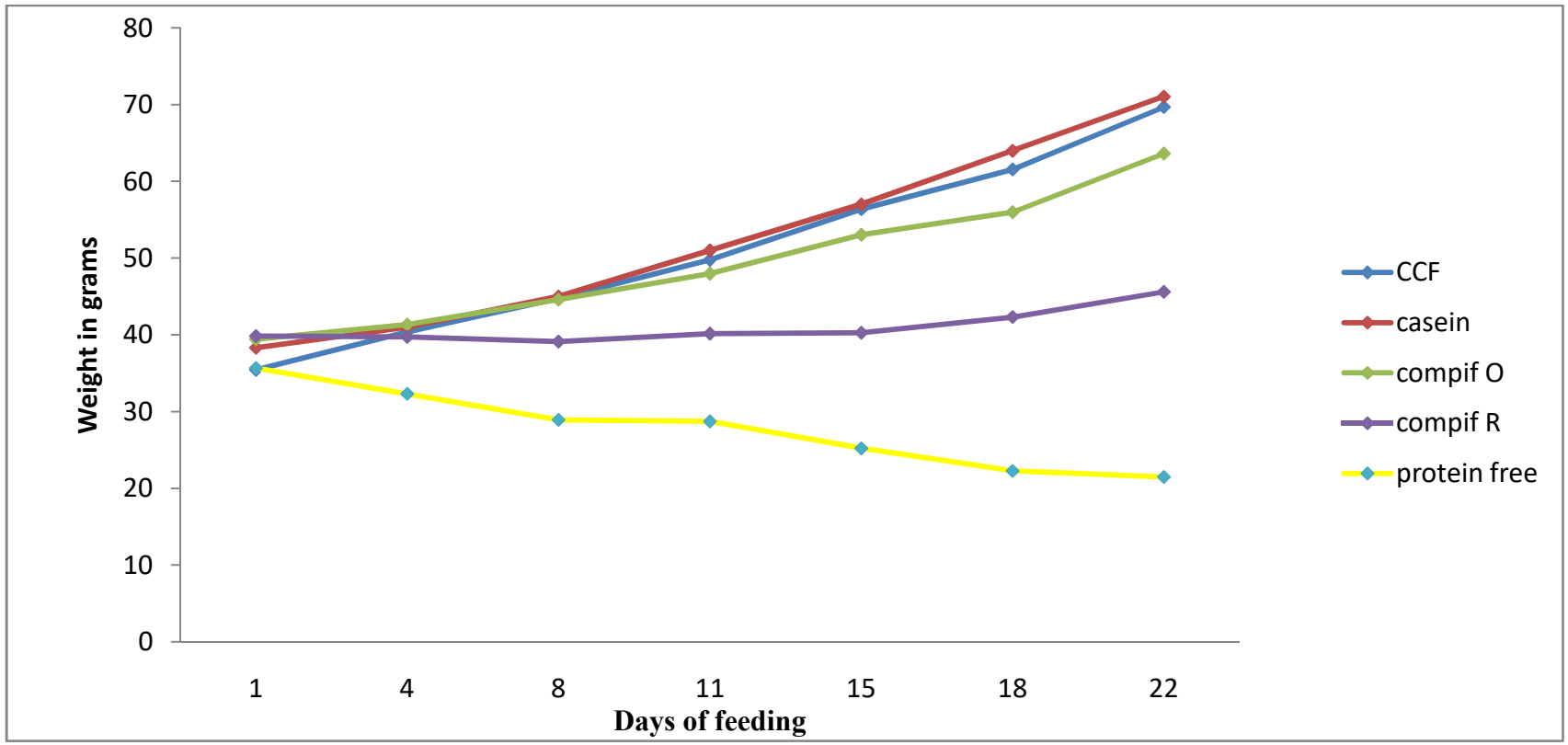


Fig 4.2 Weight changes of experimental rats during 21 days of feeding

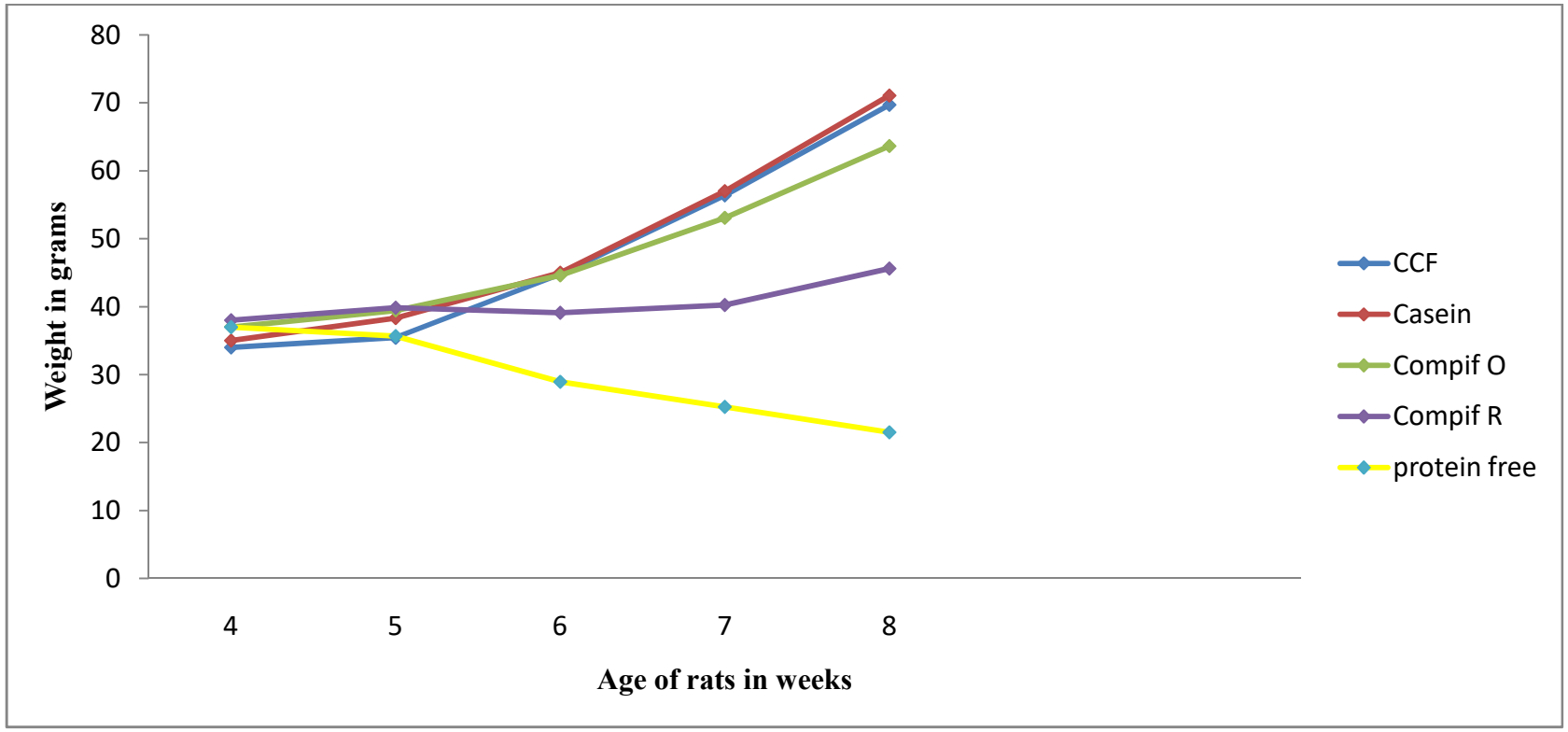


Figure 4.3 Weight for age ofexperimentalrats fed the experimental diets

4.5.4 Weight gain/loss of experimental rats during the feeding period

The mean weight gain of the rats ranged from 5.75g (Compif R) to 33.07g (CCF) (Figure 4.4). The weight gain of rats fed Compif O based diet (24.05g) was not significantly different from those of CCF (33.07g) and casein (32.74g) groups at $p \leq 0.05$. The weight gain of the experimental rats fed Compif R based (5.75g) and basal (-14.09g) diets were significantly lower than weight gain of rats from the other groups. The experimental rats fed with Compif R based diet increased their mean weight by 14% after 21 days of feeding compared to Compif O group (61%).

4.5.5Total body length gain of experimental rats fed the test diets

The effect of the experimental diets on growth of the experimental rats in terms of length is displayed in Figure 4.5. The highest mean total body length gain was recorded in casein group (5.60cm) while the lowest length gain was observed among protein-free group (1.75 cm). However, the difference between the length gain ofrats fed the two formulated foods and the control diets was not significant, but there was a significant contrast when compared with that of the protein-free group. The total mean body length gain of the experimental rats fed with Compif O was similar at $p>0.05$ with that of Compif R group. The difference in length gain was 9%.

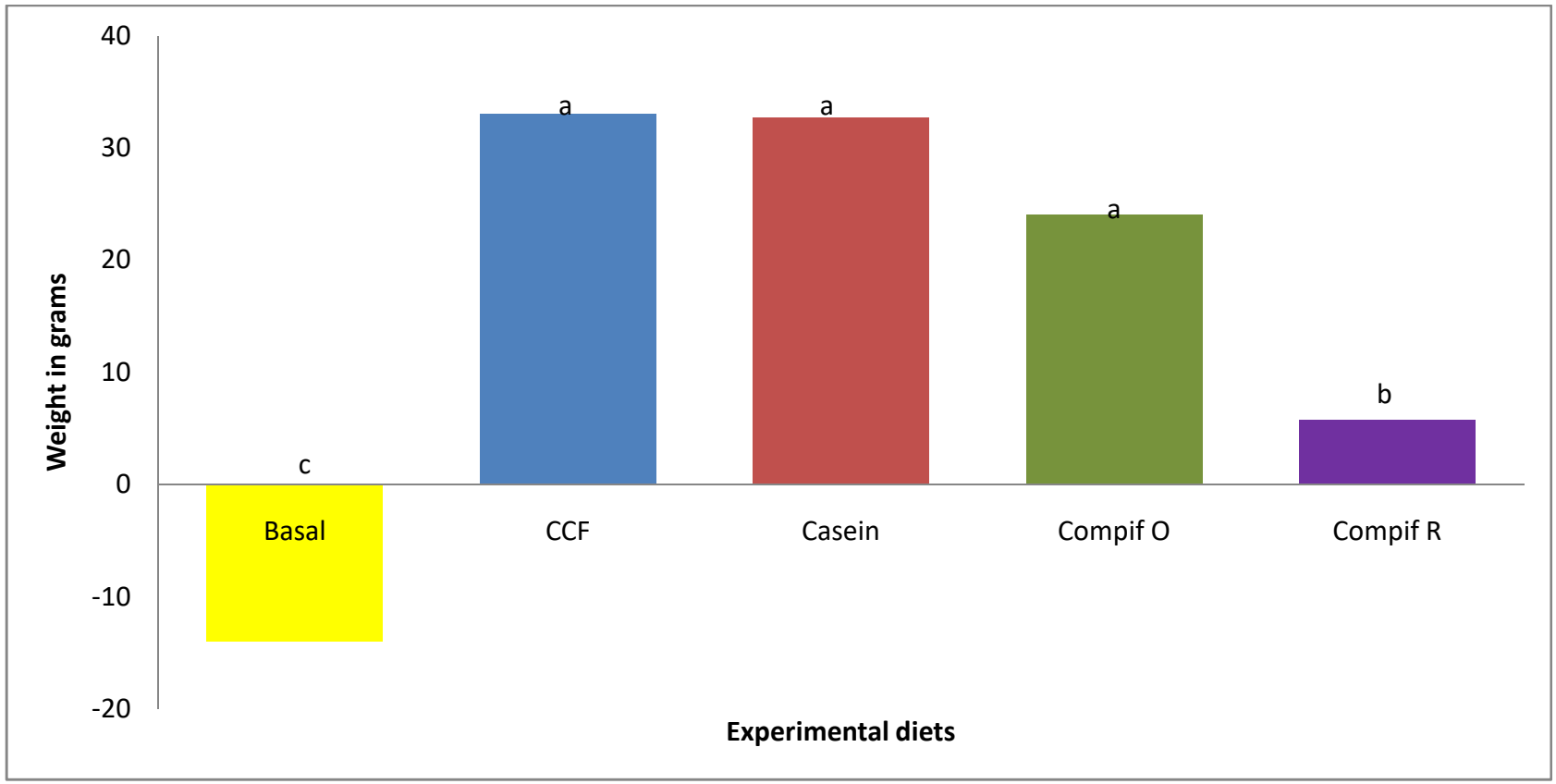


Figure 4.4 Weight gain/loss of experimental rats during the feeding period

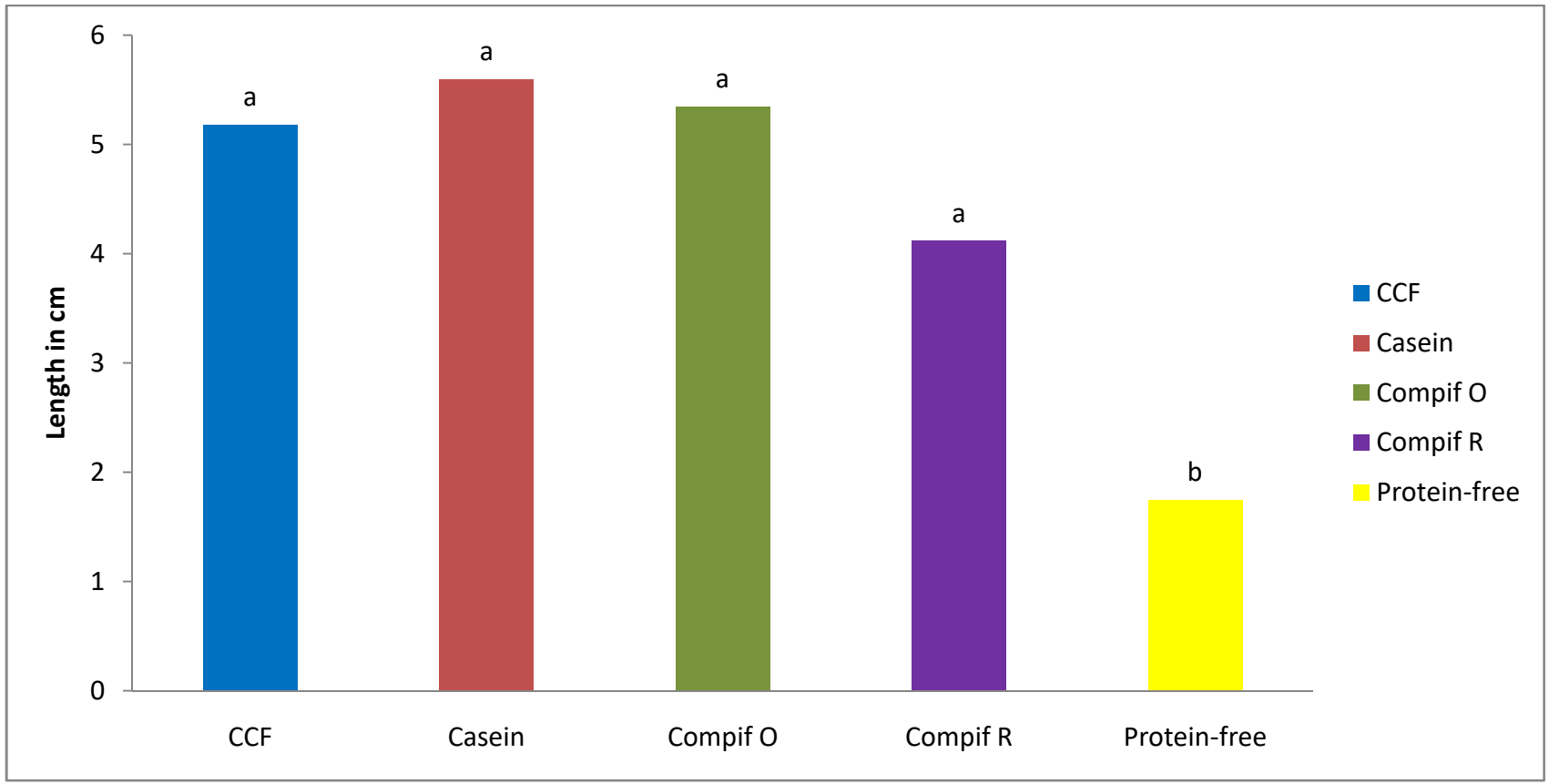


Figure4.5 Total body length gain ofexperimentalrats fed the test diets

4.5.6 Feed Conversion Ratio, Feed Efficiency Ratio and Protein Efficiency Ratios of test diets

The Feed ConversionRatio (FCR), Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) of the test and control diets are presented in Figure4.6, 4.7 and 4.8, respectively. The mean feed ranged from 71.96g (protein-free diet) to 151.13g (CCF based diet) while the mean protein intake ranged from 0.477g (protein-free diet) to 14.74g (CCF based diet) (Table 4.15).

The FCR of Compif R based diet (30.32) was significantly higher than that of Compif O (5.54). High FCR is not a desirable quality of both human and feed. The FCR of rats fed Compif O diet (5.54)was similar to those of the rats fed CCF based diet (4.57) and Casein based diet (3.64) at $p>0.05$ (Figure 4.6).

The FER of Compif O based diet (0.18) also compared favourably with those of that of the CCF based diets (0.23) but significantly lower than the FER of the Casein based diet (0.27). Compif O recorded FER that was significantly ($p<0.05$) higher than the FER of Compif R group (0.05). Low FER of food is a desirable quality.

The difference between the Protein Efficiency Ratio (PER) of Compif O group (1.69) and those of the two control diets, 2.22 (CCF) and 2.64 (Casein)was significant ($p<0.05$).Although the two formulated complementary foods did not meet the recommendation of 2.1, Compif O had better PER (1.69) than Compif R (0.48).

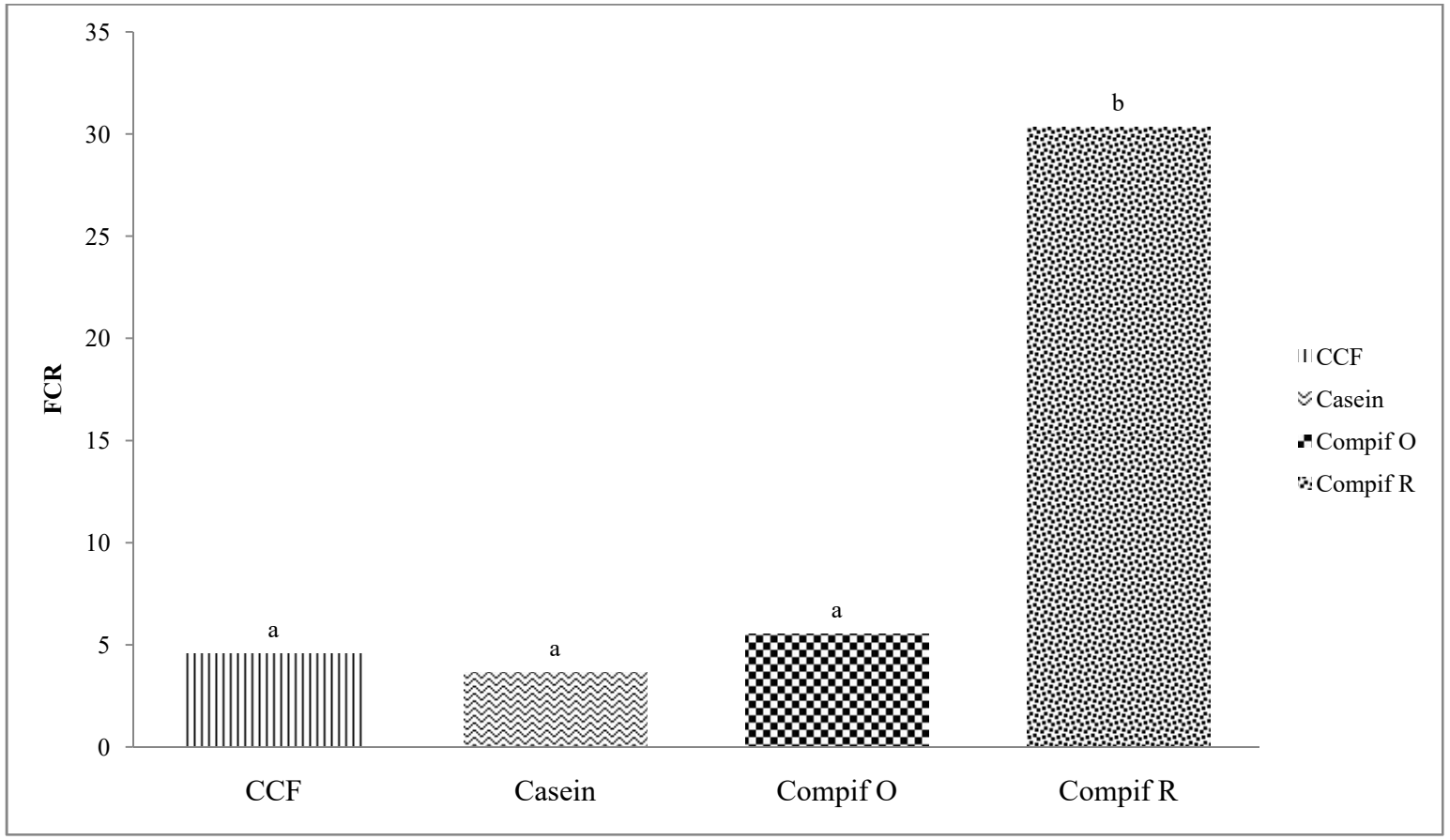


Figure 4.6 Feed Conversion Ratio (FCR) of the formulated and control diet

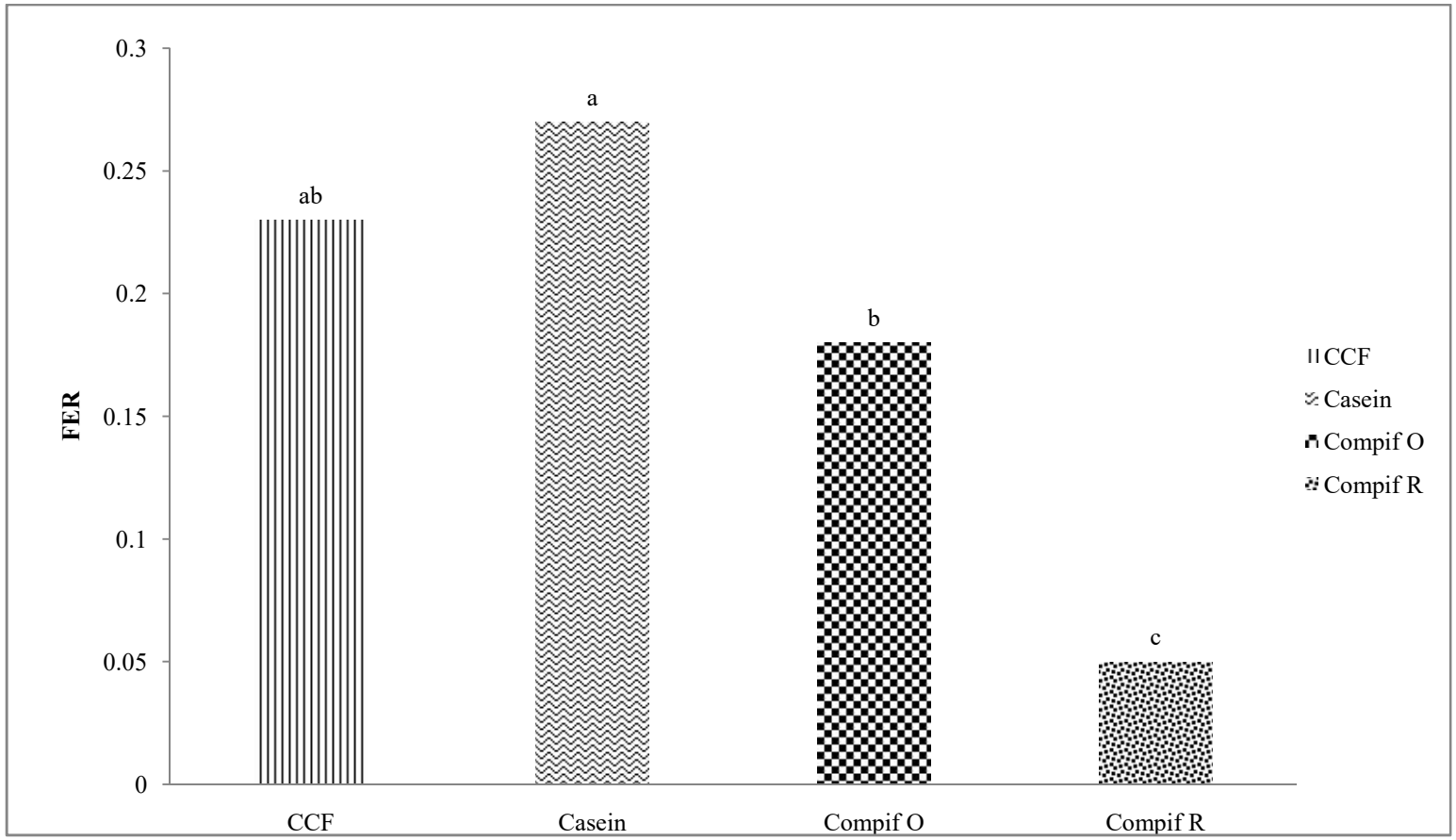


Fig. 4.7 Feed Efficiency Ratio (FER) of the formulated and control diets.

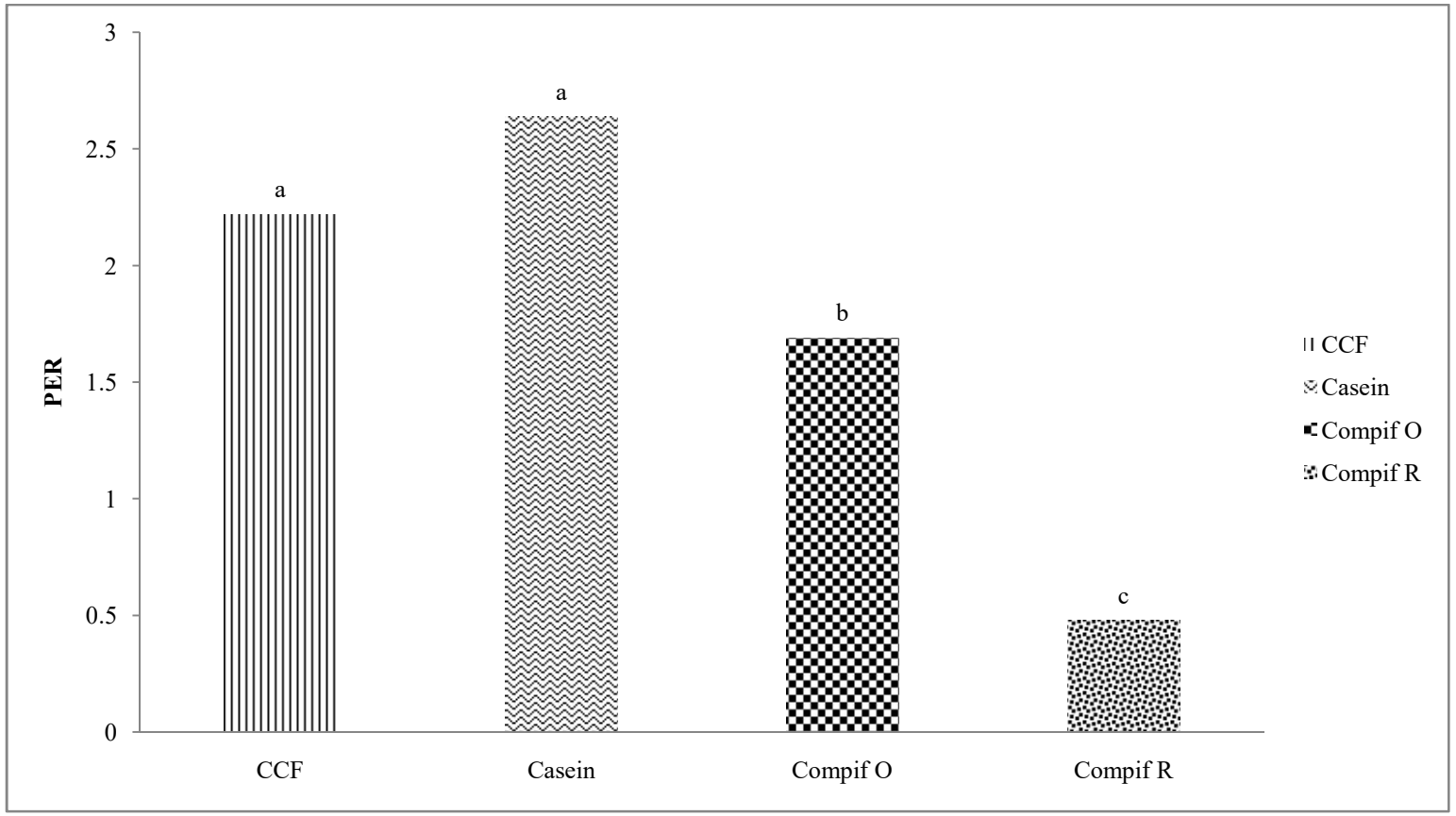


Fig. 4.8 Protein Efficiency Ratio (PER) of the formulated and control diets

4.5.7Weight of organs of experimentalrats fed the control and test diets

The effect of the diets on the weight of some organs of the rats is shown in Table 4.16. The mean weight of the hearts of rats fed Compif O based diet (0.29g) was not significantly different from those of the rats fed CCF based diet (0.32g), but significantly lower than those of the casein group (0.67g). The experimental rats fed casein based diets had the largest size of kidneys (mean weight 0.74± 0.12) followed by those of the CCF (0.67± 0.10), Compif O (0.51± 0.06), Compif R (0.38± 0.03) groups. The protein free group of rats had the least size of the three measured organs. The weights of the three organs measured in rats fed Compif O based diet were significantly higher than those of the Compif R group at $p<0.05$.

4.5.8 Protein quality parameters of the control and formulated complementary diets.

The Net Protein Utilisation (NPU), True faecal Digestibility (TD) and Biological Value (BV) values are illustrated in Figure 4.9. Compif R based diets had the lowest NPU mean value of 43.79% which was significantly different from that of Compif O (71.50%). Compif O based diet had about 79% of NPU value of reference protein, Casein (91%). The four experimental diets had comparable TD values at $p=0.318$ which ranged from 91.50% (Casein) to 92.90% (Compif R). The biological value of Compif R (47.10%) was significantly lower than that of Compif O (77.97%), 85.87% (CCF) and 99.50% (casein). The BV and NPU values of Compif O based diet (77.91%, 71.50%) were significantly higher than those of Compif R (47.10%, 43.79%). The PDCAAS of the two formulated diets (68.86%, 68.45%) weresignificantly lower than that of the casein (96%) but scored above 70% as recommended.

Table4.16 Weight of organs ofexperimentalrats fed the control and test diets (grams)

Parameters	CCF	Casein	Compif O	Compif R	Protein free
Heart	0.32 ± 0.02^{ab}	0.67 ± 0.35^{a}	0.29 ± 0.05^{b}	0.20 ± 0.03^{c}	0.18 ± 0.02^{c}
Kidney	0.67 ± 0.10^{a}	0.74 ± 0.12^{a}	0.51 ± 0.06^{b}	0.38 ± 0.03^{c}	0.28 ± 0.02^{d}
Liver	2.40 ± 0.12^{ab}	2.79 ± 0.15^{a}	1.97 ± 0.51^{b}	1.40 ± 0.27^{c}	0.99 ± 0.08^{c}

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$

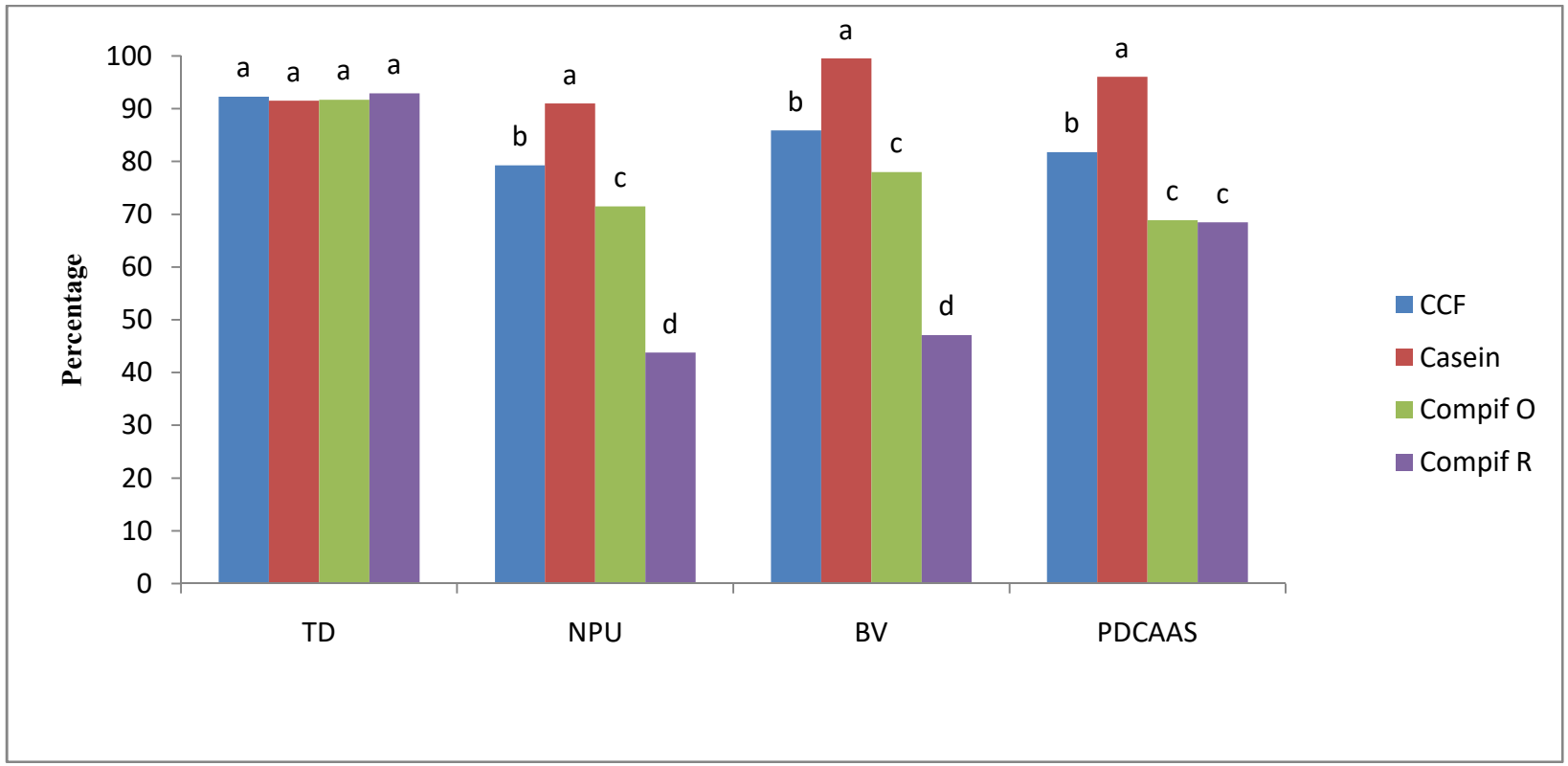


Figure 4.9 Protein quality parameters of experimental diets

4.5.9Haematological indices of experimentalrats fed the control and test diets

The figure (4.10) illustrates the influence of the diets on some blood parameters. The PCV, Hb, RBC and WBC values ranged from 27.25-45.75(%), 7.83-15.43 g/100, 4.06 - 7.60(x $10^6 mm^3$)and 3.31-5.31 (x $10^3 mm^3$)respectively (Figure 4.10). It reveals that the casein group had the highest PCV(45.75%) while the lowest value was observed in rats fed protein-free diet (27.25%). The experimental rats fed the two formulated diets and CCF were not significantly different ($p>0.05$) in terms PCV, RBC, Hb and WBC. There was no difference in the WBC of the experimental rats fed casein based, CCF, Compif O and Compif R diets. The rats fed basal diet had the highest WBC which was comparable to the WBC of rats fed casein based diets.

Figures 4.11, 4.12 and 4.13 show the effect of the experimental diets on the blood indices. The blood indices, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin (MCHC) were not affected by the experimental diets. They showed similar results including those of the rats fed protein free diet.

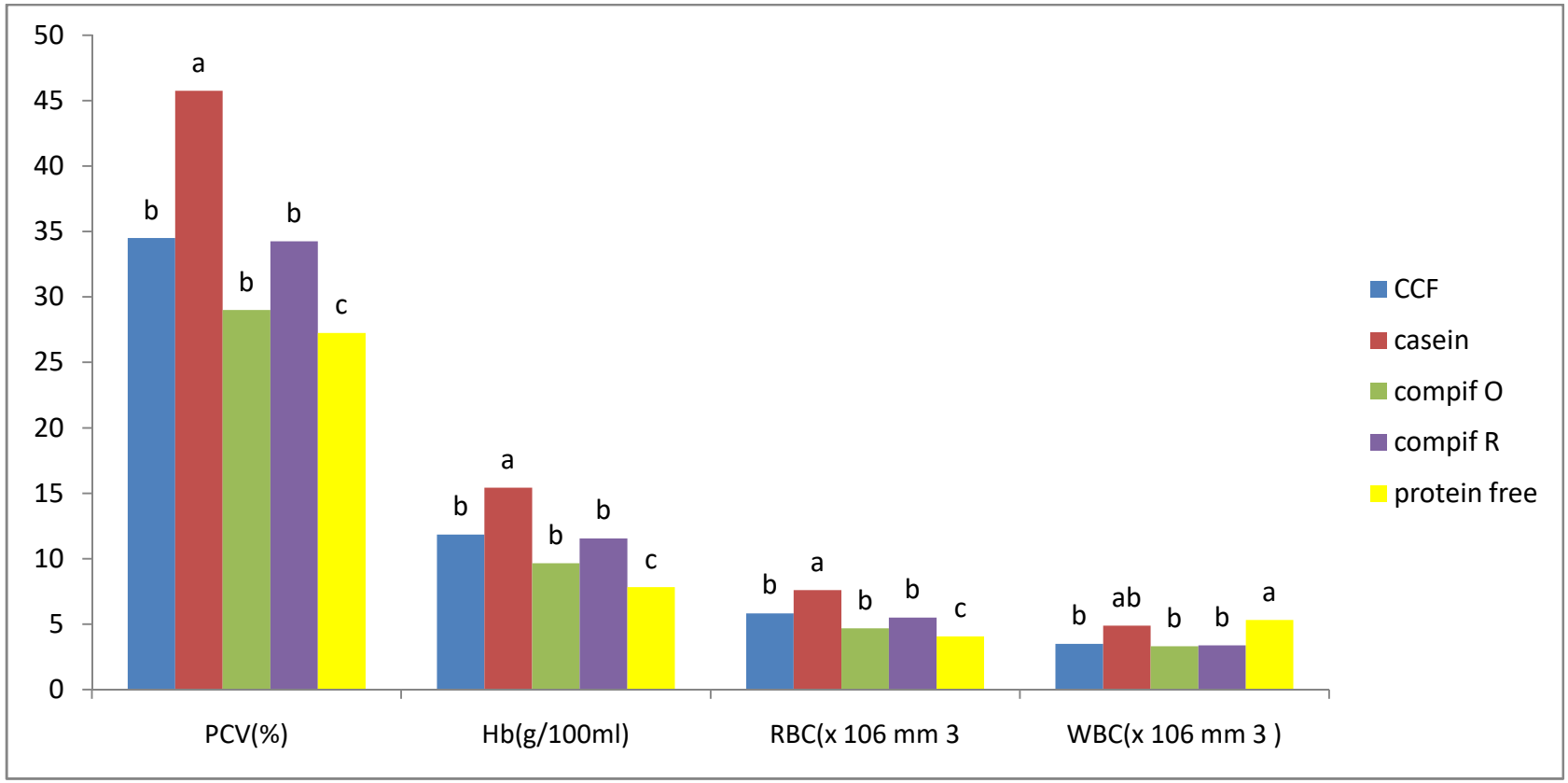


Figure 4.10 Haematological indices of experimental rats fed the control and test diets

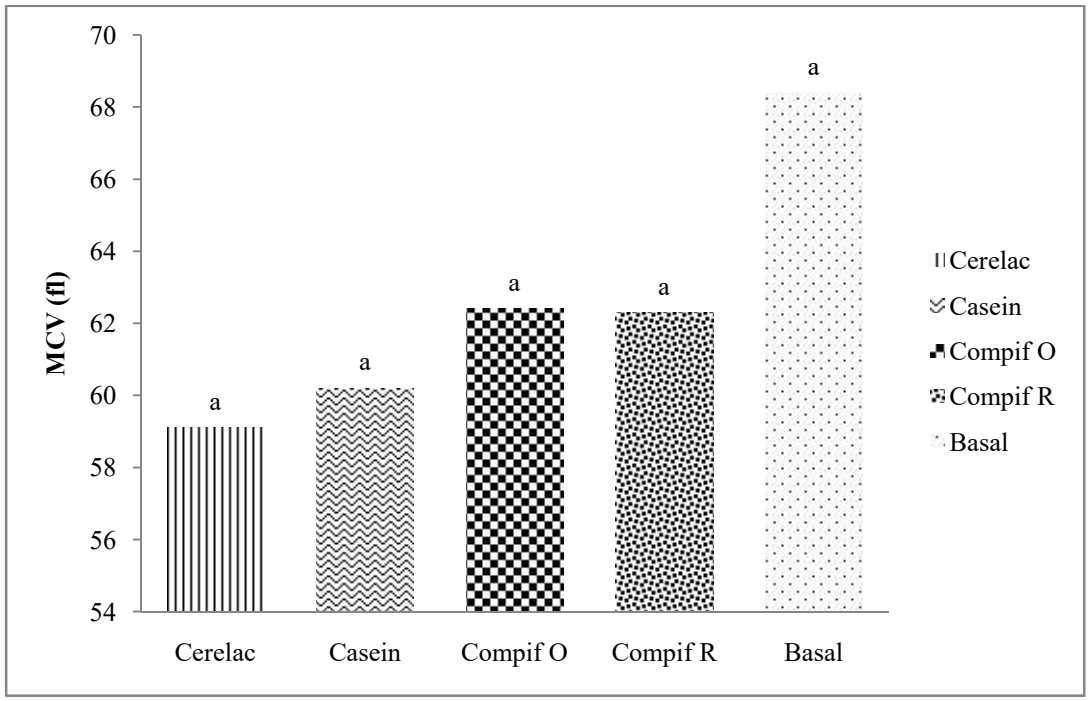


Figure 4.11 Mean cell volume (MCV)

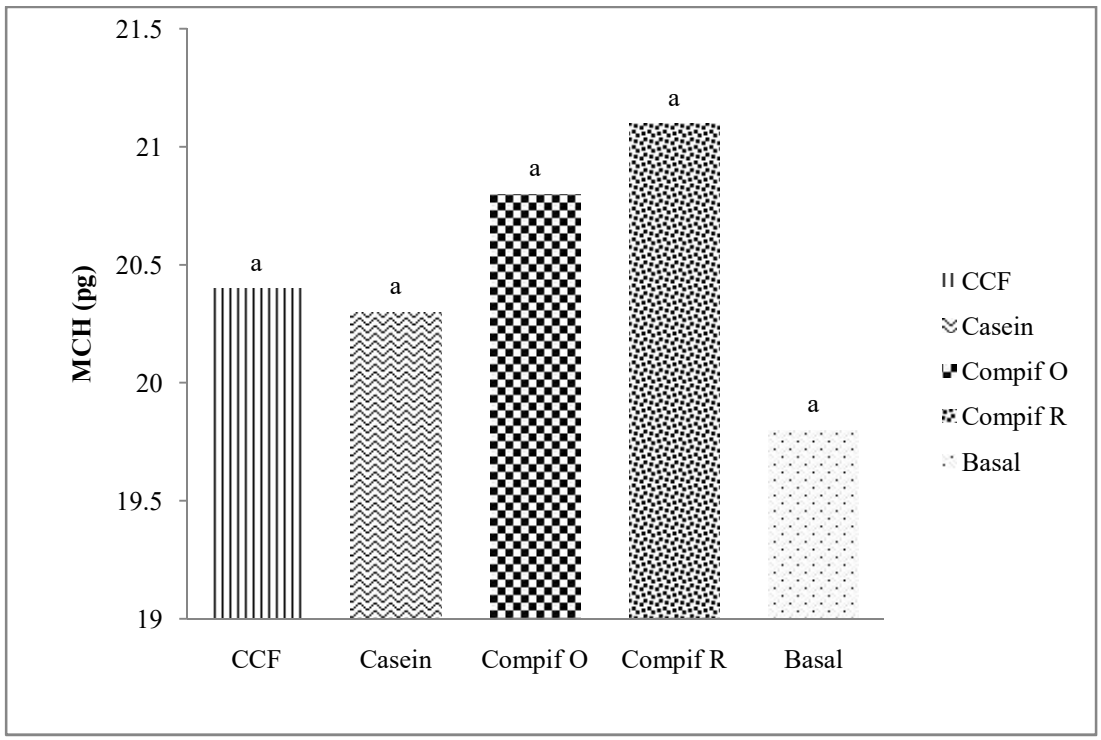


Figure 4.12 Mean cell haemoglobin (MCH)

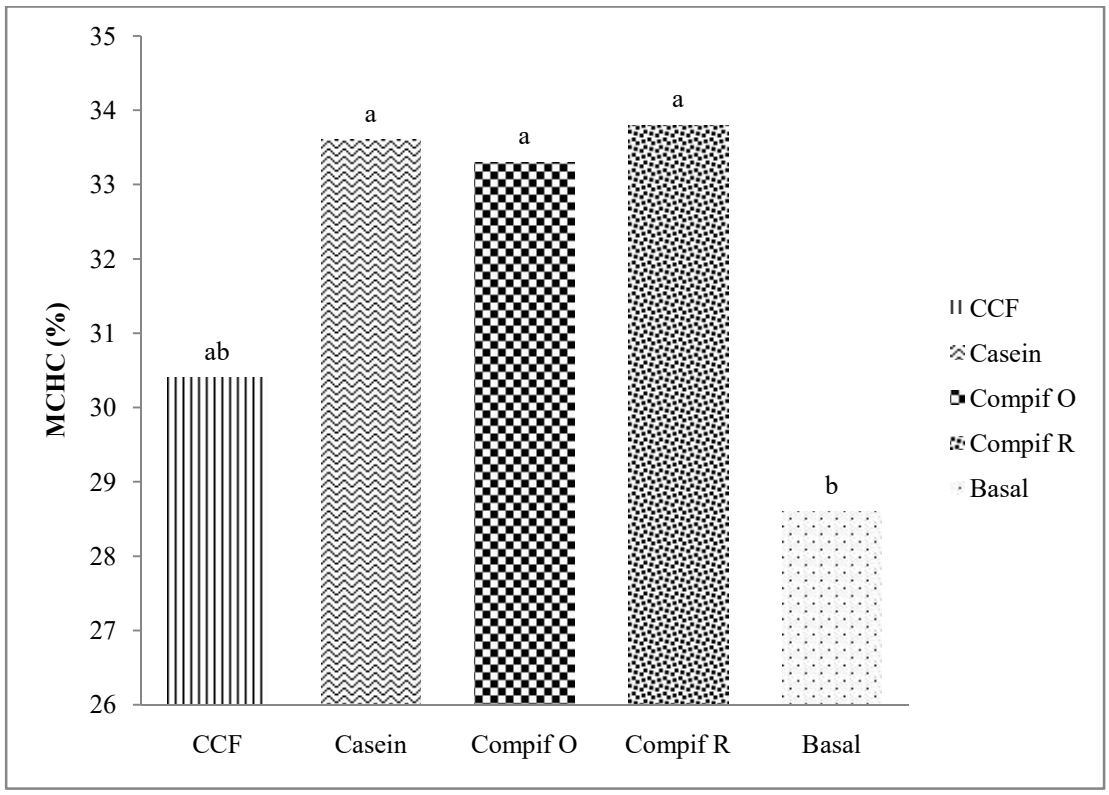


Figure 4.13 Mean cell haemoglobin concentration (MCHC) of the rats fed formulated and control diets

4.5.10Serum minerals of experimental rats fed the experimental diets

It was observed that the calcium (11± 0.51 mg/dl) and iron (3.33± 0.44 mg/dl) levels of the rats fed Compif O based diet did not differ significantly ($p>0.05$) with those of the casein group (10.78± 0.90 mg/dl; 3.25± 0.72 mg/dl) and CCF group (10.80± 0.54 mg/dl; 4.05± 0.70 mg/dl) (Figure 4.14). The levels of iron, calcium and zinc in the blood of rats fed Compif R based diet were significantly lower ($p>0.05$) than those of Compif O. CCF group had the highest blood zinc level (3.24± 0.19 mg/dl) which was higher significantly ($p<0.05$) than that of Compif O group (1.58± 0.50 mg/dl). The zinc blood level of rats fed Compif R (0.75 mg/dl) was lower than that of protein free group (1.13mg/dl).

4.5.11 Biochemical indices of experimental rats fed the test diets

The blood total protein values of the rats fed the two formulated diets were comparable and significantly higher than that of the protein free group(Figure 4.15). The casein group had significantly, the highest total blood protein (9.70%), ALB (4.55%), GLO (5.57 x 10^6) and AG ratio (0.82) values compared to the other groups. The ALB and AG RATIO levels of CCF (3.58%, 0.73), Compif O (3.38%, 0.75) and Compif R (3.23%, 0.75) groups did not differ but were significantly lower ($p<0.05$) than that of casein group (4.55%, 0.82). There was no significant differences ($p>0.05$) in the values obtained for AST and ALT for all groups but values for ALP and BUN of the protein-free group differed from the other four groups. There was no significant difference at $p>0.05$ in all the measured parameters of the Compif O and Compif R. (Figures 4.15-4.21)

4.5.12 Serum lipid profile of experimental rats fed the test and control diets

Table 4.17 shows the blood lipid profile of the experimental rats fed the control and test diets. There were significant differences ($p<0.05$) in the total cholesterol and HDL cholesterol blood levels of the two test diets and the control diet. The experimental rats fed Compif R based diet had the highest mean total blood cholesterol levels (73.75 mg/dl), followed by that of the rats fed Compif O based diet. The LDL cholesterol values observed for the two test diets were higher than that of the CCF group but not significantly ($p>0.05$).

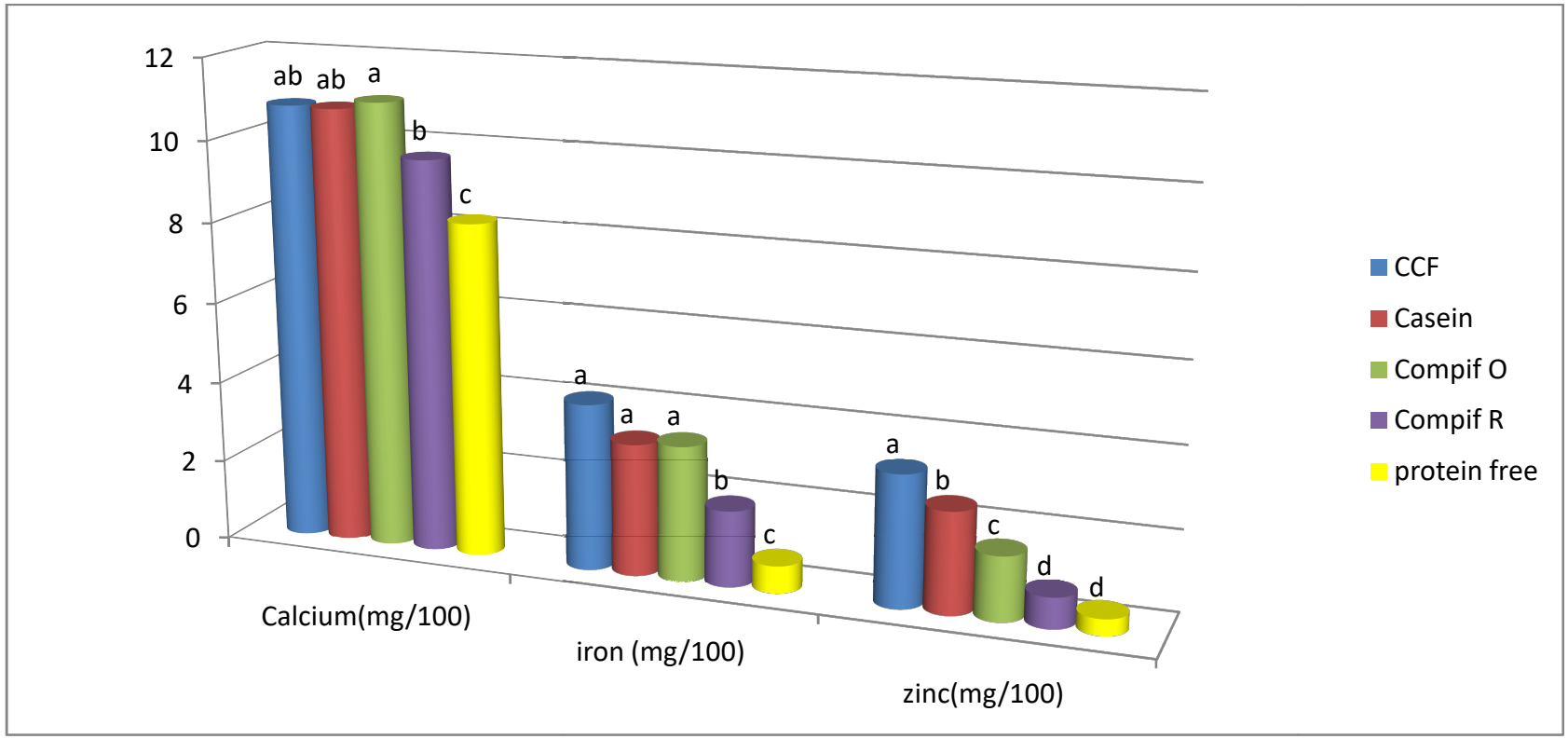


Figure 4.14 Serum minerals of experimental rats fed the test and control diets

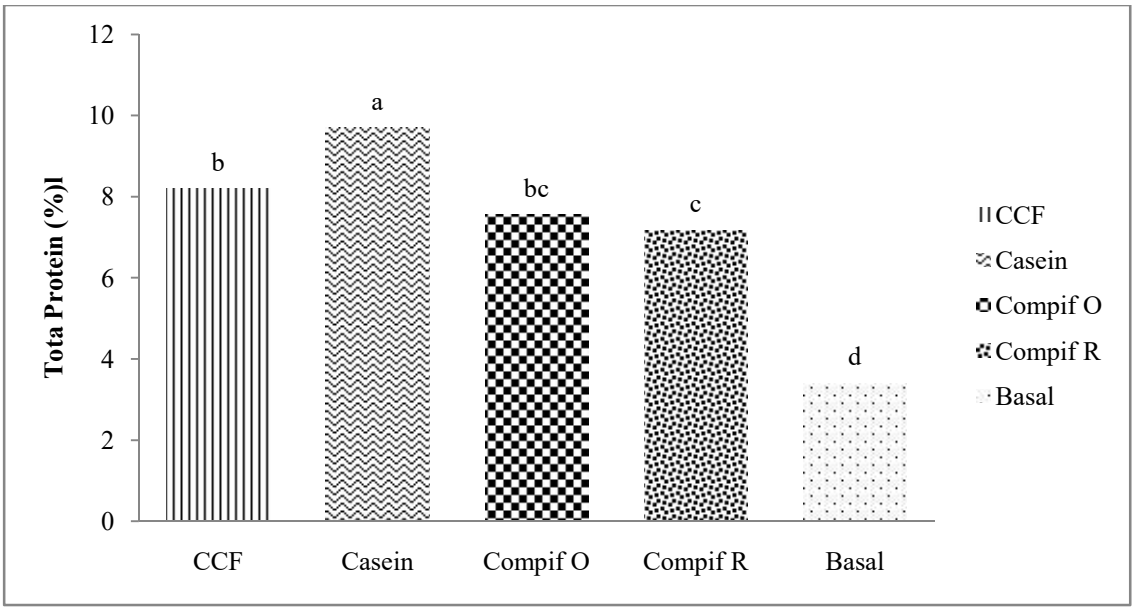


Figure 4.15 The total protein values of experimental rats fed the diets

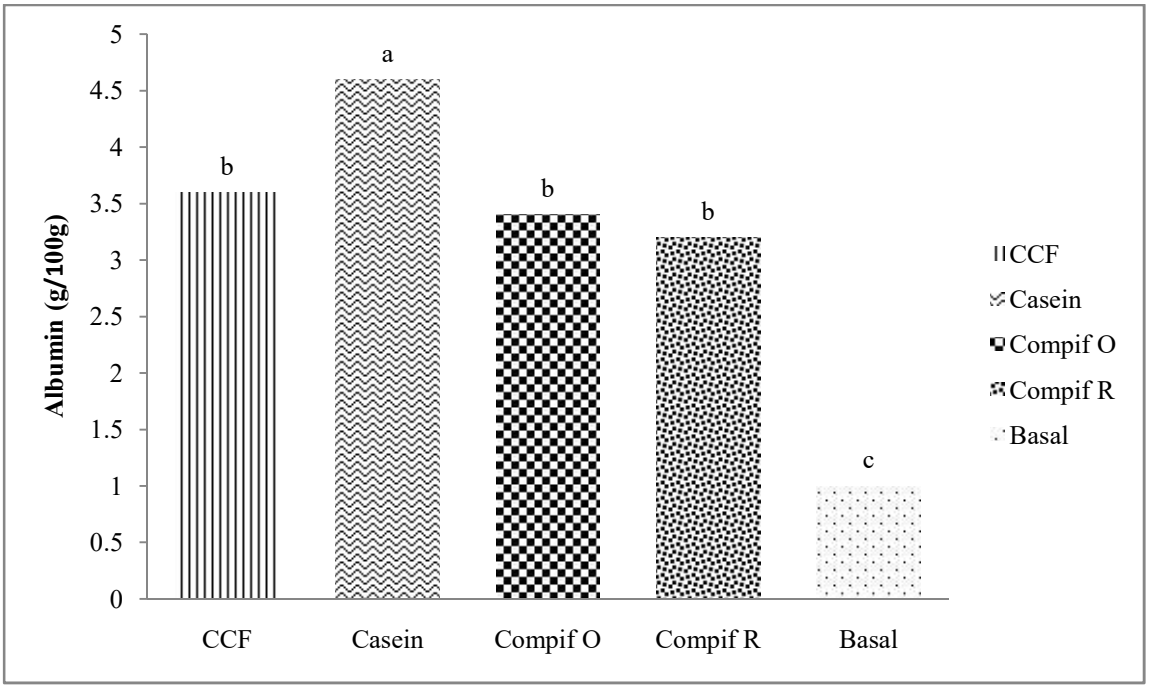


Figure 4.16 The Albumin (ALB) values of experimental rats fed the diets

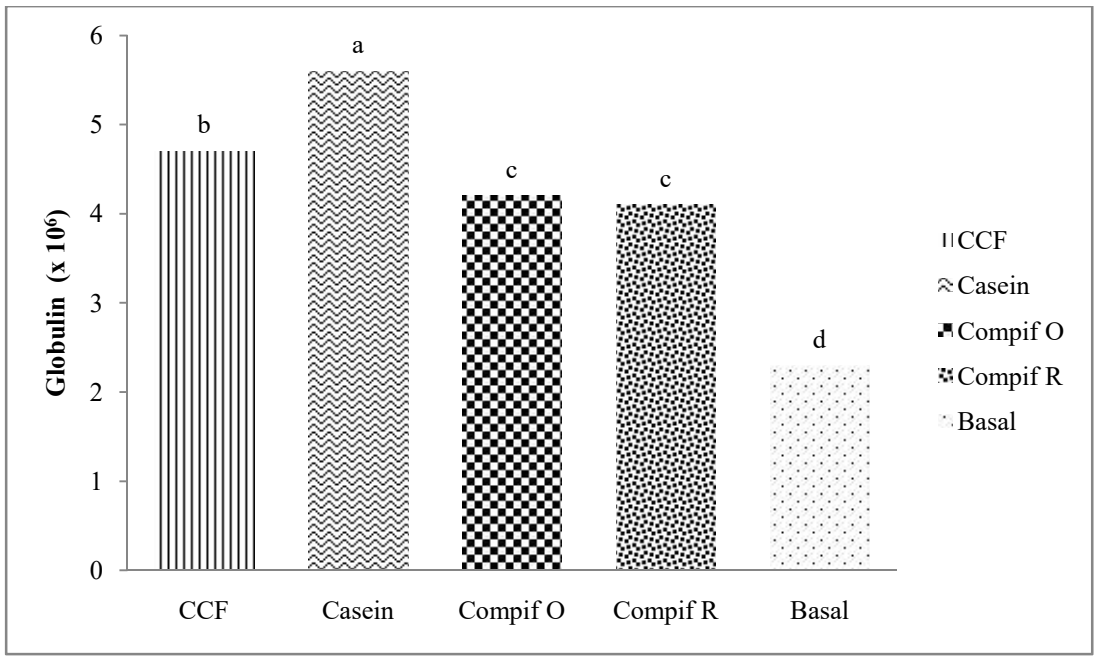


Figure 4.17 The Globulin (GLO) values of experimental rats fed the diets

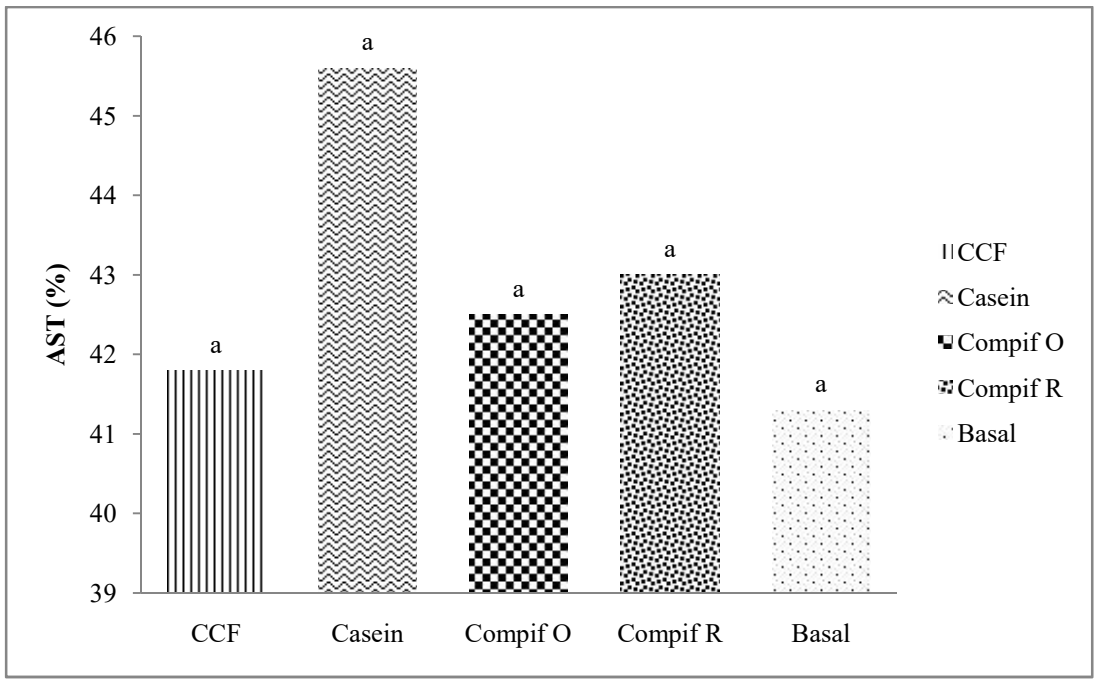


Figure 4.18 The Aspartate Amino Transferase (AST) values of experimental rats fed the diets

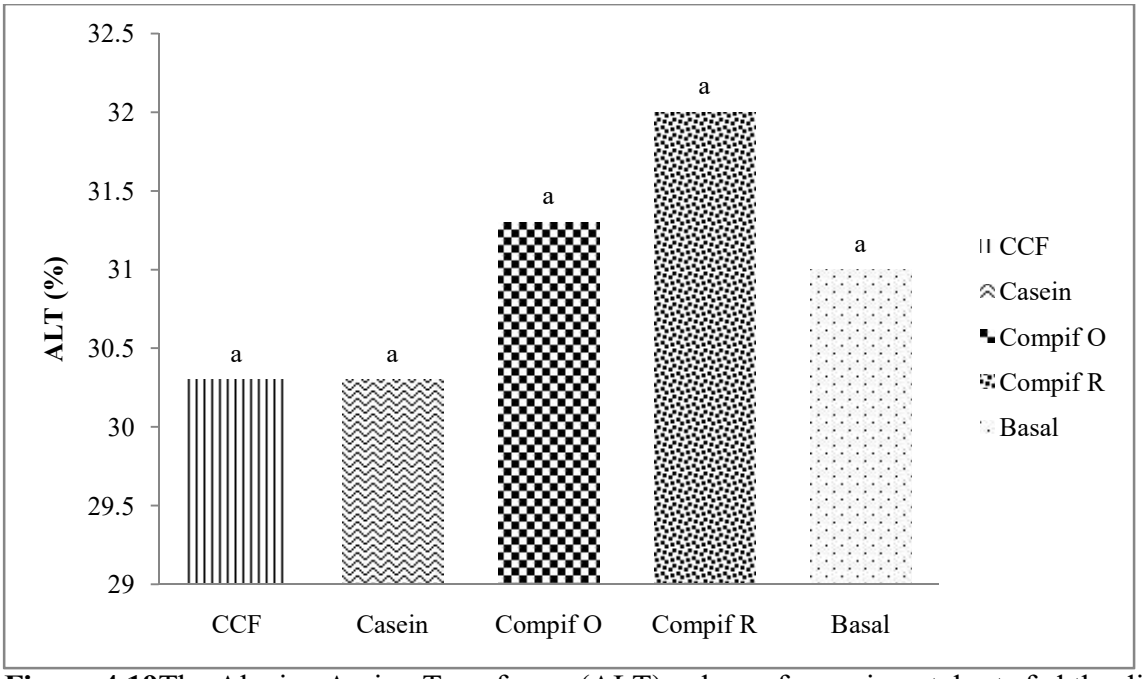


Figure 4.19 The Alanine Amino Transferase (ALT) values of experimental rats fed the diets

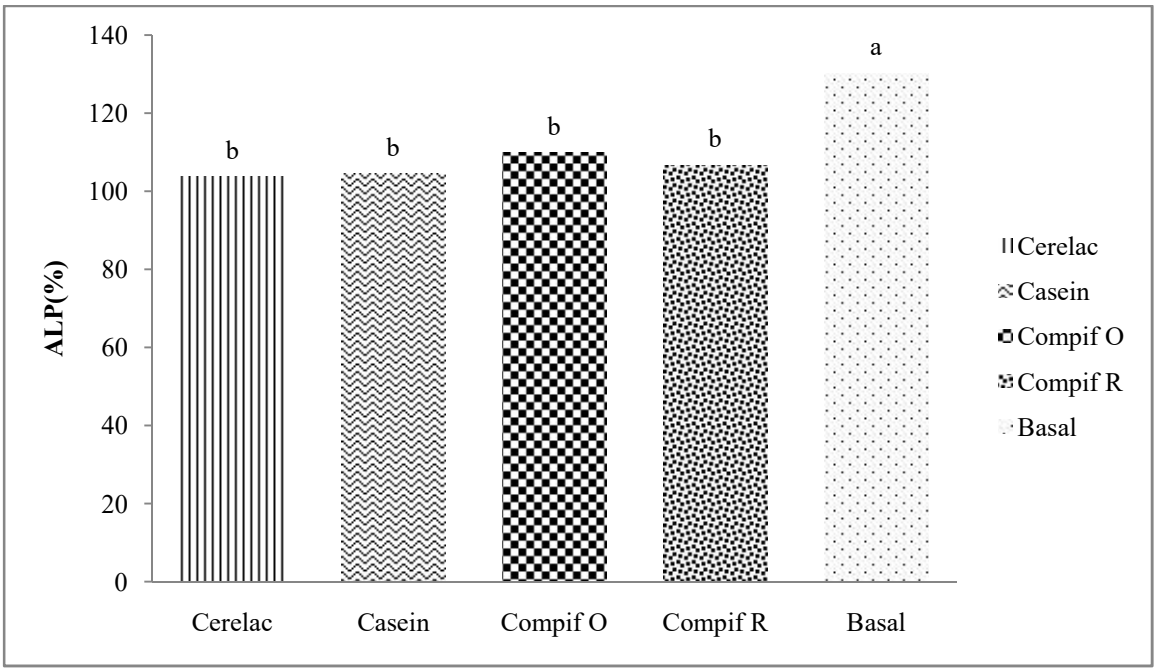


Figure 4.20 The Alkaline Phosphatase (ALP) values of experimental rats fed the diets

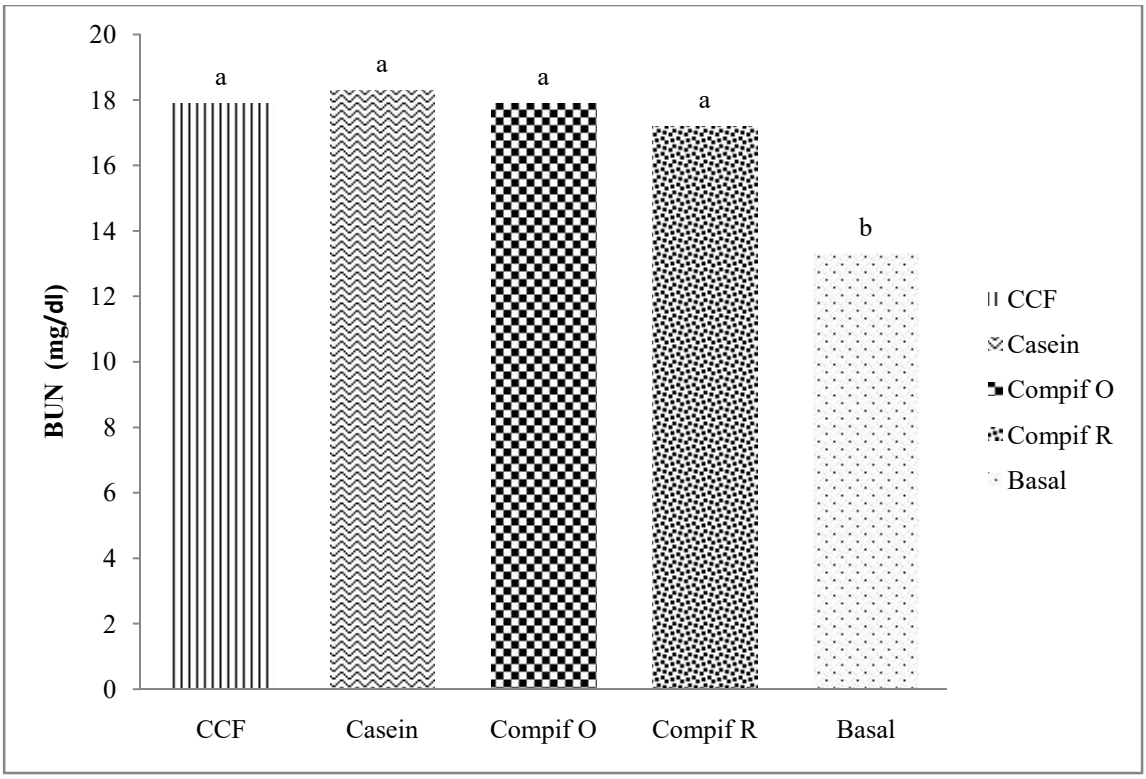


Figure 4.21 Blood urea nitrogen (BUN) of rats fed the formulated and control diets

Table 4.17 Serum lipid profile of experimental rats fed the control and test diets (mg/dl)

Parameters (mg/dl)	CCF	Casein	Compif O	Compif R	Protein free
Total chol	60.25^{b}±2.63	51.75^{c}± 4.35	68.75^{a}±4.79	73.75^{a}±4.09	36.50^{d}±5.20
Triglyceride	41.75^{c}±2.75	59.00^{a}±1.41	46.00^{bc}±6.38	54.25^{ab}±9.54	24.75^{d}±3.86
HDL-chol	30.00^{b}±3.74	21.00^{c}±2.71	36.00^{a}±3.64	40.50^{a}±2.65	18.00^{c}±2.94
LDL-chol	21.90^{ab}±2.90	19.00^{bc}±3.14	25.55^{a}±3.03	25.15^{a}±2.65	13.55^{c}±6.14
VLDL-chol	8.35^{b}± 0.55	11.75^{a}±0.30	7.20^{c}±0.71	8.10^{b}±0.53	4.95^{d}±0.77

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$.

4.5.13 Histopathological examination of Kidney and liver sections of the rats

The result of the histipathological examinations of the liver and kidney sections are illustrated in Figures 4.22 to 4.31.

Kidney sections.

Protein-free diet There were multiple foci of mild degeneration (green arrow) and loss (black arrow) of tubular epithelial cells. (Figure 4.22)

Casein-based diet. There were numerous foci of marked sloughing off of tubular epithelia cells, giving the tubules a distended cystic appearance (stars) (Figure 4.23).

Cerelac-based diet: The tubules and glomeruli appeared fairly normal. There was however a few foci of mild hydropic degeneration of tubular epithelial cells (arrows)(Figure 4.24).

Compif O-based diet: There was marked congestion of the glomerular capillaries and interstitial capillaries (red arrows). There were a few foci of sloughing off of tubular epithelial cells (black arrows) (Figure 4.25).

Compif R based diet: There were a few foci of mild cloudy degeneration of tubular epithelial cells. Kidney section appeared fairly normal (Figure 4.26).

Liver sections

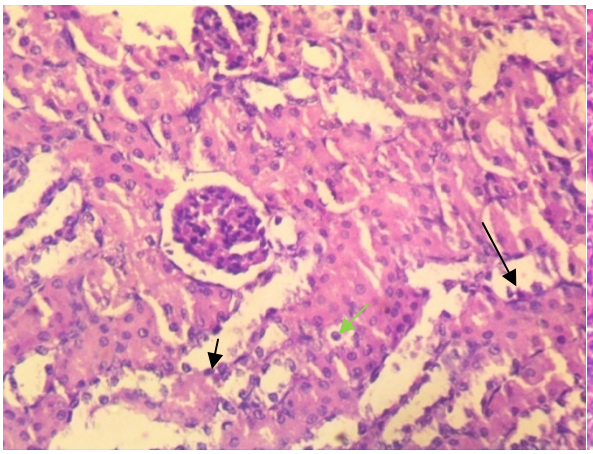
Protein free diet: There were multiple foci of moderate vacuolar change (green arrows) of periportal hepatocytes. There were random foci of single-cell hepatocellular necrosis (black arrows). There was moderate Kupffer cell hyperplasia (red arrows)(Figure 4.27)

Casein-based diet: Hepatic plates were closely-packed. There was no visible lesion with the hepatocytes (Figure 4.28).

Cerelac-based diet: There was mild congestion of the hepatic sinusoids and central veins (arrows). The hepatic plates were closely-packed (Figure 4.29)**.**

Compif O-based diet: There was moderate congestion of the hepatic sinusoids (red arrows). There were scanty foci of single-cell hepatocellularnecrosis (black arrows). There was mild Kupffer cell hyperplasia (green arrows)(Figure 4.30)

Compif R-based diet: The hepatic plates were closely-packed. There were multiple foci of marked vacuolar change of hepatocytes (arrows) (Figure 4.31).



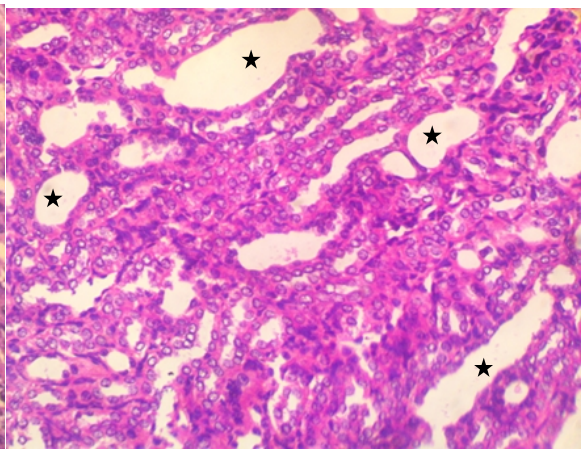
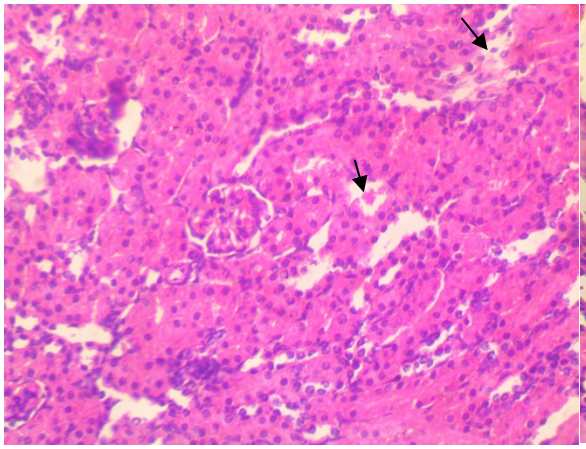


Figure 4.22 Kidney section (Protein-free diet)**Figure 4.23**kidney section(Casein-based diet).



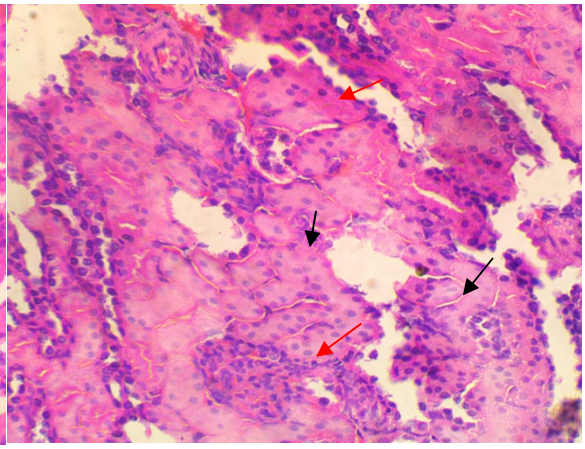


Figure4.24 Kidney section(CCF-based diet)**Figure4.25**Kidney section(Compif O-based diet)

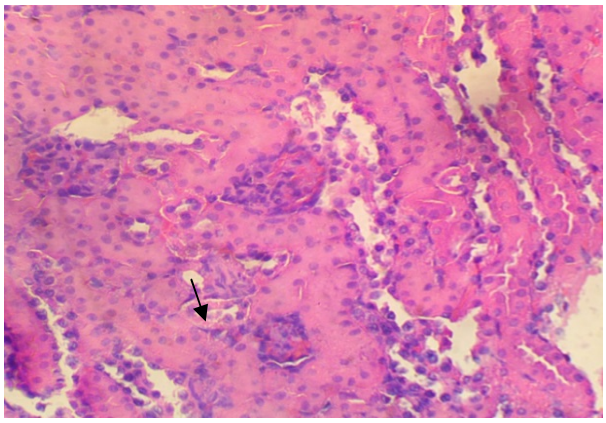
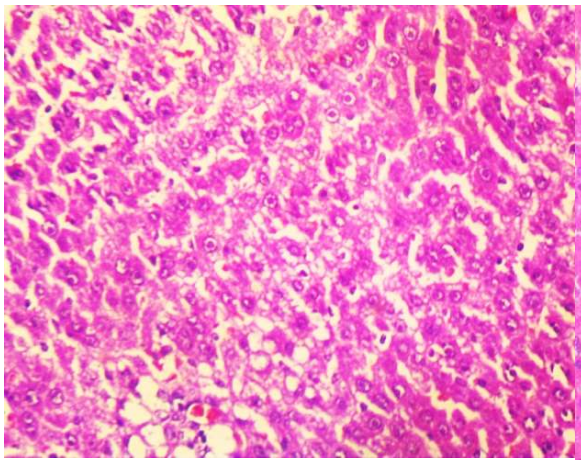


Figure4.26 Kidney section(Compif R based diet)**H&E 400X**



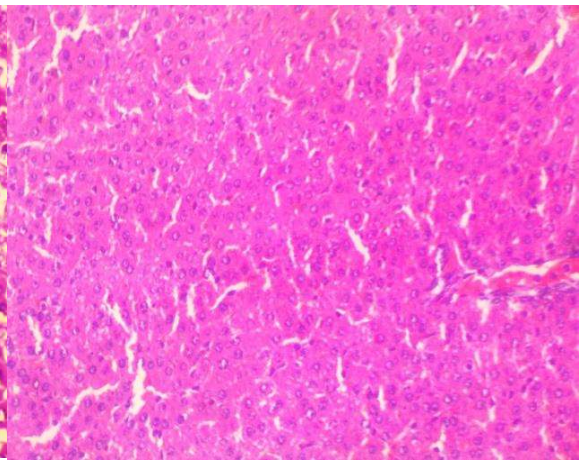
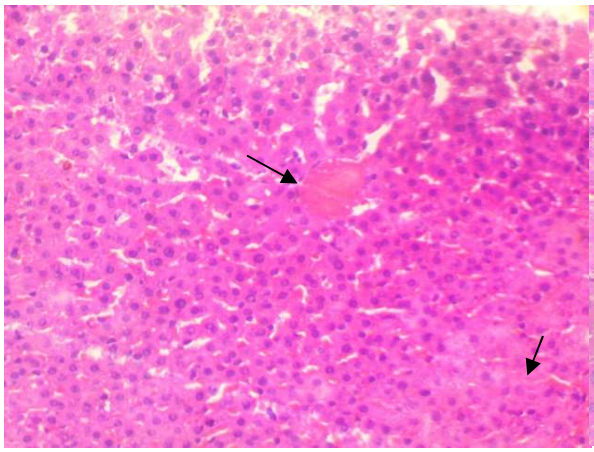


Figure 4.27 Liver section (Protein free diet)**Figure 4.28** Liver section(Casein-based diet)



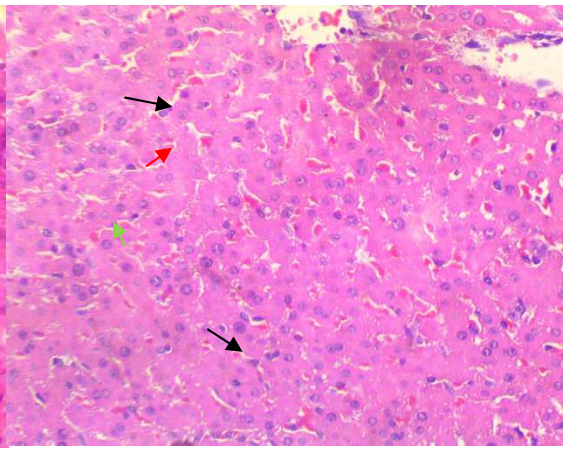


Figure 4.29 Liver section(CCF-based diet).**Figure 4.30**Liver section (Compif O-based diet)

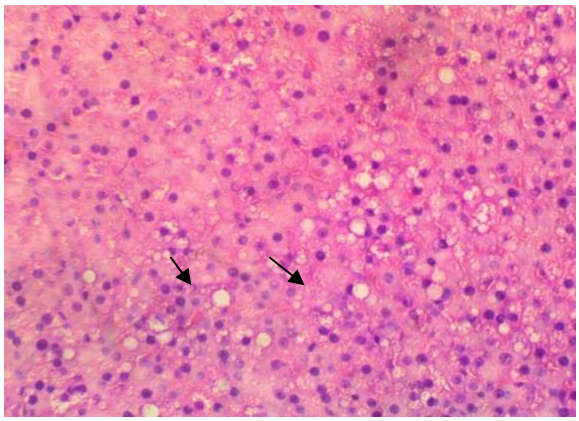


Fig 4.31 Liver section (Compif R-based diet)

H&E 400X

4.6 Research hypothesis

The null hypothesis were all rejected except for the second one where only one satisfied the condition.

H1: There were significant differences in the phytate contents of maize and pigeon pea seeds processed using fermentation, germination or combined germination-fermentation methods.

Ho: CompO satisfied > 70% of the recommendation while Compif R did not.

H1: The microbial load of the formulated complementary foodswere within recommended limit till the fourth week..

H1: The control diet was of better nutritional quality thanCompif R but comparable to Compif O.

H1: The nutritional quality of Compif O was significantly better than that of Compif R.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Effect of fermentation, germination, and germination-fermentation on nutrient and anti-nutrient content of maize and pigeon pea seeds

Tables 4.1 and 4.2 showed that the crude protein values ranged from 9.12-10.44% and 21.20-24.49% for maize and pigeon pea flours, respectively. These values agreed closely with the values reported by Tufa *et al*., (2017) for maize (7.34-10.61%) and Onweluzo and Nwabugwu (2009) for pigeon pea (20.15-22.04%). Fermentation increased the crude protein levels of maize and pigeon pea as also observed in maize by Tufa *et al*., (2017); in millet byInyang and Zakari (2008);in millet and pigeon pea by Onweluzo and Nwabugwu (2009),Wakil and Kazeem (2012),Mbaeyi and Obetta (2016) and in sorghum by Kinyua *et al.,* (2016). The increase in protein level may be as a result of increased synthesis of certain amino acids by the fermenting seeds (Bora 2014). Germination did not have any effect on the crude protein of maize (10.04%), while combined germination and fermentation methods decreased it (9.92,9.12%). This finding agreed with the observation of Ikujenlola and Adurotoye (2014) in maize,but contrasted with the works of Inyang and Zakari (2008), Ocheme and Chinma (2008), and Kinyua *et al*. (2016) who reported an increase in crude protein content of millet and sorghum with combined germination-fermentation and germination, respectively. The difference could be due to varietal and geographical variation as well as varying conditions of treatment and analysis. Total ash, iron, and zinc levels were reduced significantly in maize and pigeon pea flour in all the treatments. Similar results were reported byIkujenlola and Adurotoye (2014) and Tufa *et al*., (2017). The loss in mineral content could be attributed to probable leaching of some

volatile compounds to the fermenting medium and degradation of dry matter (Nnam and Obiakor 2003). However, the losses were less in fermented pigeon pea and combined germinated-fermented maize.The reduced ash content for maize and pigeon pea flour with fermentation was in contrast with the finding of Mbaeyi-Nwaoha and Obetta (2016), who reported increased ash content of fermented millet and pigeon pea flours. They also reported a significant increase($p<0.05$) in the iron leveland decrease in zinc content of fermented pigeon pea flour, while iron level decreased and that of zinc increased in the fermented millet.

Carbohydrate content increased significantly ($p<0.05$) from 74.82% (control) to 78.35% in combined germinated-fermented maize (72/48 hours) while it decreased from 64.66% (control) to 62.13±0.03% in fermented pigeon pea but increased with germination (65.02%). The increased carbohydrate levels observed in maize contrasted with several works mentioned above, which reported decreased levels of carbohydrate with fermentation and germination. This could be due to reduced moisture content of the treated samples when compared to control as a result of the drying process. The reduction in pigeon pea carbohydrate levels agreed with literature reports including the works of Falmata *et al.* (2014) and Mbaeyi and Obetta (2016). This observation could be due to increased activities of microbial enzymes such as alpha-amylases which hydrolysedthe complex carbohydrate into simple sugars (Mugula *et al.,* (2003); Sade (2009) and Bora (2014).

Fermentation, germination and combined germination-fermentation (72/48 hours) significantly decreased the crude fibre and crude fatlevels in maize whilecombined germination-fermentation (72/24 hours) increased the crude fibre level from 1.41% to 1.59%. Ikujenlola and Adurotoye (2014) also observed reduction in the crude fibre of maize (5.08 to 4.51%) with germination but increased fat levels (1.80 to 2.35%),while Tufa *et al., (*2016) and Onweluzo and Nwabugwu (2009) reported increased fat levels ofmaize (4.35 to 4.70%) and millet (1.50 to 4.50%), respectively.In pigeon pea, the content of crude fat increasedsignificantly with all the treatments while the crude fibre level (2.2%) reduced with fermentation (1.60%) and germination (1.00%)(Table4.2).This

finding was in agreement with that of Onweluzo and Nwabugwu(2009) who reported that crude fibre (2.2%) and fat (2.7%) levels of pigeon pea changed to 1.4% and 3.60%, respectively. Change in fat levels may be as a result of the increased activities of lipolytic enzymes which hydrolyse fats to fatty acid and glycerol (Mbaenyi and Obetta, 2016).

Fermentation, germination, and combined germination-fermentation reduced the anti-nutrient contents of maize and pigeon pea particularly phytate, tannin and oxalate contents (Tables 4.3 and 4.4). Similar findings were reported by several researchers including Kinyua *et al.,*(2016), Tufa *et al*, (2016), Bora (2014), Gbadamosi,*et al.,*(2017) among others. It has been noted that the germination of cereals reduced phytate levels significantly, but that mineral contents were lowered and increased differently depending on the type of food (Abdurahaman *et al.,*2007). Larson and Sandberg (1992)also observed that the combination of sprouting and soaking for 17 hours at 49^{o}C (120^{o}F) removed about 98% phytate from cereals. Increased period of germination (Agostini *et al.,*2010) and temperature (Liang *et al.,*2008) result in more phytate reduction. This reduction has been attributed to the activities of endogenous and microbial phytases in the germinating and fermenting seeds, respectively. Passive diffusion of water-soluble phytates could have contributed to the phytate reduction (Thompson and Amoroso 2006;Bora 2014). The natural lactic acid fermentation offers favourable pH conditions for the enzymatic degradation of phytates to organic phosphate, inositol, and other simpler forms. Based on the findings of the current study, fermentation was more effective in the anti-nutrient content removal in legume while germination and combined germination-fermentation were more effective in maize. However, Liang *et al.,* (2008) observed that fermentation was generally more effective in reducing phytic acid compared to malting. High anti-nutrient content of foods is detrimental to health as it chelates vital minerals such as calcium, zinc,and iron including proteins to form complexes and make the unavailable (Codex Alimentarius Commission, 2013). Above 800mg per day of phytic acid is discouraged. The recommended dietary intake of phytic acid varies among countries. In the United Kingdom,Sweden, Italy, Finland and the United States of America, the recommendations are 740mg, 180mg, 219mg, 370mg, and 631mgper day, respectively (Bora 2014).Germination of maize significantly reduced tannin, phytate, oxalate,

polyphenol, and hemaglutinin. However, the contents of ash, iron, and zinc were negatively affected. Crude fibre and carbohydrate levels increased in maize while crude protein content was not affected.In the current study, germination of pigeon pea also resulted in reduced quantities of anti-nutrients as fermentation but the effect on the nutrients such as calcium, iron, fat, and protein was less favourable,

Combined germination and fermentation (72/24 hours)method reduced all the anti-nutrients in maize particularly phytate and the effect on crude protein, calcium, iron, and ash more favourable than the other germination methods (Table 4.5).Combined germination (72hrs) -fermentation (24hrs) in addition to reducing above 90% of phytate in maize retained more nutrients. Combined germination methods had no effect on the phytate content of pigeon pea based on the current study (Table 4.5).

The protein content of pumpkin leaves was comparable to that of pigeon pea (Table 4.6), but not usually regarded as a rich protein source even though both are plant proteins. However, pumpkin leaves had the highest phytate content (629 mg/100g) (Table 4.7). This fact is an important consideration in nutrient bioavailability. Dried fish is very rich in protein. It is an animal source food that is rich in quality protein and haem iron. Carrot powder had the highest quantity of beta-carotene (retinol equivalent). Carrot is a known rich source of vitamin A. The inclusion of dried fish (ASF), carrot (vitamin A rich source) and ugu leaves (vegetable) to the cereal (maize) and legume (pigeon pea) supports diet diversity. The lower moisture content and higher crude fat contents of the roasted ingredients could be as a result of increased temperature during processing.The roasting method was included because it is a major processing method used for the production of Tombrown, a ready-to-eat therapeutic food given to malnourished children.

5.1.2 Chemical composition and adequacy of formulated complementary foods

The moisture contents of Compif O (6.97%) and Compif R (5.94%) were observed to be higher than 5% stipulated for dry infant foods by 39% and 19%, respectively (Table 4.8) but less than 10-15% recommended for dry formulated or processed foods. These values are comparable to 6.63% reported by Ikujenlola and Adurotoye (2014) for a blend of

quality protein maize and cowpea. The moisture values of the current study are higher than those of maize-sesame-crayfish blend (3.60-5.55%) (Fusuan *et al.,* (2017), but lower than 7.26-7.36% observed by Shiriki *et al*. (2015) for maize, soybean/peanut, and leaf powder. Compif O and Compif R had moisture contents lower than 10.05-13.03% reported forwheat-soybean-carrot based complementary foods(Gbadamosi *et al.,* (2016)and 8.60-9.71% recorded by Adeola *et al*. (2017) for sorghum, pigeon pea, and soybean flour blends. The moisture content of processed foods is influenced by the drying temperature including duration of drying, loading quantity and depth of ingredients. High moisture content is not a desirable quality for infant foods because of increased susceptibility to microbial growth and spoilage. However, the moisture values of Compif O and Compif R were within the safe limit of 10% recommended for dried foods.

The fat contents of the formulated diets were higher than those of the complementary foods formulated from sorghum, pigeon pea, soyabean and skimmed milk (4%) (Addis *et al*., (2013) and sorghum, pigeon pea, soyabean flours (2.57-2.94%) (Adeola *et al*., (2017).The addition of oil could have contributed to the higher fat level. The use of oilseeds (roasted sesame) with malted maize and crayfish resulted in a blend of higher fat (25.59%) content (Fusuan *et al.,* (2017). However, the lower-fat contents of formulated diets (Compif O and Compif R) in relation to standard (10-25%) did not affect the energy values (402.51, 412.76 kcal). Moreover, the higher fat composition may facilitate rancidity and encourage contamination. It has also been stated that caution should be taken when increasing fat portions of infant foods to prevent the displacement of protein and other vital nutrients (PAHO/WHO 2003). Also, the use of low-fat fish (panla) might have contributed to the lower-fat figures obtained. The daily fat allowance would be met, since these infants are still breastfeeding and on other family diets too. Compif R had a higher content of fat even when the same quantity of ingredients including 5% oil was utilized. This could be attributed to the fact that Compif R was formulated from roasted pigeon peamaize seeds which had higher fat values than the fermented pigeon pea and germinated-fermented maize seeds. The crude fibre of the formulations was lower than the maximum value of 5% as specified in the CODEX Alimentarius guideline for processed foods for infants and young children (Codex

Alimentarius Commission, 2013). Low dietary fibre is one of the qualities of good complementary food because high crude fibre content increases bulkiness and with a limited gastric capacity of infants, energy and nutrient intake might beaffected. Besides, high dietary fibre may negatively affect the bioavailability of already low micro-nutrients. The protein values of the formulated diets are comparable to that of Addis *et al*., (2013) (16%) but lower than those of Fusuan *et al.,* (2017) (20.78-28.09%), Gbadamosi *et al.,* (2017) (22.4-25.0%) and Adeola *et al*., (2017) (21.73-22.63%). The quantity and kind of protein sources (soya bean, sesame seeds, crayfish) could have contributed to the higher protein values in these diets. The inclusion of fish and pigeon pea could have resulted in crude protein levels which were higher than the control (15.5%). The protein contents of the formulated diets were within the recommended value (6-15%). Sufficiency in protein contents of complementary foods is very essential to supply the substrates for rapid growth and development of infants and older infants.

The calcium contents were lower than expected as it had been reported that fishmeal with bone increased the calcium composition of formulated complementary foods (Perlas and Gibson, 2005; Amagloh, 2012). Although, Amagloh in his work, added soybean and included more fishmeal (17%) on weight by weight basis after mixing, cooking and drying of other ingredients. Also, the cooking of the blend before drying could not have reduced the calcium contents, since based on the findings of Fadupin *et al.,* (2017) that blanching of green leafy vegetables did not have a substantial effect on the calcium levels. The low calcium, zinc and iron levels could be upgraded by increasing the proportion of fishmeal in the formulation. Fish contains readily available haem iron, zinc, calcium, vitamin B6, Vitamin B12 as well as high-quality protein. Furthermore, small dried fish is affordable, available and can be taken in places where cultural or religious beliefs forbid meat consumption. Also, cellular animal protein boosts iron and zinc absorption from plant-based diets. However, increasing the fishmeal portion might have sensory implications. It is noteworthy, that vitamin A contents of the formulated complementary foods were low despite the inclusion of carrot powder. This might have been due to degradation during processing or analysis. The portion of the carrot could be

increased and leafy vegetables removed because of its high phytate content. Moreover, β −carotene and ascorbic acid enhance iron absorption in the presenceof phytate.

Results of the phytate analysis of the formulated foods confirmed that low phytate complementary foods could be obtained from cereals and legumes using combined germination-fermentation, fermentation and roasting methods. The major issue is micronutrient retention and sufficiency. There is a need for a post nutrient analysis after processing to detect micro-nutrients that require fortification. The phytate levels (146-179 mg/100g) were lower than that of enriched Weanimix (maize, groundnut, soybean, fish) (800 mg/100g) and sweet potatoes based complementary foods (190-230mg/100g) (Amagloh 2012). The estimated daily energy intakes from Compif O and Compif R were about 81% and 83% of the recommended 200kcal (6-8 months old infants); 67% and 69% of 300kcal (9-11 months old infants); and 73% and 75% of the recommended 550kcal (12-24 months), respectively (Table 4.10). The quantity of food to be taken may be increased by 10% across the board to meet the daily energy needs from Compif O but not Compif R because Compif O was formulated from germinated maize and therefore can accommodate more flours. Another measure such as increased oil might be considered for Compif R which would require more dilution if extra flour would be added. The deficit in energy and fat could also be corrected when other family-based foods are taken by infants and young children are recommended.

The phytate:iron molar ratios were not met by more than 100% (Table 4.11) as also observed for enriched Weanimix (Amagloh 2012). This suggests a serious inhibitory effect on iron absorption particularly for Compif R. It is noteworthy that Compif O which had higher phytate content, had lower phytate:iron,zinc molar ratios compared to Compif R because of higher quantities of the minerals. Compif O also had higher vitamin A level, a possible iron absorption enhancer and may probably be better in nutritional quality.

Although the protein contents of Compif O (17.18%) and Compif R (16.79%) were above the recommended protein level (15%) for processed complementary foods, both foods (75%, 74%) did not meet the minimum amino acid score of 80% as specified in the CODEX guideline using casein as reference protein (Table 4.12). This further

supports the fact that adequate protein quantity does not imply good protein quality. The isoleucine (150.1, 148.8 mg/g) and tryptophan (72.5, 71.8 mg/g) values of the two formulated complementary foods were very high compared to those of CCF (47.1, 15.3 mg/g) and reference standard (32, 8.5 mg/g) respectively. The isoleucine values were higher than those reported by Okafor *et al.,* (2018) for blends of maize and pigeon pea (38.8-43.5mg/g). This could be due to the addition of fish. Surprisingly, lysine was the limiting essential amino acid in the formulateddiets and commercial diet (CCF). This was not expected because of the inclusion of pigeon pea as legume and fishmeal. The combination ratio of the formulation could be modified to tackle this. The effect of the processing methods on amino acids might be responsible for the low lysine level.

5.1.3 The functional properties of formulated complementary foods

The water absorption capacity (WAC) values of the formulated diets were lower than that of control (CCF) (Table 4.13). These values (125-134g/100g) are lower than that of fermented maize flour (235 g/100g) reported by Tufa *et al.,* (2016) and 168-191 g/100g for composites of millet, pigeon pea flour and leaf powder (Mbaeyi and Obetta 2016). The WAC values observed in the current study were also lower than 275 g/100g reported for the cereal-crayfish-carrot blend(Achidi *et al.,* 2016) and 216 g/100g forthe malted maize-cowpea blend (Ikujenlola and Adurotoye 2014). The current study recorded higher WAC compared to 118 and 90g/100g reported for pap and Nutrend (Oludumila and Enujiugha (2017) and 39-96g/100g for blends of maize, sesame seeds and crayfish (Fasuan *et al.,* 2017). The lower WAC could be attributed to germinated-fermented maize and fermented pigeon pea used. Onweluzo and Nwabugwu (2009) and Ikujenlola and Adurotoye (2014) also suggested that fermentation and malting could reduce WAC.

Water absorption capacity measures the amount of water available for gelatinisation. Low WAC is a desirable functional characteristicof complementary food required to produce thin gruel. Less quantity of water would be required to gruel, thus the energy and nutrient density of the complementary foods would not be reduced.The bulk density values of the formulated diets compare favourably with those of other works (Mbaeyi and Obetta 2016;Fasuan *et al*., 2017;Oludumila and Enujiugha (2017). The two formulated foods did

not differ in bulk density but had lower bulk densities compared to the commercial complementary food. Falmata *et al*., (2014) observed that germination and fermentation decreased the bulk density of sorghum flour significantly.Low bulk density is a desirable quality for complementary foods.

Viscosities of the formulated diets were above that of the control and 120-140 cP indicated for millet-pigeon pea-leaf powder blend (Mbaeyi and Obetta 2016). However, they were lower than those of unfermented/fermented pigeon pea flour (389/380 cP) and unfermented/fermented millet flour (397/363 cP) examined in the same study mentioned. Viscosity describes the ability of food to form a gel or viscous gruel when cooked. Lower viscosities and bulk densities could be as a result of the enzymatic degradation of starch and other higher molecular weight polysaccharides to dextrins and peptides during fermentation and malting (Falmata *et al.,*2014). Less viscous gruel permits the addition of more solids with lower moisture (thin gruel) thereby increasing energy and nutrient densities. The effect of roasting on viscosity might have contributed to the higher viscosity of Compif R. The least gelation concentration values of formulated diets are lower than those of the control and 17.25-20.25% reported by Mbaeyi and Obetta (2016), but higher than the values of 4.0-8.0% obtained for unfermented/fermented millet and pigeon pea flours (Onweluzo and Nwabugwu 2009). Compif O (fermented) had higher LGC than Compif R (roasted). Aderonke et al., (2014). This implies that the fermentation process increased LGC of Compif O. Complementary foods with high LGC are recommended for infants because foods with low LGC form gel at low solid concentration and require a larger quantity of water for thinning thereby reducing energy and nutrient densities.

5.1.4 The microbial analysis of formulated complementary foods

The microbial counts of the two formulated diets were all below the recommended maximum level of 1.0 x 10^5 cfu/g (Table 4.14). Stricter control of safety and hygiene standards was enforced in the reprocessing and reformulation of diets when high TVC was observed in the first formulation. TVC increased steadily from the first week till the fourth week but the rate of increase was less in Compif R (140%) compared to Compif O

(300%). This implied that measures to improve the storage stability of formulated diets, particularly for Compif O, would be necessary. Compif R had lower TVC and no detectable mould/yeast or E. coli growth for the four weeks. This could be attributed to the higher temperature it was subjected to during roasting. Ajibola *et al.,* (2016) observed lower TVC values for toasted ogi-crayfish mix (1.2-1.8 x 10^3cfu/g) and fungi growth in the foods. Mbaeyi and Obetta, (2016) also observed higher TVC values (2.1-3.2 x 10^4cfu/g) and mould (1.0-3.0 x 10^3cfu/g) for millet-pigeon pea-breadfruit leaf powder blends. Fermentation encouraged the dominance of lactic acid bacteria which subdued the growth of harmful bacteria as also observed by Falmata *et al.,* (2014). Fermentation and roasting enhanced the microbial safety of the formulated foods. Also, the cooking and drying processes employed could have contributed to the lower microbial load.

5.1.5 The nutritional quality of the formulated complementary foods

5.1.5.1 Growth performance characteristics of Wistar rats fed the experimental diets

The effect of the formulated diets on the weight of the animals did not show till about the fifth day (Figure 4.2). The experimental rats on the control diets gained more weight than those on Compif O, however, the weight gains were not significantly different. This slight disparity could probablybe due to the type of animal protein source and mineral content among other factors. The finding is at par with the observation of Ajibola *et al*., (2016), who found that rats fed CCF and casein-based diets performed better than those fed ogi-crayfish based diets. The current study disagreed with that of Ibironke *et al.,* (2012) and (2014) who reported that ogi-crayfish and ogi-groundnut-crayfish based diets promoted growth more than CCF. CCF and casein-based diets contain milk and egg, respectively, which are rated high in protein quality and growth-promoting factors while the formulated diets had fish. However, rats fed the two experimental diets formulated with the same type and quantity of animal source food displayed quite different mean weight gains (24.05g, 5.75g) (Figure 4.4). It can be recalled that Compif O was better than Compif R in terms of phytate: mineral ratios and mineral content while their macro-

nutrients sufficiency was comparable. This difference in weight performance could be attributed to the different processing methods utilized in the production of the two complementary foods. Ajibola *et al*., (2016) also observed that rats fed toasted diet had lower weight gain. Interestingly, in the current study, the effect of these diets on length gain was dramatic. There was no significant difference ($p>0.05$) in the body length gain among the group of rats fed the CCF (5.18g), casein-based diet (5.60g), Compif O (5.35g) and Compif R (4.12g) diets (Figure 4.5).The experimental rats fed Compif R diet which gained very little weight increased in body length as the other rats, though slightly lower. These groups of rats gained significantly more body length than the rats fed the protein-free diet (1.25g). This implies that the rats fed Compif R diet had some factors which encouraged linear growth. Based on the chemical analysis, Compf R had more calcium than Compif O, met phytate: calcium and zinc molar ratios even with low zinc content and had appreciable quantities of protein in terms of quantity. Sandstorm *et al.,* (1989) opined that some sulphur amino acids, cysteine-containing peptide and organic acids (fermentation) enhance zinc absorption and can neutralize the adverse effect of phytic acid, even in the presence of a moderate quantity of zinc. Compif R's major shortfall was probably poor phytate: iron molar ratio (6.13 >1) which depicts low iron bioavailability. Despite that both formulated diets had poor phytate:iron molar ratios, Compif O had significantly better value (4.17).

Therefore, it can be inferred from this observation that calcium, zinc and protein sufficiency (quantity and quality) support linear growth while iron quantity and bioavailability are crucial for the weight gain of animals and children. Moreover, the iron daily requirement is higher than that of zinc. It is also noteworthy that using body length (nose to tail), rats fed control and formulated diets did not differ in body length gain, but a significant difference existed when tail length gain was considered (Table 4.15). This might imply that the tail length growth of rats is more sensitive to nutrient intake and availability than body length.

Low FCR (Food Conversion Ratio) including high FER (Food Efficiency Ratio) and high PER (Protein Efficiency Ratio) are among the desirable qualities of a nutritionally adequate diet. Compif O (0.18, 1.69) performed poorly when compared to a

casein-based diet (0.27, 2.64) in terms of FER and PER (Figure 4.6-4.8). The FER (0.18) and PER (1.69) of Compif O were lower than those of soy-fermented quality protein maize (0.23, 2.32) and soy-malted quality protein maize (0.28, 2.80) blends reported by Abiose *et al.* (2015). These values were also lower than those obtained for a rice-milk based complementary food (0.20, 2.11) (Salter *et al*., 2013). The FER of Compif O (0.18) observed in the current study, was lower than the FER (0.22) of malted/fermented maize-crayfish blend (Ajibola *et al*., 2016). The FCR recorded in the current study was higher than FCR values observed by Shiriki *et al*., (2015). The PER of Compif O (1.69) and Compif R (0.48) were lower than the recommended value of at least 2.1 for complementary foods stipulated by the Protein Advisory Group (PAG, 1971). This depicted low protein quality despite the inclusion of fish powder. Notably, most of the other formulated foods had milk which is superior in protein quality. A study compared the effect of soaking and/or enrichment with small boiled fish,chicken liver, egg yolk and mung bean grits on calcium, iron and zinc bioavailability of maize and rice-based complementary foods. It was observed that the addition of chicken liver and egg yolk had a greater effect on iron and zinc bioavailability (Perlas and Gibson, 2005) compared to the other food sources. They concluded that soaking and enrichment with animal protein could improve in different degrees the bioavailability of iron and zinc. Thus, an increased quantity of fishmeal or addition of milk could improve the protein quality of complementary foods.

The mean weight of the organs followed the same trend displayed by the protein quality parameters. The mean weights of the liver, kidney, and heart of the rats fed the formulated diets were significantly different from those of the rats fed casein-based diets (Table 4.16).This finding is not in line with the observation of Ajibola *et al*., (2016) who reported that the weights of the kidney, spleen, and liver of animals fed toasted and oven-dried ogi-crayfish based complementary diets compared favourably with those of casein and CCF groups. The mean weights of the liver (1.4-2.29g) and kidney (0.38-0.74g) vary with the liver (3.00-3.60g) and kidney (0.36-1.00g) of the rats fed breadfruit-soy-groundnut based complementary diets (Adepeju *et al*., 2014). This could be attributed to the longer feeding period of the later study. However, the mean weight range of the

kidneys agreed with the findings of (0.35-0.76 g) by Ijarotimi and Keshinro (2013). This finding highlighted the extent of the effect of nutrient bioavailability on organ development.

5.1.5.2 Protein quality parameters of the formulated complementary foods

There was no difference in the true digestibility (TD) of the control and test diets. Above 90% of ingested food nitrogen of the formulated and control foods were absorbed (Figure 4.9). About 50% of the food nitrogen in Compif R was utilized for growth and maintenance compared to 85% (Compif O), 93% (CCF) and 100% (Casein). A similar finding of the lower utilization rate of roasted foods was reported by Ajibola *et al.,*(2016) when toasted (66%) and oven-dried (74%)crayfish-ogi complementary diets based on germinated-fermented maize were fed to weanling rats. The difference in the net protein utilization (NPU) of Compif R (50%) and the toasted crayfish-ogi diet (66%) could be because of higher temperature (120^{o}C) used for Compif R as against 70-80^{o}C. The low protein quality parameters obtained for Compif R could be attributed to the negative effect of roasting on nutrient bioavailability. Sade (2009) observed that the protein content of pearl millet depreciated during roasting. The works of D'Souza (2013) and Kavitha and Parimalavalli (2014) also recorded decreased levels of ash in roasted cereal and legume flours. Compif O had significantly better biological value (BV) and NPU than Compif R but comparable protein digestible corrected amino acid score (PDCAAS) values. This further illustrated that the effect of processing on essential nutrients is exhibited morein roasted foods than germinated or fermented foods despitethe reduction in anti-nutrients. The protein quality values were higher than those (BV: 41.2-70.4%;NPU: 41.2-70.4%;TD: 41.1-69.9%) reported for popcorn, African locust bean and Bambara groundnut based complementary diets (Ijarotimi and Keshinro 2013). The addition of fish and vegetables to the maize-pigeon pea mix could have improved the quality. Quadruple mix complementary foods have been reported to be better than cereal-legume mix (Zotor and Amuna, 2017).

5.1.5.3 Haematological properties of Wistar rats that were fed with the test diets

The result of the haematological analysis was varied and indicated that the rats that were fedwith Compif R based diet had comparable and slightly higher haemoglobin (Hb), packed cell volume (PCV) and red blood cells (RBCs)values than the group of rats fed Compif O (Figure 4.10). This finding did not follow the trend of earlier mentioned parameters. The PCV of the rats fed with the protein-free diet (27.25%) differed significantly with those of the rats fed with Compif O (29.0%), Compif R(34.25%), CCF (34.50%) and casein (45.75%) based diets. This is in tandem with the report of Adepeju *et al.,* (2014) and Abiose *et al.,* (2015)which reported higher PCV values for the animals fed their test diets compared to the PCV values of those fed with the basal diet (protein-free diet). The PCV, Hb, and RBC of the rats fed with the casein-based diet (45.75%, 15.43g/100ml, 7,600000mm^3) were significantly higher than those of the rats fed with the formulated diets.Similar PCV levels were reported by Abiose *et al.,* (2015) for soy-malted quality protein blend (37%) and soy-fermented quality protein maize (30.63%). Values of blood protein found in this study were all lower than those reported by Ijarotimi and Keshinro (2013) and Olapade *et al.* (2015) and normal range of 34-57%except for the casein-based group. Poor PCV, RB, and RBC may indicate iron deficiency or anaemia. The white blood cells (WBC) count of the rats fed with the basal diet was the highest (5310mm^3)but not significantly different from those of the casein group (4880mm^3). This finding agreed with those of Adepeju *et al.,* (2014) and Olapade *et al.,* (2015) who also reported higher WBC in basal groups. Olapade *et al.,* (2015) recorded a WBC count of 8170 mm^3 (basal group) and 8530 mm^3(casein group) which were higher than those obtained in this study. Adepeju *et al.,* (2014)observed lower WBC values (2400-3800 mm^3)compared to values obtained in this study (3.31-5.31mm^3). WBCs are disease-fighting cells present in the blood. High values could signify increased levels in response to infection while very low values indicate poor nutrition and low immunity.

The mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of the rats fed with formulated, control and basal diets did not differ (Figure 4.11-13).This finding supports that of Olapade *et al.,* (2015). However, the mean corpuscular haemoglobin concentration (MCHC)of the rats fed with the protein-free diet was

significantly lower ($p<0.05$) than those fed with the formulated and control diets. This contrasts with the finding ofIjarotimi and Keshinro (2012) who reported higher MCH and MCV values for rats fed with basal diet. MCH. MCV and MCHC indicate the sizes of the haemoglobin cells in relation to the cell volume. In cases of iron deficiency anaemia, the MCV is reduced and if Vitamin B12 is inadequate, the cells are enlarged.Protein affects the number and size of these cells. The MCHC is very sensitive to protein quantity and quality.

Figure 4.14 illustrates that the serum iron and calcium levels of rats fed protein-diet differed significantly ($p<0.05$) from those of casein, CCF and formulated diets but did not differ significantly from the zinc levels of Compif R group. This suggests that the expected increased bioavailability of zinc due to the reduction of phytate did not reflect since the serum zinc levels of the rats fed with the formulated diets (1.58, 0.75mg/100g) were significantly different at $p<0.05$ from those of control (3.24, 2.50mg/100g). A similar finding was reported by Liang *et al.* (2008) that the expected improvement due to reduced phytate was not reflected in the levels of invitro zinc. Adu-Afarwuah *et al*., (2008) also recorded no significant effect on serum zinc levels of infants after feeding fortified diets. Lin *et al.,* (2008) observed that zinc deficiency increased from 23% at baseline to 37% at the end of the study.In the study, 6-18 months children were fed complementary foods prepared from corn porridge fortified with fish powder (FP) and peanut/soy-based fortified spread (FS). The lower iron and zinc status of the experimental rats fed with Compif R based diet might be linked to the low levels of these minerals in Compif R. As mentioned earlier, some factors in foods might enhance zinc absorption even when levels are not very adequate. The poor iron status might have negatively affected the weight status of rats fed with Compif R based diet. The higher serum mineral levels (3.24 mg/100g) of rats fed with CCF, a commercial complementary food could be because of the fortification with minerals.

5.1.5.4 Serum biochemistry of rats fed the experimental diets

As expected the rats that were fed with the basal diet which was protein-free had significantly lower blood protein values (Figure 4.15). The total protein of the formulated diets (7.55,7.18 mg/dl)was lower than those observed for rats fed 20% pigeon pea-based

diet (8.12mg/dl) and normal rat feed (8.14mg/dl) but comparable albumin values (Soetan *et al.,* 2017).There are reports of a positive correlation between serum proteins and dietary protein (Vasan 2006). Casein diet (egg protein) showed superiority in terms of blood protein indices measured followed by CCF (milk-based), Compif O and Compif R. All the experimental rats fed with the diets except those fed with basal diet (lowest)had comparable blood urea nitrogen (BUN). BUN is a byproduct of protein metabolism and very low values might imply low protein status. This affirms that Compif R was adequate in protein quantity. The effect of the diets on the liver enzymes, Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), and Alkaline Phosphatase (ALP) are shown in Figures 4.19-4.21.The values of the serum liver enzymes tally with those reported by Soetan *et al., (*2017) for Wistar rats fed with normal rat feed (AST: 42u/l, ALT: 30.8u/l, ALP: 114.6u/l). There was no significant difference at $p>0.05$ among the serum enzymes in all the treatment groups. The protein-free group had significantly higher ALP compared to the other experimental groups. This shows that there was no damage to the liver cells. Increased enzyme activity is an indication of organ toxicity and reduced protein intake. Ewuola and Egbunike (2008) observed increased serum enzymes with increased fumonism levels in the diets fed rabbits.

LDL cholesterol and total cholesterol levels regarded as bad cholesterol were significantly higher in the formulated diets than the controls (Table 4.17). Formulated diets had fish, CCF hadmilk and casein is egg-based. It can be inferred that the type of animal source food influenced blood lipids. However, formulated diets with vegetable sources (carrot and ugu leaves) had higher HDL cholesterol, a desirable quality.

5.1.5.5 Organ histopathology of rats fed the experimental diets

The liver and kidney are organs usually affected by toxic and medicinal substances. Adverse effects are displayed as a distortion of normal morphology. The histopthological finding showed no severe deviations from the normal apart from the distortion in the hepatocytes of the liver tissues of the rats fed a protein-free diet. This shows that the diets did not have any toxic effect on the organs but the liver of the protein-free group was affected by gross protein insufficiency.

5.2CONCLUSION

Under-nutrition particularly stunting and micronutrient deficiency in the first 1000 days of life is associated with a greater risk of morbidity and mortality. Poor quality of complementary foods is a major contributor to this high under-nutrition burden which is worse during the complementary feeding period. Complementary foods in Nigeria have been reported to be of low dietary diversity and phytate dense because they are mainly plant-based. In this study, the nutrition potential of a maize-pigeon pea-based complementary food with fish; carrot and pumpkin leaves powder was investigated. Also, the effect of traditional processing methods on phytate reduction wasassessed. The combined germination-fermentation method was more effective in phytate reduction in maize and retained more nutrients compared to fermentation.Fermentation removed more phytate in pigeon pea and more nutrients were retained. Therefore, combined germination (72 hours)-fermentation (24 hours) and fermentation (72 hours) were the preferred processing methods for maize and pigeon pea respectively.

Compif O formulated from fermented pigeon pea and germinated-fermented maize satisfied above 70% of the recommendation for complementary foods as stipulated by CODEX Alimentarius Commission while Compif R from roasted ingredients did not.Compif O was better in nutritional adequacy than Compif R.

Water absorption capacity, bulk density and swelling capacity of the formulated diets were comparable and of desirable levels. The least gelation concentration and viscosity values of formulated complementary foods were higher than the control. Compif O had better functional properties than Compif R.Thus, fermentation improved the functional properties of formulated complementary food more than roasting. The microbial counts of the formulated complementary foods were within safe limits until the third week. Although fermentation enhanced microbiological safety of Compif O, roasting (Compif R) had a better effect on microbial safety and storage stability.

Compif O compared favourably with the commercial complementary food in terms of growth support while Compif R was rated poor.The complementary food formulated from fermented pigeon pea and combined germinated-fermented maize seeds

was of better nutritional quality compared to complementary food formulated from the roasted seeds.

5.3Recommendations

Fermented pigeon pea is recommended for use in formulating quality complementary food rather than roasted pigeon pea.Mothers should be encouraged to include pigeon pea when processing complementary foods. Mothers should be enlightened on the fermentation of legumes and combined germination-fermentation of cereals; this should be part of the dietary diversity campaign.Complementary foods processedwith fermentation, germination, roasting among other methods would have reduced anti-nutrient content. However, micronutrient composition is usually affected negatively. Therefore, there is a need for a post nutrient analysis after processing to detect micro-nutrients that require fortification. Measures to ensure micronutrient sufficiency in processed complementary foodmust be part of infant feeding enlightenment programmes. Some of the measures include increased portions of animal source foods in plant-based processed complementary foods and micronutrient supplementation.Also, the addition of enhancers such as orange juice and vitamin A rich sources (banana, carrots) to ready to eat feeds should be intensively promoted since they promote iron bioavailability.More than 10% of fish inclusion should be considered in complementary food formulation for improved protein quality. For other animal source foods, there is a need to determine their respective inclusion levels at which complementary food of desirable protein quality would be achieved.

Further studies should be undertaken in other ways of improving the bioavailability of protein and micronutrients in processed plant-based complementary foods. This may includeexamining the effect of adding other animal source foods singly such as milk or egg or combined such as egg and fish on the nutritional quality of the maize-pigeon pea-carrot-pumpkin blend. There is a need for further investigation ofthe amino acid content of maize-pigeon pea-fish based complementary food.More research is required in the area of standardising right temperature of roasting cereals and legumes for processed

complementary foods since most mothers prefer it because of less time and energy consumption.Finally, longer periods of animal study (three or more months) may give a clearer growth assessment particularly on the effect of complementary foods on linear growth and blood parameters.

5.4Contribution to knowledge

- **The study has contributed the following to the knowledge of nutrition:**

- It has established that germination-fermentation of maize and fermentation of pigeon pea reduce above 90% phytate content and retain more nutrients.
- This study revealed that pigeon pea, when fermented and cooked, could be combined with maize, fish, carrot, and pumpkin leaves to develop quality complementary food.
- The study further showed that oven-dried complementary food grits formulated from fermented pigeon pea and combined germinated-fermented maize could be safe for consumption for three weeks.
- The study demonstrated that maize-based complementary food fortified with fermented pigeon pea supports growth.
- The findings of this study established that roasted pigeon pea fortified food does not support growth.

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Appendix 1. Performance characteristics of Wistar rats fed control and test diets.

Parameters	CCF	Casein	Compif O	Compif R	Protein free
Weight gain/loss(g)	33.07 ±9.81[a]	32.74 ± 7.50[a]	24.05 ±4.94[a]	5.75 ± 3.63[b]	-14.17±6.56[c]
Tail length gain(cm)	2.44 ±0.38[a]	2.68 ± 0.04[a]	2.46 ± 1.58[a]	1.82 ± 0.81[b]	0.53±0.36[b]
Total body length gain(cm)	5.18 ±1.26[a]	5.60 ± 0.85[a]	5.35 ± 2.02[a]	4.12 ± 1.08[a]	1.25±0.44
Total Feed intake/rat(g)	151.12±10.70	124.32 ±9.55	133.36 ± 11.81	106.76 ±s13.80	71.96±8.53
Protein intake/rat(g)	14.74	12.53	14.33	11.32	0.477
FCR	4.57 ± 0.90[a]	3.64 ± 0.50[a]	5.54 ± 0.34[a]	30.32 ± 27.25[b]	
FER	0.23 ± 0.05[ab]	0.27 ± 0.34[a]	0.18 ± 0.01[b]	0.05 ± 0.03[c]	
PER	2.22 ± 0.47[a]	2.64 ± 0.35[a]	1.69 ± 0.10[b]	0.48 ± 0.26[c]	
Corrected PER	2.10	2.50	1.60	0.46	
NPR	3.20	3.79	2.67	1.76	
R-NPR	3.55	4.20	3.00	1.95	

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$.FCR= Feed conversion ratio; FER= Feed efficiency ratio, PER=Protein efficiency ratio, NPR= Net protein retention.R-NPR= Relative net protein retention. Period of feeding = 21 days.

Appendix 2. Protein quality and digestibility parameters of the test and control diets

Parameters	CCF	Casein	Compif O	Compif R	Protein free
Total Feed intake(g)	51.07	40.59	46.63	36.20	30.07
Total Protein intake(g)	5.21	4.26	4.98	3.72	0.20
Total Nitrogen intake(%)	0.83	0.68	0.80	0.61	0.03
TD(%)	92.28 ± 1.21[a]	91.50± 0.35[a]	91.70±0.67[a]	92.90±0.28[a]	
BV(%)	85.87±0.64[b]	99.50±0.71[a]	77.97±0.54[c]	47.10±0.16[d]	
NPU(%)	79.24±0.67[b]	91.00±0.57[a]	71.50±0.19[c]	43.79±0.15[d]	
PDCAAS(%)	81.76±0.00[b]	96.00±0.70[a]	68.86±0.68[c]	68.45±0.35[c]	

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$. True digestibility (TD), Biological value (BV), Net protein utilization (NPU), Protein digestibility corrected amino acid score (PDCAAS). Assay period= 7 days

Appendix 3. Haematological indices of Wistar rats fed the control and test diets.

Parameters	CCF	Casein	Compif O	Compif R	Protein free
PCV (%)	34.50^{b}± 7.33	45.75^{a} ± 2.75	29.00^{b} ± 6.16	34.25^{b} ± 3.69	27.25^{c} ± 2.22
Hb (g/100g)	11.85^{b} ± 2.42	15.43^{a} ± 1.70	9.65^{bc} ± 2.11	11.55^{b} ± 1.22	7.83^{c} ± 1.47
RBC ($x10^{6}$ mm^{3})	5.83^{b} ± 1.19	7.60^{a} ± 0.39	4.67^{bc} ± 1.14	5.50^{b} ± 0.61	4.06^{c} ± 0.66
WBC($x10^{3}$ mm^{3})	3.50^{b} ± 0.840	4.88^{ab} ± 1.72	3.31^{b} ± 0.68	3.38^{b} ±1.08	5.31^{a} ± 0.92
PLT ($x10^{4}$ mm^{3})	15.30^{a} ± 3.54	10.98^{b} ± 1.10	18.35^{a} ± 3.51	12.73^{b} ± 1.38	12.85^{b} ± 0.34
LYM (%)	59.00^{a} ± 3.11	60.25^{ab} ± 6.50	65.50^{ab} ± 5.07	67.25^{ab} ± 4.03	59.50^{b} ± 6.46
NEUT (%)	36.75^{b} ± 3.50	39.00^{a} ± 4.16	29.75^{b} ± 4.50	29.00^{b} ± 3.92	27.00^{b} ± 3.92
MONO (%)	2.00^{a} ± 0.96	2.00^{ab} ± 0.82	1.00^{b}± 0.00	1.75^{ab} ± 0.50	1.75^{ab} ± 0.50
EOS (%)	2.25^{a} ± 0.58	1.50^{a} ± 1.29	2.25^{a} ± 0.96	2.00^{a} ± 1.16	1.75^{a} ± 0.50
MCV (fl)	59.14^{a} ± 2.13	60.18^{a} ± 2.13	62.41^{a} ± 3.55	62.29^{a} ± 0.73	68.44^{a} ± 12.04
MCH (pg)	20.35^{a} ± 1.17	20.25^{a} ± 1.66	20.75^{a}± 1.10	21.09^{a} ± 2.03	19.78^{a} ± 5.69
MCHC (%)	30.43^{ab} ± 0.50	33.63^{a} ± 2.17	33.26^{a} ± 1.20	33.83^{a} ± 3.07	28.60^{b} ± 3.18

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P< 0.05$. PCV= Packed cell volume, Hb= Haemoglobin, RBC= Red blood cell, WBC= White blood cell, PLT= Platelets, LYM= Lymphocytes, NEUT= Neutrophils, MONO= Moncytes, EOS= Eosinophil, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration.

Appendix 4. Serum minerals of Wistar rats fed the control and test diets

Parameters(mg/dl)	CCF	Casein	Compif O	Compif R	Protein free
Calcium	$10.80^{ab} \pm 0.54$	$10.78^{ab} \pm 0.90$	$11.00^{a} \pm 0.51$	$9.70^{b} \pm 0.76$	$8.25^{c} \pm 0.96$
Iron	$4.05^{a} \pm 0.70$	$3.25^{a} \pm 0.72$	$3.33^{a} \pm 0.44$	$1.88^{b} \pm 0.30$	$1.83^{b} \pm 0.42$
Zinc	$3.24^{a} \pm 0.19$	$2.50^{b} \pm 0.53$	1.58c ± 0.50	$0.75^{d} \pm 0.24$	$1.13^{cd} \pm 0.25$
Sodium	$143^{a} \pm 5.35$	$146^{a} \pm$ 1.16	$144.75^{a} \pm 3.95$	$143.25^{a} \pm 2.23$	$132.00^{b} \pm 2.16$
Potassium	$6.75^{a} \pm 0.45$	$4.73^{b} \pm 0.29$	7.15a ± 0.13	$6.45^{a} \pm 0.93$	$4.18^{b} \pm 0.82$
Phosphorus	$6.88^{ab} \pm 2.03$	$6.53^{ab} \pm 0.78$	$8.75^{a} \pm 2.40$	$6.48^{ab} \pm 1.60$	$4.65^{b} \pm 0.64$

Values are expressed as mean **±**SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P < 0.05$.

Appendix 5: Biochemical indices of Wistar rats fed the control and test diets

Parameters	CCF	Casein	Compif O	Compif R	Protein free
TOTPR (%)	$8.20^{b} \pm 0.62$	$9.70^{a} \pm 0.71$	$7.55^{bc} \pm 0.70$	$7.18^{c} \pm 0.35$	$3.38^{d} \pm 0.70$
ALB (g/100g)	$3.58^{b} \pm 0.25$	$4.55^{a} \pm 0.45$	$3.38^{b} \pm 0.38$	$3.23^{b} \pm 0.21$	$1.00^{c} \pm 0.68$
GLO ($x10^{6}$)	$4.68^{b} \pm 0.32$	$5.57^{a} \pm 0.29$	$4.18^{c} \pm 0.43$	$4.05^{c} \pm 0.17$	$2.30^{d} \pm 0.38$
A.G RATIO	$0.73^{b} \pm 0.05$	$0.82^{a} \pm 0.12$	$0.75^{b} \pm 0.10$	$0.73^{b} \pm 0.05$	$0.23^{c} \pm 0.10$
AST (%)	$41.75^{a} \pm 1.26$	$45.50^{a} \pm 8.54$	$42.50^{a} \pm 3.51$	$43.00^{a} \pm 3.83$	$41.25^{a} \pm 2.22$
ALT (%)	$30.25^{a} \pm 1.71$	$30.25^{a} \pm 4.11$	$31.25^{a} \pm 3.30$	$32.00^{a} \pm 2.16$	$30.50^{a} \pm 6.19$
ALP(%)	$103.75^{b} \pm 3.40$	$104.50^{b} \pm 2.08$	$110.00^{b} \pm 12.25$	$106.75^{b} \pm 8.26$	$130.25^{a} \pm 2.63$
BUN (mg/dl)	$17.93^{a} \pm 0.38$	$18.30a \pm 0.25$	$17.68a \pm 0.86$	$17.23^{a} \pm 0.49$	$13.28^{b} \pm 1.22$
CREAT(mg/dl)	$1.05^{ab} \pm 0.21$	$0.60^{bc} \pm 0.08$	$0.85^{a} \pm 0.24$	$0.75^{ab} \pm 0.08$	$0.43^{c} \pm 0.05$
GLU(mg/dl)	134.00 ± 17.22	127.50 ± 6.03	125.50 ± 14.36	132.50 ± 18.56	115.25 ± 6.90

Values are expressed as mean ±SD (n=6). Means in the same row with different superscripts are significantly different from each other at $P < 0.05$. TOTPR= Total protein; ALB= Albumin; GLO= Globulin; AGRATIO= Albumin/globulin ratio; AST= Aspartate Amino transferase; ALT=Alanine Amino transferase; ALP= Alkalline phosphatase; BUN= Blood urea nitrogen; CREA= Creatinine and GLU=Glucose.

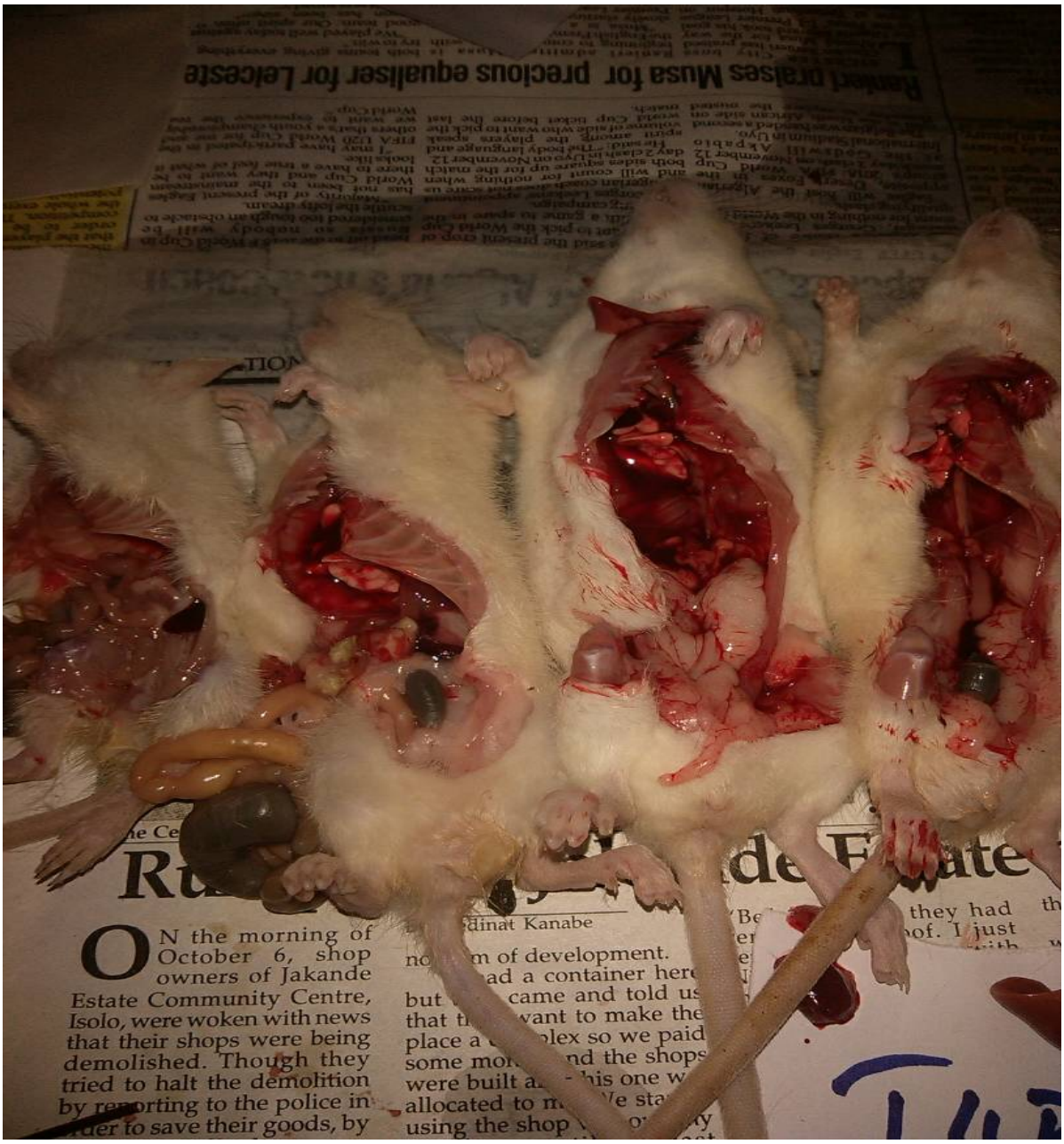
Appendix 6: Spectorophotometric determination of amino acids

using Ninhydrin chemical reaction

AMINO ACID	WAVELENGTH	COLOUR
ARGININE	505nm	LIGHT BLUE
ALANINE	620nm	BLUE
ASPARTIC ACID	465nm	PURPLE
CYSTEINE	600nm	BLUE
GLUTAMIC ACID	560nm	PURPLE
GLYCINE	525nm	PURPLE
HISTIDINE	460nm	PURPLE
ISOLEUCINE	580nm	LIGHT PURPLE
LEUCINE	590nm	PURPLE
LYSINE	450nm	ORANGE YELLOW
METHIONINE	525nm	GREENISH YELLOW
ORNITHINE	570nm	PURPLE
PHENYLALANINE	545nm	YELLOW
PYRROLYSINE	455nm	YELLOWISH BLUE
PROLINE	470nm	YELLOW
SERINE	485nm	BLUE
THREONINE	615nm	BLUISH GREEN
TYROSINE	530nm	GREENISH BLUE
TRYTOPHAN	565nm	YELLOWISH BLUE
VALINE	490nm	GREENISH BLUE

Appendix 7: Raw and fermented pigeon pea seeds NSWCC 34 (I&ART)


Fermented
Pigeon Pea (24A)


Ranked praises Musa for precious equaliser for Leicester
ON the morning of October 6, shop owners of Jakande Estate Community Centre, Isolo, were woken with news that their shops were being demolished. Though they tried to halt the demolition by reporting to the police in order to save their goods, by
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