POTENTIAL AND SAFETY OF COMPLEMENTARY FOODS DEVELOPED FROM SELECTED CEREALS AND LEGUMES CULTIVATED IN GONDAR PROVINCE, ETHIOPIA TO ALLEVIATE PROTEIN-ENERGY MALNUTRITION

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Potential and Safety of Complementary Foods Developed from Selected Cereals and Legumes Cultivated in Gondar Province, Ethiopia to Alleviate Protein-Energy Malnutrition

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DECLARATION

This thesis is my own work, and has not been presented for a degree in any other university

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To everyone who supported me especially my wife Alemtsehay Yigzaw Takele for her love and, understanding &support. Also to my mom for her endless prayer for my success.

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ABBREVIATIONS AND ACRONYMS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AOAC	Association of Analytical Chemists
AST	Aspartate Aminotransferase
BV	Biological Value
CE	Catechin Equivalent
SCN	(United Nations) Standing Committee on Nutrition
CRD	Complete Randomized Design
DSA	Diazotized Sulfanilic Acid
EDHS	Ethiopian Demographic Health survey
FAO	Food and Agricultural Organization
FER	Feed Efficiency Ratio
GI	Gastrointestinal
GOD –POD	Glucose Oxidase-Peroxidase
Hbc	Haemoglobin Concentration
HDL	High Density Lipoprotein
HFA	Height-for-Age
HPLC	High Pressure Liquid Chromatography
IDA	Iron Deficiency Anaemia
IQ	Intellectual Quotient
LDL	Low Density Lipoprotein
LSD	Least Significance Difference
MAM	Moderate Acute Malnutrition
MCH	Mean Concentration Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MUAC	Mid-Upper-Arm Circumference
NPU	Net Protein Utilization

PAG	Protein Advisory Group
PCV	Packed Cell Volume
PEM	Protein Energy Malnutrition
PER	Protein Efficiency Ratio
RBC	Red Blood Cells
RDAs	Recommended Dietary Allowances
SAM	Severe Acute Malnutrition
SD	Standard Deviations
TC	Total Cholesterol
TD	True Digestibility
TG	Triglyceride
UNICEF	Unite Nation Children's Emergency Fund
WBC	White Blood Cells
WFA	Weight-for-Age
WFH	Weight-for-Height
WHO	World Health Organization

ABSTRACT

In a bid to address the challenge of childhood malnutrition caused by poor complementary feeding, this study was aimed to formulate accessible complementary foods from selected cereals and legumes. Evaluation of the foods was then done haematologically, biochemically and nutritionally using white albino rats. Four multimix complementary foods were prepared from selected local foods that comprised red teff (*Eragrostis tef* (Zucc.), maize (*Zea maize*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), oat (*Avena sativa*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), beans (*Vicia faba*), soya beans (*Glycine max*), sesame (*Sesamum indicum*) and spinach (*Spinacia oleracea*). The four complementary blends were formulated based on the protein and energy of the commodities. The formulated diets were subjected to chemical analysis-along with a commonly used commercial formula (Cerifam) as a control. This is usually used as a complementary food in Ethiopia. Standard official procedures (AOAC) were used to determine the macronutrient composition of the developed diets and ingredients. High-performance liquid chromatography was used to detect and quantify anti-nutritional factors and some of the vitamins, while minerals were analyzed using an Atomic Absorption Spectrophotometer. The nutritional qualities of the formulated blends were assessed biologically by feeding white albino rats in order to determine feed intake, the growth rate, protein quality parameters, biochemical and haematological properties. Analysis of variance (ANOVA) was used to establish any significant difference in the analytical data for the formulated and control mixtures using SPSS Version 20. The overall proximate results indicate that protein content was ranged between12.20 to17.14%. While fat and carbohydrate values were in the range of 2.44 to 38.88% and 35.29 to 73.41%, respectively. The crude protein, fat and energy values of all blended foods were statistically ($p= 0.02$) higher compared to the control value and met the recommended dietary allowance for protein, energy and micronutrients of public health concern including zinc, iron and vitamin A based on an estimated daily intake of 65 g of the foods for 6-12 month infant. All sample diets had antinutrient/mineral molar ratio below the cutoff values, hence, mineral absorption was not inhibited in the formulated diets. Rats placed on Diet 3, Diet 4, casein, and Cerifam consumed more food than those fed on Diet 1, Diet 2, and Diet 7. The higher food intake in Diet 3 and Diet 4 might be attributed to the improved flavor and palatability due to the presence of aromatic amino acids. The mean growth rate of rats fed on Diet 3 and Diet 4 was significantly ($p=0.04$) higher than that of those placed on Diet 1, Diet 2 but similar to those fed with commercial diet and casein. The protein quality evaluation of the diets showed that the protein efficiency ratio ranged from 1.20 to 2.43 while the biological value was in the range of 54.53 to 69.48%. The net protein utilization and true digestibility ranged from 65.62 to 70.21% and 59.01 to 64.01%, respectively. Serum total protein, albumin, and globulin levels in rats given Diet 3 and Diet 4 were comparable to the controls and within the normal range. The creatinine and urea levels of rats fed on the control and formulated mixtures were also the normal range (0.2-0.8 and 7-20 mg/dL) respectively. The serum alanine aminotransferase values of rats fed on

the control and formulated foods ranged from 22.03 IU/L in Diet 2 to 37.76 IU/L in Diet 4 and were not significantly different from each other. In this study, the packed cell volume and haemoglobin values of the control and formulated foods were within the recommended range. Evidently, Diet 3 and Diet 4 gave the best growth performance after the feeding trials. Better growth performance attained by rats fed on Diet 3 and Diet 4 may be attributed to the inclusion of chickpea and soybeans that contains high quality protein that is not found in cereals. In conclusion, the growth response and protein quality (PER, BV, NPU, TD) evaluation revealed that these diets were nutritious enough and comparable to standard casein and commercially available baby food (Cerifam). Besides, absence of toxic substances in these diets, as evidenced by liver function tests, biochemical and haematological findings, could also reveal that the diets could provide adequate nutrients and support higher growth rates in infants without causing any harmful effects.

Key words: Cereals, Complementary, Legumes, Nutrient Requirement, serum enzyme, bioassay, protein quality, albino rat, biological value

CHAPTER ONE

INTRODUCTION

1.1 Background information

In spite of plentiful worldwide food production, malnutrition continues to be among the main public health problems in poor resource countries. Malnutrition essentially means bad nourishment: It could be over nutrition when the diet contains too many calories or under nutrition if the diet does not provide adequate calories and protein for growth and body maintenance. This is also referred to as Protein–Energy Malnutrition or protein calorie malnutrition. In this study the malnutrition referred to is under nutrition. It is internationally the key risk factor for ill health and mortality among communities in resource poor settings, with hundreds of millions of pregnant women and young children predominantly affected (Temesgen, 2013). According to WHO 2019 data, 155 million children under the age of five are stunted, 52 million are wasted, and 17 million are severely wasted globally. Forty-five percent of deaths in children under the age of five were due to being underweight for their age (Kramer and Allen, 2015). According to the Standing Committee on Nutrition (SCN) of the United Nations system, malnutrition is directly and indirectly associated with over 50% of infant mortality and contributes to disease development in developing countries (SCN, 2018).

In 2011, of the 8.8 million global deaths of children under 5 years of age, 93% occurred in the developing countries of Africa and Asia, where the highest childhood mortality rates are found in

Sub-Saharan Africa whereby one in seven children die before their fifth birthday (Walton and Allen, 2011). It is estimated that there are 148 million underweight children: 78 million live in South Asia and 36 million in sub-Saharan Africa. In the developing countries 20% of children are underweight and 3.5% (19 million) severely malnourished (Walton and Allen, 2011). In addition to being associated with high

morbidity to causing diseases, stunted growth and high deaths, malnutrition causes long-term developmental problems and poor educational attainment affects the ability to work, reducing the potential for national development (Wolton and Allen, 2011; Alawode *et al*., 2017).

These nutritional problems are mainly attributed to poverty, low nutritional quality traditional Complementary foods, inappropriate complementary feeding practices, high prevalence of communicable diseases and the high cost of fortified nutritious proprietary formulas (Alozie *et al.*, 2009; Eka *et al*., 2010; UNICEF, 2013). Over one-third of child mortality below five years is caused by malnutrition associated with inappropriate complementary feeding practices. The main global and national food-based efforts to address these problems include dietary diversification, fortification of staple foods, modification of traditional diets, and nutritional supplementation (Müller & Krawinkel, 2005). In addition, improving the value of local complementary foods has been identified as one of the least costly methods of reducing morbidity and mortality and improving infant health (Semahegn *et al*., 2014).

Diet and health in the early years have a major impact on the growth and development of children in to adulthood. During the first 2 years, the growth of an infant is very rapid and breast milk alone is not sufficient to meet their nutritional requirements after the age of six months (Fikiru *et al*., 2017). Hence between six and 24 months, complementary foods should be introduced in addition to breast milk. However, such food is often not nutritionally adequate. Chronic malnutrition generally occurs during this critical transitional period when children are transferred from liquid to semi-solid foods (Victora *et al*., 2010; Ibironke *et al*., 2014). Consequently, timely introduction of good quality and calorie-dense complementary foods that are affordable to low income families during this window period is necessary for both health and developmental reasons (Agostoni *et al*., 2008; Semahegn *et al*., 2014).

Despite current improvements, child malnutrition continues to be the main public health problem in Ethiopia. The country has a high prevalence of child malnutrition that is

associated with 53% of infant and child mortality (Temesgen, 2013). According to EDHS (2019), 37% of children under 5 are stunted and 12% are severely stunted. The 2019 EDHS also shows that 21% of all children are underweight, and 6% are severely underweight. Furthermore, 7% of children across the country are wasted, and 1% are severely wasted. Generally, children of rural and uneducated mothers are more likely to be underweight, wasted or stunted than others and the highest burden of this condition occurs at the aged of 6–23 months (Derso *et al*., 2017). Protein-energy malnutrition and lack of micronutrients such as vitamin A, vitamin C, iron, zinc and iodine are the major nutritional problems documented in Ethiopia (Temesgen, 2013). A combination of diseases, inappropriate feeding practice, and nutritionally inferior diets account for the occurrence of children malnutrition in Ethiopia (Temesgen, 2013).

For economic reasons, foods of animal origin and commercially fortified foods are often too expensive and unattainable for most low-income families. Therefore, they usually depend on traditional complementary foods, usually cereals that are not supplemented with legumes and/ or tubers. These foods are very low in nutritive value and are characterized by low protein and energy density and high bulk (Ajani, 2010). In most developing countries, more than 70% of the protein in the diet is supplied by cereals that are of poor protein quality as they are limited in some essential amino acids, particularly lysine and tryptophan (Anigo *et al*., 2010). In contrast, most legumes are rich in these particular amino acids, though they are deficient in methionine. Thus, the processing and blending of cereals with locally available protein rich legumes and modification with some vegetables improves the protein content and quality of cereal-legume mixtures by mutual complementation of their individual amino acids (Mensa-Wilmot *et al*., 2003).

The formulation of nutritious formula supplements using household level approaches has received much attention from local and readily available materials in many low-income communities. The technologies applied are inexpensive, sustainable and can be adapted to different cultural, dietary traditions and locally feasible strategies (Griffith *et al*., 1998). Many researchers suggest that supplementation of cereals with legumes such as soybean; beans, pea and chickpea provide high quality complementary foods

(Ajanaku *et al.*, 2013). The selection of these specific legumes was based on their high protein content and well balanced amino acids. And introduction of sesame and spinach leaf powder into the blend to increase its protein, vitamin and mineral content. Hence, there will be improvement of the nutritional value of the food as well as the nutritional status of the infant if both cereals and legumes are blended with spinach and sesame during the preparation of the food. In addition, the mentioned cereals, legumes and vegetables are locally produced and this may make the food product very affordable. Thus, in view of the nutrition problems associated with the traditional complementary foods in Ethiopia, infant diets require appropriate processing and blending of locally affordable food commodities for improvement of their dietary quality and consistency. The aim of this study is to develop complementary foods based on composite blends of local cereal and legume grains in Gondar province, Ethiopia, and evaluate their nutrient composition, effect on weight gain and lipid profile and liver function tests using albino rats.

1.2 Statement of the problem

Ethiopia has a high prevalence of child malnutrition that contributes to 53% of infant and child mortality (Temesgen, 2013). Protein-energy malnutrition and lack of micronutrients are the major nutritional problems recorded in Ethiopia (UNICEF, 2019). Low nutrient-dense local complementary foods and poor complementary feeding practices are among the major causes for the high incidences observed among children in Ethiopia (Temesgen, 2013). WHO has established guidelines for infant and young child feeding practices for children aged 6–23 months by incorporating the minimum acceptable diet (MAD) as one of the eight core indicators of complementary feeding (USAID, 2011). It is the result of a combination of low dietary diversity and low meal frequency. Low dietary diversity and meal frequency practices are risk factors for health and growth in children under the age of two. According to recent demographic and health survey reports from ten Asian and African countries, including Ethiopia, the proportion of children fed a minimum acceptable diet ranges from 7% in Ethiopia to

36% in Nepal. This suggests that providing a minimum acceptable diet is a major issue both globally and in developing countries (Guirindola, 2018; WHO, 2013). Tassew *et al*. (2019) found that only 6.1 percent of 2919 children aged 6–23 months were fed MAD in an Ethiopian study. On the other hand, the proportion of children with the least amount of dietary diversity and the least amount of meal frequency was 11% and 42%, respectively.

Deficiencies in essential micronutrients such as iron, calcium, zinc, iodine, and vitamin A are common nutritional problems in Ethiopia that affect all segments of the population, particularly children under the age of five (Hassen *et al*., 2020). According to the Ethiopian Demographic and Health Survey, the prevalence of anemia among children under the age of five was 54% in 2005, 44% in 2011, and 57% in 2016 (EDHS, 2016), indicating no decrease despite the implementation of various intervention strategies. According to Hassen *et al*. (2020), the early neonates had the highest prevalence rate of VAD, with 84,547.1 per 100,000. However, children aged 5 to 9 years had the highest prevalence rate of Iodine, with 926.1 new cases per 100,000. All these deficiencies are caused by diets with little variety, low bioavailability, frequent meal skipping, a lack of access to micronutrient-rich and fortified foods, and a low intake of vegetables and fruits (Sheehy *et al*., 2019).

Many commercially prepared foods produced by roller-drying and extrusion technologies marketed in Ethiopia and other countries are too expensive for middle and lower income rural and urban parents. Consequently, many families depend on insufficiently processed and low quality traditional complementary foods for their children. Thus, one approach to bridge the food gap that leads to protein-energy malnutrition among young children in poor-resource countries is to develop low-cost complementary foods to improve infants' nutrition.

In Gondar area, Ethiopia traditional complementary foods are prepared mainly from a limited choice of unprocessed cereals, which are not supplemented with legumes and are

thus limiting in the required level of micronutrients, and protein quality. This coupled with the viscosity, fiber concentration and anti-nutrients are major constraints in providing infants with adequate nutrients. Therefore, there is a need for complementary foods with appropriate formulation and processing using local food-based approaches. The objective of this study was to use indigenous foods to formulate composite mixtures that meet the nutritional needs of infants and children and are readily available and affordable to both poor rural and urban mothers.

1.3 Justification of the study

A number of researchers recommend the use of nutritious locally available foodstuffs such as cereal, legumes, and vegetables to prepare composite blends for infants and children (Muhimbula *et al*., 2011; Kuyper *et al*., 2010). WHO also suggests the use of low cost affordable local foodstuffs in complementary feeding. North Western Ethiopia experiences support the production of different crops that can be used in the development of such low cost complementary foods. Apart from the development of such foods being sustainable in the region, they are also acceptable to the community. In addition to address nutrition problems of infants, the development of these foods will also improve food diversification and eating habits. Various processing techniques (combined strategy) was applied to reduce the anti-nutrients and thus increase the bioavailability of the minerals. Thus, processing techniques being applied are cheap, and can be administered in resource poor settings. This information can be used in designing appropriate policies for improvement of complementary feeding practices. Therefore, formulation and evaluation of nutritionally adequate legume supplemented complementary foods from local ingredients is promising.

1.4 Objective of the study

1.4.1 General objective

To develop complementary foods based on composite blends of local cereal and legume grains in Gondar Province, Ethiopia, and evaluate their nutrient composition, and effect on weight gain, lipid profile and liver function tests of albino rats fed on them

1.4.2 Specific objectives

1. To formulate complementary diets based on composite blends of selected cereals, legumes and vegetables commonly cultivated and consumed in Gondar Province.
2. To assess the macro- and micro-nutritional value of the complementary foods from various composite blends, and compare their nutrient profiles with RDAs and reference formula
3. To determine the anti-nutrient content of the developed complementary foods
4. To determine the protein quality and weight change of rats fed on the different complementary diets
5. To determine the biochemical and haematological indices of rats fed on the different complementary diets
6. To determine the effect of the different complementary diets on the lipid profile and liver function tests of albino rats.

1.5 Study hypothesis

1. **Null hypothesis:** There is no significant difference in macro and micronutrient compositions of the formulated diets in comparison to the commercial control (cerifam).

2. **Null hypothesis:** There is no significant difference in weight gain, lipid profile and liver function among albino rats fed complementary diets formulated from local foods in Gondar Province, Ethiopia, in comparison to those fed on cerifam and casein protein (control diets).

3. **Null hypothesis:** There is no significant difference in the haematological and biochemical indices, among albino rats fed complementary diets formulated from local foods in Gondar Province, Ethiopia, in comparison to those fed on cerifam and casein protein (control diets).

1.6 Significance of the study

Agriculture is the leading economic sector in Ethiopia and Northwestern Ethiopia is the largest producers of teff, chickpea, pea, beans and other crops (Temesgen, 2013). Thus, the development of complementary foods by the complementation of cereals and legumes through local food-based approach will have a great contribution not only to alleviate PEM but also by substituting the import of infant foods. Utilization of locally affordable resources can enhance income generation and provide employment opportunity in the region. Furthermore, the results of this study will provide answers to the questions (a) whether such traditionally processed formulations can meet the various nutritional recommendations for infants and young children; and (b) whether they can substitute expensive infant formulas sold in the markets. The outcome of this study provides information necessary for designing appropriate policies for improvement of complementary feeding practices in the study area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of malnutrition

Malnutrition is defined as a condition when the body does not have enough of the required nutrients (under-nutrition) or has too much nutrients (over-nutrition) (UNICEF, 2010). The macronutrient components of the food consist of protein, carbohydrates and fats, whereas the micronutrients are minerals and vitamins (UNICEF, 2010). In poor resource settings, carbohydrates are the major part of the diet (80%) and the main source of energy. Fats are also an important component which makes up about 10% of the diet in these communities. Proteins which are derived from both plant and animal sources are required to build new tissue and cells. Proteins from animal sources contain essential amino acids that cannot be produced by the body but must be consumed in the diet. Proteins from cereals alone do not provide sufficient essential amino acids in the right balance to meet physiological requirements. In order to obtain the correct balance of essential amino acids from plant sources, cereals and pulses must be correctly combined when planning a meal (UNICEF, 2010).

Childhood malnutrition is a global public health problem and a major risk factor to child morbidity, mortality, intellectual and physical disability (Kabir *et al*., 2013). Globally an estimated 165 million, 10 million and 52 million of children below five years of age are stunted, underweight and wasted, respectively (UNICEF, 2012). Globally, the amount of undernutrition among children is significantly high throughout the world, with a large number of children living in the developing world (Muhimbula *et al.*, 2011). In addition, malnutrition in sub-Saharan Africa is associated with about 60% of infant mortality below 5 years (Kandala *et al*., 2011).

Malnutrition is not a single problem with simple solution. Diverse/complex and interrelated factors are involved in the process and development of malnutrition and similarly a series of multifaceted and multi-sectorial approaches are needed to tackle the problem (Alawode *et*

al. 2017; UNICEF, 2020). Conceptual framework for the determinants of malnutrition was developed by UNICEF in 2020 to address the nutrition problem. Based on this conceptual frame work, determinants of malnutrition are complex and multiple linkages, embracing socio-economic, food, health and caring practices. The framework is used at national, regional and local levels to plan effective measures to improve nutrition. It serves as a guide for assessing and analyzing the causes of the nutritional problem and helps determine the most appropriate mix of measures. Inadequate food intake and illness are immediate causes of malnutrition and lead to a vicious cycle in which illness and malnutrition worsen each other. It is known as the Malnutrition Infection Complex as shown in (Fig. 2.1). Therefore, both insufficient food intake and diseases need to be addressed to support recovery from malnutrition.

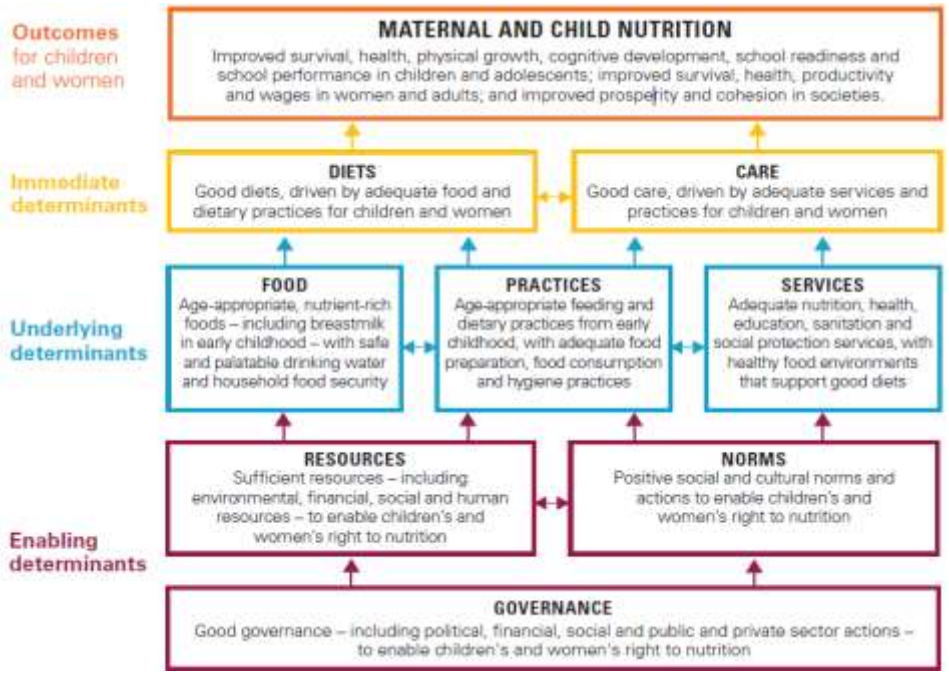


Figure 2.1: Conceptual framework on the determinants of maternal and child Nutrition (UNICEF, 2020).

The three underlying causes of malnutrition are insufficient household food security at home (restricted access of food), restricted access to health services and/or limited

environmental conditions and limited social and care environment in household and local level particularly with regard to women and children. The basic causes of community malnutrition lie at regional and national levels, where policies and strategies that affect resource allocation (human, economic, political, and cultural) affect what happens at community level. The geographical isolation and the lack of access to the markets due to the poor infrastructure have a significant negative impact on food security.

2.2 Malnutrition trends in Ethiopia

Even though, Ethiopia has shown improvements in reducing children mortality in the last decades, malnutrition among infants and young children continues to be common problem in the country (EDHS, 2016). Undernutrition in the country can be described as a long term year round phenomenon due to chronic food inadequacies combined with high levels of illness in under-five children (Yisak *et al*, 2015). According to the Ethiopian DHS 2019 data, 37% of under-five children are stunted, and 12% are severely stunted. Generally, the prevalence of stunting increases with age from 22% among children 6-8 months up to 45% of children 24-35 months, and it is higher in males than female children (40% versus 33%). Furthermore, stunting is higher in children of rural areas (41%) than urban areas (26%). Stunting varies among regions, which ranges from high (49%) in Tigray to low (14%) in Addis Ababa. Stunting, wasting and underweight decreases as the mother's educational level and wealth increases. High proportion of children (42%) born to uneducated mothers are stunted compared to those children born from mothers who have more than secondary education (17%). In general, 7% and 21% of Ethiopian children under-five are wasted and underweight respectively (EDHS, 2019). The highest percentages of wasted children are observed in Somali (21%), Afar (14%), and Gambela (13%), and the lowest in Addis Ababa (2%) and Harari (4%). While the highest number of underweight children are recorded in Somali and Afar regions (both 32%), while the lowest percentage is observed in Addis Ababa (5%) (EDHS, 2019).

Some of the most widespread nutritional problems in Ethiopia are micronutrient deficiencies such as iodine, vitamin A, iron and protein-energy malnutrition. The promise of the government of Ethiopia to alleviate childhood undernutrition is questionable. While the government has adopted a multisectoral approach to nutrition issues in the last years, there are no specific intervention programmes and nutrition policies. Even, the departmental responsibility is unclear (Rural Development or Ministry of Health).

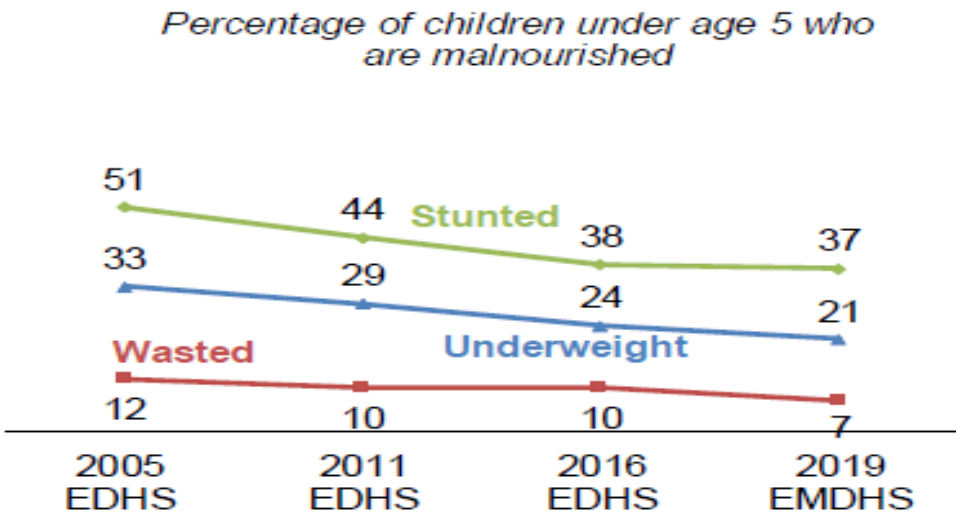


Figure 2.2: Trends in nutritional status of children (Source: EDHS, 2019)

2.4 Nutritional interventions to solve malnutrition

Children and infant malnutrition interventions are usually targeted at the age of 6 to 24 months. Because, this is a critical period whereby peak incidence of growth faltering, micronutrient deficiencies and infectious diseases occur in developing countries. After the age of two years, it is much more difficult to reverse the effects of malnutrition such as stunting, and other functional permanent deficits (Dewey and Adu-Afarwuah, 2008). Most of the time ignorance and food taboos are reasons for poor nutritional quality of infant foods in East Africa. The interventions generally include multiple approaches related to the Guiding Principles for Complementary Feeding of the Breastfed Child.

There are about ten guiding principles. (1) duration of exclusive breastfeeding and age of introduction of complementary foods; (2) maintenance of breastfeeding; (3) responsive feeding; (4) safe preparation and storage of complementary foods; (5) amount of complementary food needed; (6) food consistency; (7) meal frequency and energy density; (8) nutrient content of complementary foods; (9) use of vitamin-mineral supplements or fortified products for infant and mother; and (10) feeding during and after illness.

According to Dewey and Adu-Afarwuah (2008) interventions were clustered into five categories depending on the main strategy used: (1) education about complementary feeding as the main treatment (2) complementary food or a food product offering extra energy (with or without added micronutrients) provided as the only treatment (3) provision of food combined with some other strategy, usually education for mothers (4) fortification of complementary foods (centrally processed fortified foods or home-fortification products) with micronutrients (with no difference in energy provided to intervention vs. control groups), and (5) increased energy density and/or nutrient bioavailability of complementary foods through the use of simple technologies.

2.5 Complementary feeding

Scientifically, exclusive breastfeeding is recommended for the first 6 months of an infant's life because breast milk is uncontaminated and contains all the nutrients and several immune factors such as immunoglobulin, T lymphocytes, phagocytes, and enzymes like lysozymes, an infant needs to keep their optimal growth and health (Temesgen, 2013; Abeshu *et al*., 2016). Infants in their first six months of life are usually healthy due to nutritional adequacy and quality of breast milk (Moges, *et al*., 2016). The early introduction of solid food is discouraged for a number of reasons. First, it exposes infants to an increased risk of chronic diseases, particularly diarrheal disease. Second, it decreases infants' intake of breast milk and therefore suckling, which

reduces breast milk production. Third, supplementary foods are often nutritionally inferior in poor socioeconomic environment (EDHS, 2016).

Complementary feeding is defined as the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants and therefore other foods and liquids are needed, along with breast milk. Therefore, at this period the introduction of complementary diets are necessary since breast milk can't provide sufficient amounts of particular nutrients, including minerals, protein vitamins, and carbohydrates. Infants are particularly susceptible to malnutrition and infection during the complementary feeding period. It is the critical period for growth faltering, certain micronutrient deficiency and high occurrence of some childhood illness. The nutrient requirement for growth and development between the ages of 6 and 24 months is higher per kilogram of body weight than at any other time in life (WHO/UNICEF, 2002). Because, childhood is a period of rapid optimal growth as well as physiological, mental and immunological development when adequate nutrition needs are fulfilled. Essential nutrient substances such as protein, zinc, and plant origin essential fatty acids are vital requirements for their brain development for the first two years from birth (Akinola et al., 2014). If not addressed, the above nutrient deficiencies and infection can lead to death or long term irreversible consequences on future learning ability, economic productivity, immune response, and reproductive outcomes (WHO/UNICEF, 2002). Most tooth development and maturity in the first five years also require calcium, phosphorus, and important vitamins such as A, C, and D. Deficiency of these nutrients can therefore have negative consequences, some of which may be long-lasting or even death. Complementary feeding plays a vital role in filling these nutrient deficient gaps.

The ideal age range for introduction of complementary food is between 6 to 23 months with continuous breastfeeding, where most infants and young children reach a muscle and neurological development stage such as chewing, swallowing, digestion, and excretion which enables them to be fed other foods along with breast milk. Complementary diets could be specially designed transitional foods which are needed to meet the nutritional and physiological needs of infants or general family foods, which

are likely to tackle the gaps between the daily nutrient and energy needs of infants and toddlers (Abeshu *et al*., 2016). In most developing countries complementary diets are formulated from basic cereals and root crops, followed by fruits and vegetables (Muhimbula *et al*., 2011). While the option of a particular food item differs considerably among populations, due to ease of access, tradition, and availability (Kuyper *et al*., 2013). Although high-quality commercial foods are available, they are usually costly and therefore unaffordable by poor rural households. Various approaches are needed to provide families with the abilities to feed their babies with improved formulations using inexpensive and locally accessible staples (Muhimbula *et a*l., 2011). Based on the recommendations of WHO, complementary diets should have similar nutrient composition with the human milk and that they should be consumed two to three times per day for 6-8 months and three to four times per day for 9-11 months of infants (Friel *et al.*, 2010). The Global Strategy of WHO)/UNICEF for Infant and Young Children Feeding put emphasis on the utilization of appropriate locally accessible foods. Low-cost, widely available and locally appropriate complementary feeding recommendations that take into account cultural diversity are more likely to result in further improvement in complementary feeding methods than general recommendations (Fahmida *et al*., 2014).

The complementary foods developed from cereals and starchy root have been linked with the occurrence of protein-energy malnutrition among infants (Temesgen, 2013). This accounts for more than 25% death rate in infants of the developing world. It is also known that complementary foods produced from cereals are inadequate in certain essential amino acids which are required for the sufficient growth and healthy living of infant. Besides, cereal-based complementary diets have a high dietary bulk and high viscosity which affect the quantity of food a child could consume per meal; this regularly affects the quality of the nutrients available to the children. The local-based complementary foods that are expected to support growth and maintain good healthy should contain enough nutrients and must be of low viscosity. The problem of high dietary bulk and viscosity could be prevented using malting, soaking, germination and

other processes such as extrusion due to the redution of the high fiber component (Abiose *et al*., 2015).

2.6 Complementary foods in Ethiopia

Complementary foods can be generally developed using household technologies such as soaking, sprouting, germination, popping, fermentation, roasting and milling and modern food processing technologies such as roller drying and extrusion cooking. Depending on specific cultures, beliefs of food taboos, previous experience with feeding patterns, family's eating habits and nutritional knowledge different types of weaning foods are developed (Lutter & Rivera, 2003). Development of complementary food is guided by high nutritional value to supplement breast feeding, acceptability, low price and use of local food items. The recommendation for complementary feeding formulas for children aged 6 to 23 months in Ethiopian depend on simple and locally available foods that are not nutritious enough to fill the gap between calorie, protein, and micronutrient needs. The concept of improved feeding of infant and young children is not well understood by most families in Ethiopia. The point at which infants begin the actual grain based solid food varies significantly with the degree of urbanization, ethnicity and the socio economic status of the families (Suhasini and Malleshi, 2003). Generally, rural areas of infants starts very late from 8 to 12 months of age while urban infants begin at about 5 months (Melaku *et al*., 2005).

In general, local weaning foods in Ethiopia are mainly based on cereals and mostly an extension of family foods. It is made of cereal crops and/or starchy tubers such as sorghum, millet, maize, *teff*, oat, yam, rice and, potato, and barley (Abeshu *et al*., 2016). Mostly traditional weaning foods are prepared in the form of tin porridge or gruels from starchy foods, like wheat, maize, barley, tef, rice, oat, millet and sorghum (Lutter & Rivera, 2003). However, in some regions viscous porridges which are hard for children to consume are produced from carbohydrate roots or tubers (Ramakrishna *et al*., 2006). As a result, local mothers usually dilute the porridge with water to reduce its bulk. Such dilution, however, ultimately reduces the energy density of the food. Furthermore,

children consuming these foods grow poorly and have higher mortality rates. Proper combination and complementation of cereals and legumes to increase the nutrient density of complementary foods is the best strategy recommended for improving child nutrition.

2.7 Nutritional drawbacks of traditional complementary foods

In the developing world the use of low nutrient density weaning foods cause childhood acute malnutrition (Mahmoud and Anany, 2014). In poor resource settings, commercial weaning foods are too expensive for the average family, so mothers/caretakers often rely on plant-based traditional complementary diets that are low in nutritive value. These plant based diets have common features of low protein, high viscosity, low energy and micronutrient density in particular iron and zinc (Dewey, 2005). Due to its starch content cereal grains will get gelatinized and swollen while cooking, thus making the food bulky and viscous, so that it gives the stomach of the baby and young children huge work to do since their stomach capacity is not developed very well (Akinsola *et al*., 2017). Thus, traditional baby foods from these cereal crops often fail to meet the nutritional needs of the infants. They are therefore, known to poorly support growth and development (Frias *et al*., 2005).

The presence of anti-nutritional factors in cereal-based food is another problem which negatively affects the bioavailability of nutrients. The best documented being phytic acid which forms insoluble complex with Ca, Fe, Zn and possibly other metals, while oxalic acid forms oxalate precipitates with dietary calcium. For example the relatively poor availability of the fairly high Fe content of cereals is mainly due to their correspondingly high phytate composition (Ijarotimi and Keshinro, 2012). One of the common methods for predicting the bioavailability of minerals is the phytate: mineral mole ratio. Gibson *et al*. (2010) reported that 62% of native and 37% of processed complementary foods in low-income countries has a phytate: mineral mole ratio that exceeds the recommended values for mineral bioavailability. Furthermore, cereal-based diets are generally inadequate in a few essential amino acids particularly lysine and tryptophan, which is

necessary for the old infants and young children physical growth and development (Ijarotimi and Keshinro, 2012; Muhimbula *et al*. 2011).

The poor processing techniques and hygiene problems due to lack of knowledge of simple processing techniques to improve the nutritional quality are also other factors responsible for low nutrient density in local infant foods (Mariam, 2005). Encouraging the use of low cost and affordable plant protein sources for instance legumes in infant feeding is the best ways of improving the nutrient status (Mahmoud and Anany, 2014). Many researchers have revealed that the complementation of cereals and legumes with appropriate vegetables rather than a single diet will increase the efficiency of proteins for infant growth (Akinsola *et al.,* 2017; Ramakrishna *et al*., 2006). In addition, different processing technologies either singly or in combination have been proposed among other means to ensure higher nutrient density in complementary foods.

2.8 Formulation of high nutritive value local complementary food

The use of high nutrient local and readily available foodstuffs such as cereals, legumes and vegetables to prepare complementary foods for infant feeding has been advocated by a number of researchers (Mensa-Wilmot *et al*., 2003). In low income countries like Ethiopia, complementary foods could be improved by combining locally available carbohydrate sources with legumes that complement each other in such a way that the new patters of amino acids created by this combination are similar to that recommended for infants. Cereals are deficient in lysine but fair in sulphur containing amino acids that are limiting in legumes. Therefore, the combination of cereals and legumes has been found to produce amino acid patterns that adequately promote growth (Anigo *et al*, 2010; Mensa-Wilmot *et al*., 2003). However it is evident that trace minerals and vitamins are very low in cereals and legumes. This could therefore provide significant quantities of the nutrients if properly processed and blended with appropriate vegetables.

2.8.1 Utilization of legumes for complementary foods

Soybean (Glycine max) belongs to the family Leguminosae and is grown in many regions of the world. It is an important source of protein (40%), carbohydrates (32%), lipids (20%), minerals/ vitamins (5%), and fiber (3%) for human nutrition (Etiosa *et al*., 2017). Soybeans also contain biologically active or metabolic proteins, such as enzymes, trypsin inhibitors, hemagglutinins, and cysteine proteases. Soybean has many advantages over animal protein sources due to the fact that it contains low amount of saturated fatty acids and of course, cholesterol free. Soya bean is also of particular interest as a vegetable protein source because of its cholesterol lowering abilities in patients with type II hyper lipoproteinamia (Etiosa et al., 2017). It lowers the cholesterol level by improving the catabolism of Low Density Lipoprotein fraction (LDL), increasing billiard cholesterol excretion and gastrointestinal (GI) lipids absorption may be slow with soy protein. There is increasing evidence in the health promoting components of soybean as it reduces blood serum cholesterol, cancer, heart disease, osteoporosis, chronic renal disease, oxidative stress, and others (Ekor *et al*., 2010). Soy protein is a complete protein, and thus contains all the protein related components to sustain normal growth for 2-5 year old child. Elimination of oligosaccharides, anti-nutrients, beany flavor, and the reduction of the viscosity is the major challenge in using soybean flour as an infant food. Soaking and roasting treatments have been applied to overcome this problem.

Faba beans (*Vicia faba* L.) is one of the most ancient crops cultivated in many areas of the world for its edible seeds, which are used green or dried, fresh or canned (Jensen *et al*., 2010). This pulse contains a significant amount of proteins and also an important source of fiber, starch, choline, lecithin, and vitamins (Abu-Reidah *et al*., 2014). It is also a good source of minerals like iron, calcium, phosphorus, potassium and sulfur. The bean is fairly high in the content of protein, and has a balanced pattern of essential amino acids with the exception of methionine level. Nevertheless, the presence of anti-nutrients such as hemagglutinins, trypsin inhibitors and tannins in the seed negatively affects the biological value of the legume (Vidal-Valverde *et al*., 1998). Inexpensive and

adaptable technologies such as soaking, germination, fermentation and cooking modify the nutritional quality and bioavailability of minerals by eliminating anti-nutrients. The above traditional treatments, have been also used to improve the nutritional quality of the legume (Kayodé, 2006).

Chickpea (*Cicer arietinum L.*) is classified under the Fabaceae family and is one of the ancient and most widely consumed legumes in the world as a staple food crop across the tropical and subtropical regions. Ethiopia is ranked eight worldwide in 2005 in chickpea production which serves as a multi-purpose crop (Shiferaw *et al*., 2007). Among the many purposes chickpea can be used to improve the nutritional status and human health due to its medicinal purpose and as a major source of protein, fiber, complex carbohydrates, vitamins, and minerals especially for those who cannot afford livestock products (Menale *et al*., 2009). In Ethiopia, the use of chickpea grains for human food has long history and used in different forms as green vegetable, 'Kollo, 'nifro' and 'wot' (sauces) made up of 'shiro' or blended with cereals and/or legumes for preparing of infant foods using local processing approaches. Bioavailability of macro and micro-nutrients are critical in infant food preparation beside cost for purchasing, sensory acceptability and processing approaches (Yewelesew *et al*., 2006). Chickpea contains a high content of phytate and tannin which will precipitates minerals like Fe, Zn and protein, thereby decreasing the bioavailability and digestibility unless appropriate and affordable processing techniques are implemented.

Peas (*Pisum sativum L.*) are another legume with great nutritional significance due to their high content of protein, dietary fiber, minerals, vitamins, and antioxidant compounds. The interest of pea for human consumption is lower than that of other traditionally more accepted pulses (Schneider, 2002). However, in recent years, the wealth of nutrients available from the pea and its beneficial functional properties have prompted increasing interest and demand for this legume for the food preparation oriented to geriatric and infant nutrition (Davidsson, 2001).

2.8.2 Utilization of cereals for Complementary foods

Teff (*Eragrostisis teff*) is one of the major indigenous crops in Ethiopia, where it is believed to have originated and has the largest share of area under cereal crop production. Over two thirds of the human nutrition in the country is provided by this crop. It is considered to have high in nutritional quality; however there is little information about its usefulness in infant formulations (Zhu, 2018). The crop is supposed to be an excellent source of minerals such as iron, high amount of calcium, potassium and other essential minerals than in other cereal grains. Although the reported high iron content of teff seed has been disproved by some works, the lack of anemia in Ethiopia is considered due to the available iron from Injera (Zhu, 2018). Teff flour blending in all complementary food will enrich the products with iron and other essential minerals.

Maize (*Zea mays L.*) is a cereal grain that is cultivated in many parts the world in different agro ecological environment. Among the cereals, maize has become one of the Africa's most important staple foods, since it was introduced into the continent by the Portuguese in the 16th century. It is consumed by both adults and infants in large amount and is the major sources of both macro and micronutrients. The grain is used in various forms such as whole corn, corn flour, cornstarch, corn gluten, corn syrup, tortillas, tortilla chips, polenta cornmeal, corn oil, popcorn, cornflakes, etc. It is prepared as gruel and used in feeding infants (Oluwalana, 2014). From nutrition point of view, maize is comparatively poor in the quality of its protein, because it has limiting amounts of two essential amino acids, lysine and tryptophan (Azevedo et al., 1997). However the grain is rich in vitamins A, C, and E, carbohydrates, essential minerals, dietary fiber, and contains 9% protein (Oluwalana, 2014). It has been reported that maize seeds have moisture (11.6-20.0%), ash (1.10-2.95%), protein (4.50-9.87%), fat (2.17-4.43%), fiber (2.10-26.70%) and carbohydrates contents (44.60-69.60%) (Enyisi *et al*., 2014). The regular consumption of maize helps to remove the toxic substances in food, speeds up the passage of faces through the intestine, provides protection to the digestive tract by

reducing the stomach acidity and improves the function of the gall-bladder (Elsgaard *et al*., 2012).

Wheat (*Triticum aestivum L*.) is one of the major globally produced and marketed crops which covers 15% of the total sowing regions of cereal grains in the world. It is the second most important cereal grains in the world next to rice that traded internationally (Falola *et al*., 2017). Wheat is also a strategic crop commodity in sub-Saharan Africa which generates farm income and improves food security status (Amentae *et al*., 2017). It is produced for both consumption and sale in many African countries; however the level of production and income generation is varied among countries. Ethiopia is one of the largest wheat producers in terms of total wheat area cultivated and total production (CSA, 2014). Wheat and wheat products represent 14% of the total calorie intake in the country which makes wheat the second-most important food behind maize (19%) and ahead of teff (10%), sorghum (11%) and enset (12%) (FAO, 2014). Wheat flour is used as infant supplements in Ethiopia in many regions, since infant foods are mostly an extension of family foods (Temesgen, 2013).

Barely (Hordeum vulgare)

It is one of the staple food crops for many Ethiopian highlands who treats the cereal with traditional approaches and utilize different parts of the plants for various purposes. Barley is ranked as the fifth crop after maize, tef, sorghum and wheat (Temesgen, 2013). Now a days, consumption of barley is increasing widely as foods and snacks. This is due to recent research advocates about their desirable nutritional contents like fibers which contains beta–glucans and tocotrineols chemicals which lowers cholesterol level (Lee *et al*., 2007). The crop is used as whole dehulled grain, semolina and flour. Various dishes and recipes such as bread, couscous and soups, Besso (fine flour of well-roasted barley grain moistened with water, butter or oil), Chiko (besso soaked with butter and spice), Genfo (thick porridge) and Kolo (de-hulled and roasted barley grain) are made from the grain. Traditional weaning gruels are also prepared from the barely flours. The grain is mainly utilized as carbohydrates sources. Barley contains 19 amino acids and is low in

lysine and methionine. This might be a reason that most traditional barley recipes are prepared along with legumes or animal protein sources to supplement the deficient amino acids (Temesgen, 2013).

Oats *(Avena sativa)*

Oats are generally regarded as healthy and commercially nutritious food. The discovery of the cholesterol-lowering properties of oats has led to wider appreciation for human consumption. The consumption of oat bran is also believed to lower the bad cholesterol, and possibly to reduce the risk of heart disease (Youssef *et al*., 2016). Oats have a higher energy density due to the high content of oil. It has also relatively a high amino acid balance and significant amounts of dietary fiber which is high in soluble fiber composed of β-glucan. Oats have a significant amounts of essential minerals like Mg, P, Fe, Cu and Zn and vitamins like thiamin, vitamin E, folate, niacin (Ahmad and Zaffar, 2014). Thus, blending oat in infant formulation is good sources of essential nutrients due to its low allergy, good nutritional profile, low levels of free fatty acids, flavor compatibility, cost and absence of hull in the flour (Svanberg, 1988).

Sesame seed (*Sesamum indicum L.*): is probably the most ancient oilseed crop speculated that it was native to tropical Africa. Furthermore, the significance of sesame in the economies of several African countries justifies the African continent to be the center of its origin. In this study sesame is supposed to provide essential fatty acids to the infant (Makinde and Akinosdo, 2014).

2.9 Effect of antinutriets on mineral bioavailability

Legumes and cereals are high in macronutrients and micronutrients, as well as anti-nutritional factors. Tannins, phytic acid, oxalates, Saponins, lectins, protease inhibitors, amylase inhibitors, and goitrogens are major anti-nutritional factors found in edible crops. Anti-nutritional factors combine with nutrients and become the primary source of concern due to decreased nutrient bioavailability. Other factors, such as trypsin

inhibitors and phytates, found primarily in legumes and cereals, reduce protein digestibility and mineral absorption. Anti-nutrients are one of the main factors that reduce the bioavailability of various cereal and legume components. Micronutrient malnutrition and mineral deficiencies can result from these factors. There are a number of traditional methods and technologies that can be used to reduce the levels of these anti-nutrients (Samtiya *et al*., 2020).

A variety of antinutritional factors in food and feed products can impair protein digestibility and amino acid availability. Antinutritional factors like glucosinolates in mustard and rapeseed protein products, trypsin inhibitors and hemagglutinins in legumes, tannins in legumes and cereals, phytates in cereals and oilseeds, and gossypol in cottonseed protein products can occur naturally. Similarly, high tannin levels in cereals such as sorghum and grain legumes such as fababean (Vicia faba L.) can result in significantly reduced protein and amino acid digestibilities (up to 23%) in rats, poultry, and pigs. Phytase supplementation of swine or poultry production rations has provided indirect evidence that normally encountered levels of phytates in cereals and legumes can reduce protein and amino acid digestibilities by up to 10% (Gilani *et al*., 2005).

2.9.1 Tannins

Tannins are polyphenolic compounds that are abundant in plants. They are either condensed or hydrolyzable tannins. Condensed tannins are mostly found in legume forage and some seeds. The majority of tannins found in legumes are condensed tannins, which are formed through polymerization of flavan-3-ols or flavan-3, 4-diols. Tannins are secondary compounds produced by plants in their leaves, fruits, and bark (Timotheo and Lauer 2018). The amount of tannins in a plant is determined by the variety, maturity level, and extraction method. One of these compounds' properties is the ability to precipitate proteins. Tannins typically reduce protein digestibility and essential amino acid levels by forming reversible and irreversible tannin-protein complexes between the hydroxyl group of tannins and the carbonyl group of proteins (Raes *et al*. 2014).

2.9.2 Phytic acid/phytates

Phytate is an abbreviation for phytic acid (myo-inositol hexaphosphate), which is composed of an inositol ring with six phosphate ester groups, and its salts: magnesium, calcium, or potassium phytate. It is worth noting that myo-inositol phosphates with fewer than five phosphate groups (i.e., IP-1 to IP-4) have no negative effect on mineral absorption Sandberg *et al*.(1999), whereas myo-inositol phosphates with fewer than three phosphate groups do not inhibit iron absorption (Reddy, 2002). Phytate is the primary phosphorus storage form in cereals, legumes, and oleaginous seeds. The phytate content of cereals ranges from 0.06 percent to 2.22 percent, with polished rice having the lowest (Reddy, 2002). In most cereals, phytate is concentrated in the bran (aleurone layer), but in maize, it is concentrated in the germ.

The phytate content of whole legumes ranges from 0.17 percent to 9.15 percent and is uniformly distributed throughout the cotyledons, where it is associated with protein. As a result, removing the hull or seed coat of legumes increases their phytate concentration. Because of the lack of intestinal phytase enzymes, phytic acid chelates metal ions, particularly zinc, iron, and calcium, but not copper Egli *et al*. (2004), forming insoluble complexes in the gastrointestinal tract that cannot be digested or absorbed in humans. Endogenously secreted minerals such as zinc and calcium Egli *et al*. (2004), are also complexed by phytate, rendering them unavailable for reabsorption into the body.

2.9.3 Oxalates

Oxalic acid and its salts are metabolic byproducts found in a variety of plant tissues. Because oxalates bind calcium and other minerals, eating these plants may have negative consequences. While oxalic acid is a normal byproduct of mammalian metabolism, excessive oxalic acid consumption may result in stone formation in the urinary tract when the acid is excreted in the urine. Soaking and cooking oxalate-rich foods will reduce the oxalate content by leaching. Vegetarians who eat more vegetables will have a

higher oxalate intake, which may reduce calcium availability. This may be a greater risk factor for women, who require more calcium in their diet. Diets low in calcium and high in oxalates are not recommended in humans, but the occasional consumption of high oxalate foods as part of a nutritious diet poses no particular risk (Noonan, 2002).

2.10 Indigenous practices/methods to enhance the quality of complementary foods

Indigenous processing methods have been shown to have significant effect on the viscosity, dietary bulkiness, and nutrient density of most traditional weaning foods. Starch is the major component of cereal-based complementary foods. It is also considered to be the major water-binding component in these foods and to a large extent determine the dietary bulk properties. Traditional processing technologies which includes soaking, sprouting, germination, dehulling, roasting and milling plays an important role since it influences the bioavailability, utilization of nutrients and also improves palatability that may results in enhancing the digestibility and nutritive value (Sandberg & Andlid, 2002).

2.10.1. Fermentation

Fermentation is an old economical processing method, which was practiced in Ethiopia and elsewhere. In this process food components are subjected to the action of enzymes or micro-organisms so that desirable biochemical changes cause significant modification of food. This is important in plant foods to increase the nutritional quality and remove undesirable compounds. Fermentation involving lactic acid bacteria offers potential for widespread applications, particularly with respect to the preservation of cereals, legumes and root crops and the provision of safe, low-cost weaning foods for developing countries (Wanjala *et al*., 2016). Fermentation of cereal-legume blend is potentially important processing method that can be expected to improve nutritional value of complementary foods by reducing water binding capacity of cereal flour. This allows the porridge to have a free-flowing consistency even with a high proportion of flour

(Wanjala *et al*., 2016). The poor starch and protein digestibility of cereals is caused by phytic acid and polyphenols that bind to enzymes in the digestive tract and thus inhibit utilization of proteins and carbohydrates. The fermentation process increases protein composition and boost protein quality of the cereal-based food by improving its palatability and digestibility. It also offers better essential amino acid profile. In addition to this, it also lowers the tannins content; increases the vitamin content and appetizing flavors are developed. During fermentation process, proteins are broken down to amino acids, starches are converted into simple sugars, riboflavin and niacin contents increase (Ameny & Hegsted, 2006). In addition, vitamin B is synthesized and some amount of available iron is increased because some anti-nutrients such as phytate which chelate vitamins and minerals are removed.

2.10.2 Roasting

Roasting is one of the simple and easily adaptable technologies to reduce the bulkiness, improve the flavor, color, texture, increase shelf life and overall acceptability of the product (Gernah *et al*., 2011). It also significantly reduces the moisture content of most cereals. The roasting process should be uniform and properly controlled because it does not only contribute to development of flavor and aroma but also to the color of the product. Color is an important quality indicator of the roasting process. Development of roasted flavor and aroma depends upon the temperature and time of roasting beside the type of grains and techniques applied (Temesgen, 2013). Traditionally roasting of cereals and legumes is used primarily for reduction of anti-nutritional factors.

2.10.3 Soaking

Soaking has been an indigenous technique practiced in Ethiopia for cereal and legumes before cooking. The practice makes the grains soft and readies them to be cooked faster. Soaking cereals and legumes in water can result in passive diffusion of water-soluble phytates, which can then be removed by decanting the water (Hortz and Gibson, 2007).

The extent of the phytate reduction depends on the species, pH, and length and conditions of soaking (Hortz and Gibson, 2007). Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking. Soaking under optimal conditions (about 12 hours at 30^{o}C) also activates naturally occurring phytases in cereals and results in varying degrees of phytate hydrolysis, depending on the kind of cereals (Lindsay and Ahluwalia, 1997).

In general soaking, reduces phytic acids, tannins and polyphenols, improves the body's ability to absorb minerals such as iron, zinc and calcium, makes proteins more available for absorption and decreases cooking time and improves food texture (Gupta *et al*., 2015).

2.10.4 Dehulling

Dehulling is a physical treatment which remove the seed coat (hull) that contain undesirable substances like tannins and high-lignin fibers present in the seed coat. Therefore, removal of the hulls would reduce off-flavors. The removal of the hull involves soaking of the whole beans in water for some times and removing the hulls manually and drying the cotyledons (Wang, 2016).

2.10.5 Germination

Germination is a natural physiological process resulted by the action of natural enzymes. It was an indigenous technique practiced by the local people to improve the taste of germinated cereal-based beverages. Now it is recognized that, the practice also improves the nutritional value of many foods. Germination is a cost-effective and sustainable process for the development of infant foods with reduced viscosity, and high energy and nutrient density (Temesegen, 2013). It is also the processing method of legume seeds to increase nutritive value and health promoting qualities. These include legumes like soybean, beans and, cereals such as wheat, barley and oats (Gahlawat & Sehgal, 1993). The practice increases the endogenous phytase activity in plant seeds through de novo

synthesis and intrinsic phytase activation. Tropical cereals like wheat, corn, and barley have less endogenous phytase activity. Therefore, germination has a reducing effect of phytic acid on these grains Gupta *et al*., 2015).

2.10.6 Combined methods

There is little information on the use of combined processing methods in the production of food products, particularly food supplements. Combined processes would not only produce food products adequate in nutritive value, but would be well accepted and tolerated by the targeted groups (Hotz and Gibson, 2007). It will also increase food security, diversification, and alleviate protein-energy-malnutrition thereby, reducing infant morbidity and mortality. Therefore, the above discussions reveal that an integrated approach that combines multiple strategies is likely to be superior. The applicability of this combined strategy is more appropriate in plant-based diets in resource-limited settings. Application of such multiple strategies can almost completely remove phytic acid. This is important because phytate is a potent inhibitor of iron absorption, even at low concentrations (Hurrell, 2004). Despite the advocacy of household-level food processing and other food-based strategies to improve nutritional adequacy, there has been little effort to assess their impact in well-designed trials (Hotz and Gibson, 2007). Further studies of the efficacy of these strategies to determine their impact on nutritional status is needed thus further justifying the current study.

2.11 Nutrient requirement of complimentary foods

Complementary diets are expected to fill the gaps in nutrients and energy daily requirements for young children and infants (Abeshu *et al*. 2016). Consequently, the foods should be highly energy dense, good protein composition with all essential amino acids, minerals (iron, folic acid, and calcium), required vitamins, and no (safe level) ant-nutritional components, and with good palatability qualities (UNICEF, 2013).

2.11.1 Energy requirement

Complementary diets are expected to provide adequate energy for the growing child to meet their daily energy requirement. The number of kilocalories of energy in a given diet per milliliter per gram of this diet is called energy density. Human milk is assumed to have an energy density of about 0.7 kcal/ml (WHO, 2002). The recommended minimum energy density in infant formula diet is 0.8 kcal/g higher than in breast milk. In reality, the energy density in infant formulas usually is between 0.6 and 1.0 kcal/g and may even fall to 0.3 kcal/g in watery and dilute foods. Thus, the amount of supplemental foods needed to bridge the energy gap is the energy density in the diets offered (WHO, 2009).

Energy dense foods are critical for children with waste since they have an increased energy need for catch-up growth. Low energy density diets have been associated with protein-energy malnutrition (Abeshu *et al*. 2016).

The estimated total energy requirement for healthy breastfed infants is approximately 615 kcal/day at 6 to 8 months, 686 kcal/ day at 9 to11 months, and 894 kcal/day at 12 to 23 months (Dewey, 2001). For babies in developing countries with "average" breast milk intake, the energy requirement of supplemental food increases from 200 kcal / day after 6 to 8 months to 300 kcal / day after 9 to 11 or 12 to 23 months. This corresponds to 29, 55, and 71% of the total daily energy requirement, which coinciding with the reduce intake of breast milk in old age. These values may vary with the level of daily breast milk intake (Dewey, 2001; WHO, 2001). The amount of food required per day to meet their energy needs is a function of the amount of energy needed from supplemental foods and the energy density of the food (i.e., kilocalories per gram). For supplemental foods with an energy density of 0.6–1 kcal/g, the amount (gram or volume) of food needed to provide the energy requirement is between 200 to 333 g/ day for 6 to 8 month-old, 300–500 g/day for 9 to 11 month-old, and 550–917 g/day for 12 to 23-month-old children. Energy-dense foods have an energy density of 1.07–1.46 kcal/g. For such

foods, the approximate amount of supplemental food that would meet the energy requirements described above is 137-187 g / day (Dewey, 2001; WHO, 2001).

2.11.2 Protein requirement

Protein is an important nutrient constituent in infant complementary diets. Although our body preferentially utilizes carbohydrate and fats, they are the major sources of indispensible amino acids and energy during energy deprivation. An appropirate amount of dietary protein intake is essential for infants to ensure their normal health and growth as well as maintaining cellular function and integrity. Low protein intake is usually associated with a lack of energy and leads to protein-energy malnutrition, the most prevalent form of malnutrition in the world. Cereal grains, legumes and oilseed meal alone or preferably mixed may constitute good sources of protein.

The protein requirement of infants and toddlers increases with age. The daily requirement of protein (in grams per day) to satisfy their nutritional need is 9.1 g for 6–8 months, 9.6 g for 9–11 months, and 10.9 g for 12–23 months. Breast milk supplies a significant part of the daily protein needs of infants and young children. The amount of protein needed from complementary foods is 1.9 g/day at 6–8 months (21%), 4.0 g/day at 9–11 months (42%), and 6.2 g/day (57%) at 12–23 months, when average breast-milk intake is assumed (Dewey, 2001; WHO, 2001).

2.11.3 Fats/lipids requirement

Dietary fats comprise an essential part of nutrients obtained from foods we consume. They are an essential source of energy, fat-soluble vitamins (A, D, E, and K) and essential fatty acids for growing infants and young children. Additionally, dietary fats have a significant role in promoting good health and enhancing the sensory qualities of the foods (Abeshu *et al.*, 2016). In the first 6 months of baby life, fat accounts for about 50% of breast milk energy and serves as a primary source of energy. With the beginning

of infant food, however, fat is step by step overtaken by carbohydrate as the primary energy source (Abeshu *et al*., 2016).

If the proportion of energy from fat is assumed at least 30%, the quantity of fat required from weaning diets to satisfy daily needs depends on breast milk intake level. Complementary diets should give dietary fats appropriating to 34, 38, and 42% of daily energy requirements for 6–8, 9–11, and 12–23 months, respectively, for those infants with low breast milk intake. With sufficient intake of breast milk, however, the necessity from complementary foods is 0 g/day (0%) for 6–8 months, 3 g/day (5–8%) for 9–11 months, and 9–13 g/day (15–20%) for 12–23 months (WHO, 2001)

2.12 Micronutrients of public health importance

Micronutrients are minerals and vitamins that are required by the body in small quantities. They form components of enzymes or cofactors needed for metabolic reactions in the body (Devlin, 1997). They are essential for infant growth, development, and prevention of illness (WHO, 2009). Sufficient intakes of these nutrients, for example, zinc, calcium, and iron are vital for ensuring growth, optimal health, and development of infants and young children. In well-nourished mothers, breast milk is having generous amounts of folate, iodine, vitamin A, B, C, and selenium. Consequently, the quantity needed from complementary diets before 12 months is zero (Dewey & Brown, 2003). However, other several micronutrients in breast milk are comparatively low. The total daily requirement of micronutrients needed from complementary foods in percentage ranges from 30 to 97%. For example 86% of zinc, 97% of iron, 76% of magnesium, 81% of phosphorus, 73% of sodium, and 72% of calcium during 9–11 months are expected from infant complementary foods (Dewey & Brown, 2003). The World Health Organization (WHO) and UNICEF have identified deficiencies of iron, vitamin A, iodine and zinc as the four-micronutrient disorders of greatest importance. These deficiencies are linked with the high prevalence of childhood diseases and mortality. The deficiencies are also supposed to have negative impact on general growth and development, reproduction, maturity and immunity responses

2.12.1 Vitamin A

Vitamins are organic substances synthesized in the body of the animal or found in plants in nature, which are required in minute quantities for normal cellular metabolism. Vitamins are generally grouped in to water soluble (B-vitamins and vitamin C) and fat soluble vitamins such as vitamins A, D, E and K. Now days, vitamin A has received a wide attention as a result of its high deficiency prevalence particularly in infants and young children. Vitamin A occurs in various forms as aldehyde (retinal), alcohol (retinol), esters (retinyl acetate or palmitate) and vitamin A carotenoids (β-carotene, α-carotene). Vitamin A is relatively unstable, mostly due to many double bonds that are subjected to degradation. Plants contain vitamin A as carotenoids (provitamin form), which animals transform into vitamin A after ingestion in the diet (Wirakartakusumah and Hariyadi, 1998).

Dietary sources of vitamin A are dairy products, meat, egg, liver, fish, green leafy vegetables and pigmented fruits (papaya) and vegetables (carrots). However, the low income of poor countries and families has limited this important animal dietary source of the vitamin A particularly in children. Hence, efforts to ensure adequate vitamin A intake for the primary prevention of corresponding disorders still continue to receiving a significant attention in many developing countries. The recommendations in these countries are focused on the consumption of fortified/supplemented diets that deliver adequate vitamin A to promote growth, prevent night blindness and strengthen corneal structure and immune function in children (Wirakartakusumah and Hariyadi, 1998).

Vitamin A contributes significantly to immune functioning by differentiation of epithelial cells and the normal visualization of our eyes. It also plays a role in ensuring the normal and optimal functioning of the epithelial surfaces and its deficiency impairs mucosal function in the gastrointestinal, genitourinary and respiratory tracts. Its deficiency results in loss of cilia from the respiratory mucosal lining, loss of microvilli in the gut mucosa and loss of mucin and goblet cells in the mucosal lining of all these

organs. Generally, Vitamin A deficiency can lead to growth retardation, susceptibility to infection, and night blindness (Abrha *et al*., 2016). In Ethiopia the prevalence of vitamin A disorder is extremely high, estimated to 27 percent. Despite the availability of foods rich in vitamin A such as spinach, pumpkin, mangos, carrots, eggs, and organ meats, the percentage of Ethiopians who consume these foods is relatively low, especially in rural areas (EDHS, 2005).

2.12.2 Iron

Iron is vital element present in our food which has several physiological functions, mainly the transport of oxygen from the lungs to the cells and the synthesis of haemoglobin. Many enzymes that are involved in maintaining the integrity of the cells such as peroxidases, catalases and oxygenases contains iron in nature (Barragán-Iba˜nez *et al*., 2015). Iron deficiency states are the most common micronutrient malnutrition in the world, as it affects greater than 3.5 billion people worldwide, of which almost 2 billion are women and children (UNICEF, 1998). Though iron deficiency anaemia (IDA) is a public health problem in both the developed and developing countries, the prevalence is higher in the tropics. However, women, the elderly, vegetarians, infants, young children and migrant groups of the developed countries are also at risk of iron deficiency. Iron deficiency can occur in all life stages, and is more prevalent in high-risk individuals, such as pregnant women and children between the ages of 2 and 12. Around 50% of all cases of anaemia worldwide are thought to be caused by iron deficiency (Barragán-Iba˜nez *et al.*, 2015). Women with IDA are vulnerable to low birth weight infants and pre-mature birth. This is why iron is required during pregnancy to increase RBC production, which compensates for the relatively hypoxic (low oxygen) intrauterine environment, to supplies oxygen to the fetus for development. Sufficient iron transport across the placenta ensures the birth of a full-term, normal weight infant. Iron is also necessary for postnatal growth, as it increases red cell volume and builds lean body mass (Chaparro, 2008).

The main cause of IDA is an imbalance between tissue iron requirements and body iron stores. The leading causes in order of prevalence are: (1) inadequate iron intake particularly in the paediatric population; (2) abnormal iron loss (chronic bleeding), especially in adolescent and adult women with vaginal bleeding such as dysfunctional uterine bleeding or bleeding secondary to uterine myomatosis (Haidar, 2010). In adults of both sexes, the causes are chronic diseases involving gastrointestinal bleeding, such as erosive gastritis, bowel polyps, intestinal diverticulosis or colon cancer. The dietary sources of iron include fish, eggs, liver, meat, leafy vegetables, fruits, cereals and legumes. Milk is also a poor source of iron. The prevalence of iron deficiency anemia in Ethiopia is 53.5% in children of 6–59 months and 26.6% in women (Haidar, 2010) . However, the prevalence of IDA in Ethiopia is not as high as in some other Sub-Saharan African countries, possibly due to Ethiopians' consumption of teff, a local grain rich in iron (Haidar, 2010). Iron deficiency anemia leads to lower productivity and decreased ability to concentrate, both of which have costly economic consequences.

2.11.3 Zinc

The deficiencies of only three nutrients namely iron; iodine and vitamin A are generally supposed to be of public health importance and targeted for prevention in development programmes. However, the role of zinc in human nutrition was recognized only more recently (Berhe *et al*., 2019). Zinc is the second most abundant metal next to iron and is engaged in a wide range of physiological functions. It is an important component of many enzymes that supports metabolic process such as immune system, brain development, cell division, wound healing, DNA and RNA synthesis, sexual maturation, normal growth and development during pregnancy among others (Bahijri, 2001). Due to its role in the biochemical processes of growth and developmental, zinc is considered as one of the most essential mineral elements in foetal, infant and early childhood development. For instance, the essentiality of zinc in normal brain development suggests a clinical function for the element during the prenatal period (Prasad, 1982).

Zinc plays an important role as antioxidant in the body as well as the synthesis and action of insulin hormone. It regulates the actions of many genes; some of these genes are involved in fatty acid synthesis and signal transduction. The body does not store zinc and hence the diet is an important source to meet the body's zinc requirements. It is also an essential component the enzyme that converts provitamin A into retinol, hence its deficiency interferes with vitamin A metabolism (Ross, 1999). This seems to support the notion that any vitamin A supplementation will require an increase of zinc.

Many animal and plant origin foods are the source of zinc. However, the bioavailability is highly dependent on the type of the foods. Animal and marine sources of foods are readily available compared to plants origin foods. Beef, lamb meats, fish, pork, poultry and cheese are good sources of zinc. Red meat, liver, shellfish, and oysters are one of the richest sources of zinc. While wholegrain cereals, nuts and legumes are important sources of zinc in the diet, roots and tubers and fruits and vegetables are poor sources of dietary zinc.

Zinc deficiency is a global problem which affects 17% of the world's population with the highest risk occurring in Sub Saharan Africa and South Asia (Wessells and Brown, 2012). In developing countries, children and pregnant women are the most vulnerable groups of zinc deficiency. In infants and children the deficiency is always associated with increased risk of infections. Low plasma zinc level increases the risk of diarrhoeal disease and respiratory illness in children, and several studies have demonstrated the benefits of zinc supplementation in children. Zinc supplementation is known to reduce the risk of infection as well as the duration and severity of illness during an acute infectious episode. Zinc supplements elicit beneficial responses even during an acute episode of illness; for instance, they decrease the duration of the common cold.

2.12.4 Iodine

Iodine is one of the essential micronutrient that is required for the production of thyroid hormone. Thyroid hormone is required to support various physiological functions for normal growth. It is particularly important for cognitive and neurological development during fetal life, infancy and childhood (Skeaff, 2011). Iodine deficiency is a wordwide public health problem which affects Europe, Asia, east Africa and south and Central America (Gizak *et al*., 2017). Pregnant women and school children are extremely affected group of iodine deficiency. Based on the WHO estimates almost 37% of school age children are at risk of insufficient iodine intake at global level (Skeaff, 2011).

Although its disorders can be prevented by the use of iodized salt a simple and cost-effective intervention, iodine deficiency disorders affect large segment of the population in Ethiopia. The prevalence varies in different regions in the country, and in some areas the goiter rate reaches 71% (Cherinet, and Kelbessa, 2010).

2.13 Biochemical and haematological indices

Exposure to hazardous or toxic substances can have a variety of effects on the body. When chemicals and other hazardous substances are absorbed, they travel through the various body systems and can affect specific organs, known as the "target organ." Fortunately, the body has mechanisms to process and eliminate many of these substances, primarily in the liver and kidneys. This ability to eliminate toxic substances has the potential to lessen the effect(s) on the target organ. Toxic substance elimination is just one of the many functions of the liver and kidneys. The kidneys, for example, maintain blood volume and regulate mineral content in the bloodstream. The liver is responsible for converting nutrients into energy, forming proteins, and storing carbohydrates. Kidney and liver damage can be acute or chronic. An acute process is defined as a relatively short period of time (hours to weeks) between toxin exposure and the onset of symptoms or medical findings. A chronic process is defined as a long period of time (years) between toxin exposure and the onset of symptoms or medical findings.

Depending on the type of toxin and the extent of exposure, detecting an acute or chronic process or disease can be difficult.

2.13.1 Liver function tests

Rather than testing for the toxic substance itself, medical screening for chronic liver and kidney disease usually entails a test that measures how well these organs function. While there are a number of reasons for this approach, the main one is that many substances that cause chronic liver and kidney disease are difficult to detect in the body. A variety of tests are available to detect abnormalities. Blood tests, also known as liver and kidney function tests, are among the most commonly used. Six liver function tests and two kidney function tests are performed in a typical blood chemistry profile (Oluwajuyitan and Ijarotimi, 2019).

With the exception of bilirubin, all of the liver function tests are measurements of enzyme levels. These enzymes are normally found in liver cells, and there is a "normal" level of these enzymes in the bloodstream. When the liver becomes inflamed or damaged, the damaged cells release abnormal amounts of these enzymes into the bloodstream. As a result, levels in the bloodstream are elevated (Hasan *et al*., 2018). Another substance commonly measured in the blood to detect liver disease is bilirubin, which is produced by the breakdown of red blood cells. Once again, a normal bilirubin level is maintained because the liver constantly removes bilirubin from the body. However, if the liver is damaged, bilirubin is not removed, and the level in the bloodstream rises. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamic transpeptidase (GGT), Lactic dehydrogenase (LD or LDH), Alkaline phosphatase (ALP), and Bilirubin are the kidney function tests (Hasan *et al*., 2018).

2.13.2 Kidney function tests

The kidney function tests do not look for enzymes, but rather the breakdown products of normal body processes. Protein breakdown results in the production of blood urea

nitrogen (BUN). BUN is produced in the liver and transported through the bloodstream to the kidneys for elimination. Creatinine is a byproduct of the breakdown of a specific type of muscle protein. It is produced in specific muscles and then transported through the bloodstream to be eliminated by the kidneys. The BUN and creatinine blood levels will rise if the kidneys become impaired and are unable to eliminate the normal amount of these substances (RusulArif and Haider, 2014).

2.13.3 Serum lipid profile

Because of their clinical importance, cholesterol and triglycerides are commonly measured in human and animal blood/serum. Cholesterol is a necessary component of mammalian cell membranes that plays important roles in membrane permeability and fluidity, as well as a precursor of bile acids, steroid hormones, and fat soluble vitamins (NCEP 2002). Triglycerides are esters (derived from glycerol and three fatty acids) that play an important role in metabolism as energy sources and dietary fat transporters; they are a major component of VLDL and chylomicrons.

Cholesterol is transported in the bloodstream in association with lipoproteins, which are named based on their density; thus, in addition to total cholesterol (TC), the following are routinely measured in serum assays: High density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), which in clinical practice includes intermediate density lipoprotein cholesterol, and very low density lipoprotein cholesterol (Ihedioha *et al*., 2013).

2.13.4 Haematological profiles

Haemoglobin is an iron-containing oxygen transport metallo-protein found in all vertebrate red blood cells, and it has the physiological function of transporting oxygen to tissues of the animals for oxidation of ingested foods, releasing energy for other body functions, as well as transporting carbon dioxide out of the animal's body (Ugwuene, 2011). The haemoglobin concentration and haematocrit values indicate the severity of

anemia. The high haemoglobin concentration and haematocrit value is an indication of high iron status. Packed cell volume (PCV), also known as haematocrit (Ht or Hct), is the proportion of red blood cells in whole blood. According to Isaac *et al*. (2013), packed cell volume is involved in the transportation of oxygen and absorbed nutrients; thus, increased packed cell volume results in improved transportation and thus increased primary and secondary polycythemia.

The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) values are important indicators of anaemia risk, and a low level indicates anaemia (Adeoti *et al*., 2018). White blood cell counts are important in defending the body against infections; however, white blood cell counts cannot provide definite or specific information; however, differential counts can provide specific information about infections, toxicity, allergy, immunosuppression, and poisoning (Aboderin and Oyetayo, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Design and Data Analysis

The eleven food varieties including Cerifam (control), namely brown teff flour, maize flour, barley flour, wheat flour, oat flour, chickpea flour, pea flour, beans flour, soybeans flour, sesame flour and spinach leave powder as well as formulated diets were produced in triplicates and chemically evaluated under completely randomized design. Whereas, a complete randomized design (CRD) was used to evaluate the formulated diets using rat bioassay in comparison with a commercial control (Cerifam) as well as casein protein. Four multimix complementary diets were prepared from selected local foodstuffs of Gondar Province, Ethiopia. The foodstuffs were chosen based on their availability and acceptability in the study area. Forty-two rats of the same age (4 week) were divided into seven groups of six rats such that the average body weight in each group was not significantly different. Groups I, II, III, and IV were administered the formulated diets and served as experimental groups. While groups V, VI, and VII were received Cerifam, casein & protein-free diet and served as control groups. All of the experiments were done in triplicate.

All data obtained from the experiment on food intake, body weight, protein quality, hematological and biochemical parameters were subjected to One-way ANOVA using SPSS Version 20 and expressed as mean±SD. The means were compared using Least Significance Difference (LSD) test and Duncan Multiple Range test at $P<0.05$.

3.2 Raw materials collection and processing

The raw ingredients red teff (Eragrostis tef (Zucc.), maize (Zea maize), barley (*Hordeum vulgare*), wheat (*Triticum aestivum)*, oat (Avena sativa), chickpea (*Cicer arietinum*), pea (Pisum sativum), beans (Vicia faba), soya beans (Glycine max), sesame (Sesamum indicum) and spinach (Spinacia oleracea) were purchased from local market in Gondar

city, in enough quantities (4 kg for each samples). Whereby the commercial formula (Cerifam) was used as a standard control during the chemical analysis and feeding trials using rat model because it was plant based complementary food, have well balanced nutrients and the most commonly used commercial baby formula in Ethiopia. Cerifam is made with wheat flour, soya flour, milk powder, sugar, vitamins (A, B1, B2, B6, B12, C, D, Nicotinic Acid, and Folic Acid), minerals (Iron, Iodine, and Calcium Phosphate), palm oil, and fruits like apple, banana, orange, and strawberry.

3.2.1 Processing of the raw materials

A combination of traditional methods of soaking, germination, dehulling and roasting of grains was adopted prior to conducting the experiments to process into their respective flour as shown in **Figure 3.1**. Dehulling of legumes reduced the anti-nutrients such as phytates and tannins. Germinating was practiced since it increases the digestibility of the food and the density of the nutrient by lowering the bulk viscosity. Roasting was done in order to enhance the flavor and protein digestibility of the food. All the combined processing approaches significantly increased the nutritional quality, mineral bioavailability, and protein digestibility of legumes.

The techniques of Nakitto *et al*. (2015) were adopted for the processing of bean, pea, chickpea, and soybean flours. Impurities such as dusts and defective (spoiled) grains in the sample were removed and 1000 grams of each grain were soaked in distilled water (1:10 w/v) for a period of 12 h. The soaking water was removed, and samples were allowed to germinate in a dark places for 48 h by keeping it between thick layers cotton cloth. Distilled water was added daily to moisten the seeds. Then the seeds were simply dehulled manually since nearly all the seed coats had split open due to the germination. Finally, the samples were then allowed sun-dried for 24 h and, the dried materials were roasted at 350°C on stainless steel iron pan, with minor modification, reducing the roasting time to only 5 min. Then the grains were milled (Model No: GSB 1514) and passed through a 0.5 mm sieve, packed into an airtight polythene bags for storage in a cool place (about 4°C) until used.

Teff grains were cleaned for impurities such as broken grasses, other mixed grains by handpicking and all other undesirable components such as dusts were removed by washing with tap water. This was followed by soaking the grains in distilled water for 12 h. The water was then removed and the grains samples were placed between moistened cotton cloths and allowed to germinate at room temperature for 24 h. After 24 h of germination, the samples were sun-dried for 8 h and ground to fine powder using an electrical mill (Model No: GSB 1514) (Badau *et al*.,2006). Maize grains were cleaned by handpicking and floatation to eliminate broken seeds and unwanted materials. Grain samples were cleaned with deionized water to remove dirt and dusts followed by steeping in clean water for 8 h at room temperature. The soaked samples were cleaned in water and spread evenly between thick layers of cotton cloth for germination in the dark for 72 h and fresh water was added daily to moisten the seeds. The malted samples were then sun-dried for 24 h and ground to fine flours then sieved (60 mesh), and packaged in polyethylene bags (Abiose *et al*., 2015).

Wheat, barley and oat grains were processed following the methods described by Fikiru *et al*. (2017). The grains were cleaned for extraneous material, separately washed with distilled water and air-dried overnight. This was followed by steeping and germination of the grains to prepare malt. The dried rootlets, husks and acrospires were removed by polishing the cured samples then milled and sieved (0.5 mm). The flour samples were packed in airtight polythene bags, and then kept in a cool place until use.

Processing of Sesame seed was carried out using the method of Fasuan *et al*. (2017). The seeds were sorted, cleaned by distilled water to remove impurities and soaked in salt solution of 3% sodium chloride for 12 h. Thereafter, the grains were dehulled, washed and dried. The dried seeds were roasted at 70^{o}C for 30 minutes then milled. The obtained flour was sieved and packaged in an airtight container for further use. Spinach leaves were separately washed with clean water. This was followed by drying of the leave samples at room temperature for 48 h. Finally, the dried spinach leaves were milled (Model No: GSB 1514) into powder to obtain smooth and consistent particle sizes. Spinach is the most widely available and reasonably priced vegetable in the study

area. Furthermore, spinach contains sufficient amount of vitamin A. The practice of incorporating vegetables in to baby foods by most mothers in Ethiopia is very poor and that why we incorporate it. Soaking, dehulling and roasting was done to reduce the anti-nutritive factors and to improve upon the flavor of the final product. Malting (germination) of the grain was practiced since it increases the digestibility, lowers the bulk viscosity and thereby increases the nutrient density. All the combined processing approaches significantly increase the nutritional quality, mineral bioavailability and protein digestibility of legumes. A summary of the processing of the various foods is presented in **Figure 3.1** while the foods are presented in **Figure 3.2**.

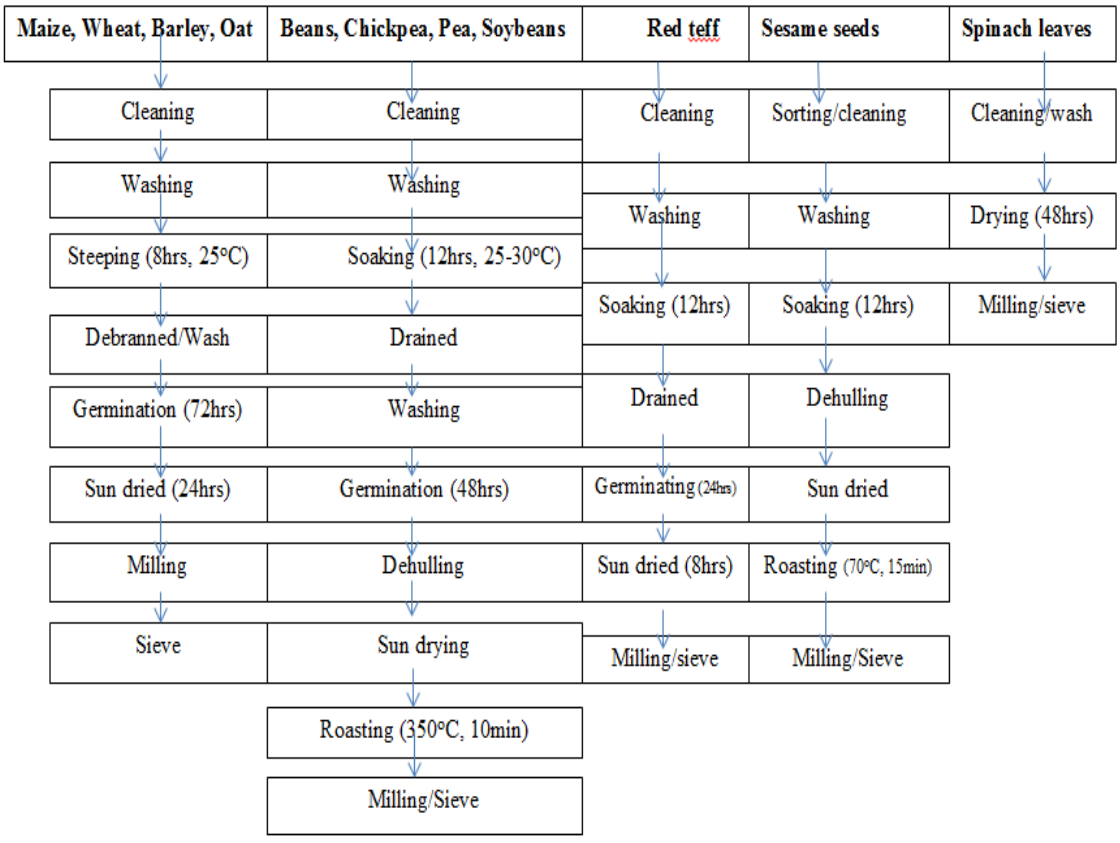


Figure 3.1: **Flow chart showing raw material processing**

Figure 3.2: Raw materials used for processing the complementary foods: a. Teff b. Maize c. Pea d. Wheat e. Beans f. barley g. Chickpea h. Oat i. Soybean j. Sesame k. Spinach

3.3 Formulation of the composite diets

The blend proportion recommendation of 75% cereal and 25% legume for infant complementary food formulation was considered at the beginning (Gopaldas *et al*., 1986; Plahar *et al.*, 2003). The ingredient ratios were designed based on material balance

method for the 0.6–1-year-old infant (FAO, 1985) and the recommendations for supplementary foods targeted for nutrition intervention (WHO, 2003) and cost considerations. Furthermore, formulation was done by referring secondary sources of nutrient compositions of the raw ingredients to meet recommended levels of vital nutrients that are recommended in various guidelines for infant and children complementary foods (Codex, 1991). A computer programme called NutriSurvey (ProNut-HIV, 2005) for Windows was adopted to estimate the nutrient proportions. A dry weight of 65g of the formulated diets were calculated and compared to recommended dietary allowance (RDA) for the same age group for infant 6-12 month (Mariam, 2005). The ten ingredients utilized for the formulations were processed separately into powdery flour forms and the multi mix composite was formulated by combining the flour obtained in different proportions (**Table 3.1**).

Table 3.1: Processed raw materials used in the formulations of the composite diets and their proportions

Formulation	Ingredients & processing methods	Proportion (% w/w)
Diet-1	Soaked, germinated-teff	40
	Soaked, germinated-maize	30
	Soaked, germinated, dehulled and roasted-pea	20
	Soaked, Dehulled and roasted-sesame	5
	Spinach	5
Diet-2	Soaked, germinated-teff	40
	Steeping, germinated-wheat	30
	Soaked, germinated, dehulled and roasted-bean	20
	Soaked, Dehulled and roasted-sesame	5
	Spinach	5
Det-3	Soaked, germinated-teff	40
	Steeping, germination-barley	30
	Soaked,germinated, dehulled and roasted-chickpea	20
	Soaked, Dehulled and roasted-sesame	5
	Spinach	5
Diet-4	Soaked, germinated-teff	40
	Steeped, germinated-oat	30
	Soaked,germinated, dehulled androasted-soyabean	20
	Soaked, Dehulled and roasted-sesame	5
	Spinach	5

Whereby Commercial formula (Cerifam) was used as a standard control because Cerifam is the most commonly used commercial baby formula in Ethiopia.

3.4 Chemical analysis of the diets

The standard official procedures (AOAC, 2000) were followed to determine the proximate composition of the developed diets and ingredients.

3.4.1 Determination of moisture content

Moisture was estimated by oven drying method (AOAC, 2000 Method #925.05). It was determined as weight-difference before and after drying to constant weight. Values were expressed as percentage moisture. The empty dish was dried in the oven at 105ºC for about 3 h and transferred to acrylic desiccators cabinet (Thermo fisher Nalgene: 5317-0120, China) for about 15 to 20 min to cool to ambient temperature. Then the empty dish and lid were weighed. About 3 grams of representative (well-mixed) flour samples was spread evenly to the empty dish in triplicate. The dishes containing samples were weighed again before drying in the oven (Electric heated thermostatic drying box: WH-71, China) at 105ºC for 3 h. After drying, dishes with a partially covered lid were transferred to the desiccators to cool the sample to room temperature after which the dish and its dried sample were weighed.

Calculation Moisture (%) = $\frac{(W1-W2)\times 100}{W1}$

where W1 = weight (g) of the sample before drying

W2 = weight (g) of the sample after drying

3.4.2 Determination of ash content

Ash values were determined by combusting five grams of samples using a muffle furnace at 550° C for 6 h (AOAC, 2000 Method # 941.12).

A porcelain crucible was ignited in a muffle furnace (Model No. F48050-33, USA) for 5 min at 550ºC, cooled in a desiccator and was weighed. Five grams of each food sample was added separately to the crucible and weighed again. The crucible contents were fired in the muffle furnace (Model No. F48050-33, USA) for 6 h at 550°C to light gray ash. They were then removed and placed directly in desiccators to cool and weigh.

The weight difference or weight loss of the crucible and samples prior to ashing gives the value of the organic substance of each food sample, while the weight difference between the crucibles alone and the crucible plus ash gives the ash weight of each sample. Values for ash were calculated and expressed in percentages as follows.

$$Ash(\%) = \frac{m_3 - m_1}{m_2 - m_1} \times 100$$

Where: m_2-m_l = sample mass in g on dry base and m_3-m_l = mass of ash in g.

3.4.3 Determination of crude protein

Crude protein content in the samples was estimated by micro Kjeldahl method (AOAC, 2000 # 979.09). The protein was determined by multiplying the total nitrogen content by a factor of 6.25 (FAO, 2003).

One gram of food sample was weighed separately on pre weighed Whatman filter paper into the digestion flask (round- bottom flask with a long neck). Then the samples were digested by addition of small volume (3-5ml) of concentrated H_2SO4 (an oxidizing agents which digests the food). About 1 g of catalyst mixture comprising Na_2SO4 or K_2SO4 with anhydrous CuSO4 in the ratio of 10:1 was added. The digestion process converted any nitrogen in the food (in the form of nitrates or nitrites) into ammonia. In acidic solution, ammonia was not liberated as gas because rather it exists as ammonium sulfate salt.

N (in food) $\longrightarrow$ $(NH4)_2$ SO4

After digestion was completed, the content of the flask was diluted by water and a concentrated NaOH (40%) solution. It was added to make the solution slightly alkaline and to liberate ammonia gas.

$(NH4)_2$ SO4 +2NaOH $\longrightarrow$ 2NH3 + $2H_2O$ + NaS_2O4

The ammonia was then distilled into receiving flask that consists boric acid (4%) for reaction with ammonia.

Titration

The borate ions were titrated with standard acid (0.1N HCI).

$NH_3 + H_3BO_3$ (boric acid) $\longrightarrow$ $NH_4 + H_2BO3$ (borate ion)

$H_3BO_3 + H^+ \longrightarrow H_3BO_3$

Calculation: Total nitrogen, percent by weight

$$= \frac{(T - B) * N * 14.007 * 100}{W}$$

Where: T: Volume in ml of the standard sulphuric acid solution used in the titration for the test material

B: Volume in ml of the standard sulphuric acid solution used in the titration for the blank determination

N: Normality of standard sulphuric acid

W: Weight in grams of the test material

Crude protein, percent per weight = 6.25 * total nitrogen

3.4.4 Estimation of crude fat

Crude fat was estimated by the Soxhlet extraction method by frequent extraction of 5 grams of samples with hexane at 60-80°C (AOAC, 2000, #Method**4.5.01**).

Five grams (moisture-free) of sample was weighed and filled into fat-free labeled thimbles. These were then plugged with glass wool and transferred in to extraction apparatus containing 160 ml of petroleum ether (b.p. 60-80°C). Clean, dry receiver flasks were also weighed and placed in the extractors. The extraction units were then assembled and cold water was circulated while maintaining the water bath temperature at 60°C. The extraction process was taken about eight hours. At the end of the extraction, the thimbles were removed with the samples and placed in an oven at 70°C for three hours and dried to constant weight.

The crude fat value was obtained as a weight difference before and after the extraction. De-fatted residues were stored in airtight sample containers for further analysis.

3.4.5 Determination of total carbohydrate

The total carbohydrate was estimated by difference with the exclusion of crude fiber (100-(% crude fat + % crude protein + % moisture + % ash) (AOAC, 2000).

3.4.6 Crude fiber determination

Crude fiber was determined according to (AOAC, 2000 # 920.169). About 1.5 grams of food sample was transferred into a 600 ml beaker and approximately 200 ml of 1.25% sulfuric acid was added to the beaker. This was then boiled for 30 min by rotating periodically. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml of 28% KOH was added and again boiled gently for another 30 min. Subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. Next, the sample was filtered and the residue was placed on crucibles, dried in an electric oven (WH-71, China) at 130^{o}C for 2 hours. Then it was cooled at room temperature for 30 min in a desiccator (Thermo fisher Nalgene: 5317-0120, China) and weighed, and finally transferred to muffle furnace (Model No. F48050-33, USA) for 30 min ashing at 550^{o}C. Afterwards, the sample was cooled again in a desiccator and re-weighed. The crude fiber content was determined using the equation:

Total Crude fiber (%) = (W1 − W2/ W3) ×100

Where:

W1 = Crucible weight after drying

W2 = Crucible weight after ashing

W3 = Sample dry weigh

3.4.7 Mineral analyses

The method indicated by Association of Official Analytical Chemists (AOAC, 2003) was used for mineral analysis. About 2 g of each flour samples was digested with concentrated nitric acid and hydrogen peroxide. Then the solutions are filtered in a 5ml volumetric flask and the filtrate was loaded to Atomic Absorption Spectrophotometer (novAA-400P, Germany). Zinc (Zn), iron (Fe), copper (Cu), calcium (Ca), and magnesium (Mg) were determined at wavelengths 324.7nm, 248.3 nm, 213.9 nm, 422.7 nm and 285.2 nm respectively. All values were expressed in mg/100 g.

3.5 Assay of antinutritional factors

3.5.1 Determination of phytates

Quantification of myo-inositol hexa-phosphate (IP6) phytates was done using HPLC as described by (Camire and Clydesdale, 1982). The HPLC was performed with a Shimadzu SCL-10A (Kyoto, Japan) system consisting of a column oven (Model: CTO-10A), and a UV-Vis detector (Model: SPD-10AV). A weighed, 0.5 gram of flour sample was extracted with 10 ml of 3% H_2SO_4. The contents were filtered and the filtrate was transferred to a boiling water bath for 5 minutes, followed by the addition of 3 ml of $FeCl_3$ Solution (6 mg of iron (III) per ml in 3% H_2SO_4. The contents were then heated for 45 min to complete the precipitation of the ferric phytate complex. It was then centrifuged at 2500 rpm for 10 min and the supernatant was discarded. The precipitate

was washed with 30 ml of distilled water, centrifuged and the supernatant discarded. To the residues 3 ml of 1.5 N NaOH was added and the volumes brought to 30 ml with distilled water. The contents were then heated for 30 minutes in boiling water bath to precipitate the ferric hydroxide. Subsequently, the samples were then cooled and centrifuged. The supernatant was transferred to a 50 ml volumetric flask. The precipitate was rinsed with 10 ml of distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. This was micro filtered before HPLC analysis. Inositol standards (Aldrich Chemical, USA) were prepared at a concentration of 25, 50, 100, 250, and 500 µg/ml for quantification. The mobile phase was 0.005N sodium acetate in distilled water, at flow rate of 0.5µL/min. Calculations were made from a standard curve, $Y = 1244.3x + 10583$ where $R^2 = 0.9995$.

3.5.2 Determination of oxalates

Oxalate content of the flour samples were determined by the HPLC method as described by Libert (1987) with modifications suggested by (Yu et al., 2002). To achieve this 0.5 g sample was homogenized in 4 mL 0.5N HCl. The homogenate was heated at 80ºC for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25mL. About 3 ml of the solution was withdrawn and centrifuged at 12000 rpm for 10 minutes. About 1 ml of the supernatant was passed through a micro filter (0.45µ) before HPLC analysis. Sodium oxalate standards were prepared at varying concentrations for quantification. The calculations were based on a standard curve (R^2 = 0.995) drawn with sodium oxalate as the standard. HPLC analysis was done using UV-IVIS detector using a solution 0.01N H_2SO_4 as a mobile phase. The flow rate was 0.6 mL/min and was detected at a wavelength of 221 nm.

3.5.3 Determination of Tannins

Amount of condensed tannins in the food sample was determined according to Vanillin-hydrochloric acid method (Burns, 1971; Price, *et al*., 1978). A processed 0.25-gram flour sample was extracted with10 ml of 4% HCl in methanol by shaking for 20 min in a

shaker (Labortechnik KS 250b, Germany) and separated using a refrigerated centrifuge (Kokusan, Type H-2000C, Japan) at 4,500 rpm for 10 min at 25°C. The supernatant was stored into a 25 ml volumetric flask and extraction from the residue was repeated with 5 ml of 1% HCl in methanol. The second supernatant was combined with the first and diluted to 25ml. Catechin hydrate standards were prepared at a concentration of 0, 10,20,40,60, 80 and 100 µg/ml. Duplicate aliquots of 1ml of sample extracts were kept into test tubes where one served as sample blank. The standard solutions and samples were reacted with 5 ml vanillin-HCl reagent prepared by mixing just before use (equal volumes of 8% HCl in methanol and 1% Vanillin in methanol) and were allowed to stand for 20 minutes. To the sample blanks, 5ml of 4% HCl in methanol was added. Absorbance for all prepared solutions were read at 500 nm and tannin content calculated as percent catechin equivalent (CE) using the standard calibration curve, $Y = 0.00063x + 0.01094$ where $R^2 = 0.995$.

3.5.4 Estimation of molar ratio of antinutrient/minerals

The mole of antinutrients and minerals were calculated by dividing the weight of antinutrients and minerals with its molecular weight (Al Hasan *et al*., 2016; Norhaizan & Norfaizadatul, 2009). The molar ratio was estimated by dividing the mole of antinutrients with the mole of minerals. Molar ratio= mole of antinutrients/mole of minerals

Molar ratios of antinutrient/minerals were used to predict the mineral bioavailability (Morris and Ellis, 1989). The recommended values used to predict the bioavailability were when the ratios exceed: calcium: phytate > 0.24, phytate: iron > 1, phytate: zinc > 15, and phytate × calcium/zinc > 200 (Al Hasan *et al*., 2016; Morris and Ellis, 1985).

3.6 Vitamin assay

3.6.1 Determination of total vitamin C

Ascorbic acid content of each sample was obtained by HPLC method (Vikram *et al*., 2005) with some modifications. To achieve this, about 2g of each diet sample was weighed and extracted with 0.8 percent metaphosphoric acid. The flour extract was centrifuged at 1000rpm. The supernatant was filtered and diluted with 10mL of 0.8% metaphosphoric acid using 0.45µ filter paper and 20µl of various concentrations of ascorbic acid standards were made to make a calibration curve. HPLC analysis was done using a Shimadzu UV-VIS detector at a wavelength of 266 nm. The mobile phase was 0.8% metaphosphoric acid and a flow rate of 1.2 ml/min was used.

3.6.2 Estimation of beta carotene

Beta-carotene content was analyzed using column chromatography and UV spectrophotometer. Extraction was done using acetone and petroleum ether as indicated by Rodriguez-Amaya and Kimura. (2004). Briefly, about 1 gram of each flour sample was weighed and placed in wooden mortar with about 10 ml of acetone. This was followed by thoroughly grinding, after which the acetone extract was transferred into a 100 mL volumetric flask. The residue was again extracted with 10 ml acetone and the extract was added to the contents of the volumetric flask. The extraction with acetone was continued until the residue no longer showed any color (red-orange). The combined extract was then made to a volume of 100 mL with acetone. About 25 mL of each extract was evaporated to dryness using a rotary evaporator (RE100, UK). The residue was dissolved with 10mL petroleum ether and the solution was introduced into a chromatographrotene column. This process was eluted beta-carotene with petroleum ether and beta-carotene was collected in flasks. The eluted beta-carotene was made to a volume of 25 ml with petroleum ether and the absorbance was read at 440 nm using a UV- Vis spectrophotometer (Shimadzu model UV – 1601 PC, K, Japan). Beta-carotene standards were prepared at a concentration of 0, 0.190, 0.407, 0.811, 1.207, 1.596 and

1.945 µg/ml used for making a standard curve. Calculations were made from a standard curve, $Y = 0.1962x + 0.01$ where $R^2 = 0.9993$.

3.6.3 Determination of B vitamins

Quantification of four water-soluble B-vitamins was done by HPLC with diode-array detection simultaneously. The issues of chromatographic interferences of cereal-legume flours were addressed by solid-phase extraction with Sep-Pak C_{18} (500 mg) cartridges that allows the separation of B-vitamins and eliminates the interfering components. The methods described by Ekinci and Kadakal (2005) was employed for solid-phase extraction of water-soluble B-vitamins. Various concentrations of the respective vitamin-B standards were used to draw the calibration curve. Standard solutions of B-vitamins were kept in dark glass flasks, to protect them from light and placed under refrigeration. A 20µL aliquot of samples and standard was injected into the HPLC system and monitored by a photodiode array detector at 234 nm for thiamine, 266 nm for riboflavin, 261 nm for niacin and 324 nm for pyridoxine. A 0.45 µL membrane filter was used to filter the mobile phase followed by degassing by sonication before use. The flow rate was 0.7ml/min and the column was operated at ambient temperature (about 25^{o}C). Identification of the respective vitamins was achieved through comparison of their retention times. A calibration curve was drawn for the respective vitamins. Calculations were made from a standard curve with correlation coefficients ($R^2 > 0.999$).

3.7 Biological assay of the formulated diets

The albino rat trials were carried out using the methods of Abiose *et al*. (2016). At the beginning of the experiment, forty two (42) white albinos (*Rattus norvegicus*) were obtained from the Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar, Ethiopia. The rats were four weeks old and weighed 33-40 g with no significance differences in weight among the groups (36.00±2.39). The rats were fed with normal diets (pellets) and water for a period of four days for proper acclimatization to the laboratory conditions before commencement of the experiments.

After the acclimatization period, the rats were then reweighed and allocated into seven groups of six rats randomly in each cages in an air-conditioned room (23± 2°C). Approval for the use of the animals was obtained from the Ethical Committee of Institute of Biotechnology, University of Gondar, Ethiopia for experimental purposes only (Ref No. IOB/490/08/2019). All animals received appropriate care according to the Institution's Guidelines for the care and use of laboratory animals.

3.7.1 Experimental treatment of the animals

A complete randomized design (CRD) was used in which forty-two rats were divided into seven groups of six rats and there were no significance differences in the average body weight per group. Groups I, II, III, and IV were administered the formulated diets and served as experimental groups (Table 3.1). While groups V, VI, and VII received Cerifam, casein & protein-free diet and served as control groups. Just before each feeding, a known amount of each diet was mixed thoroughly with sufficient boiling distilled water to a thick paste and allowed to cool before feeding the animals. Each group was provided with 150g of the respective diets and 200ml water daily for 28 days. During this period, daily records of diet, water, and weight of the animals were kept. The weighed diet was given and the unconsumed diet was collected, dried, and weighed daily. Seven days before the end of the feeding experiment, the faeces and urine were collected separately from each group. Faeces samples were dried at 80ºC for 12 hours in an oven, cooled, and weighed. A few drops of dilute sulphuric acid (H_2SO4) were added to the urine, which was kept under frozen conditions. Nitrogen in the triplicate samples of urine and faeces was determined by the micro-Kjeldahl method (Ijarotimi & Keshinro, 2012). Weight change of the animals and nitrogen was used to determine the protein quality of the blended diets such as the biological value (VB), net protein utilization (NPU), true digestibility (TD), protein efficiency ratio (PER) and feed efficiency ratio (Ijarotimi & Keshinro, 2012).

$$BV = \frac{Ni - (Nf - Nef) - (Nu - Neu) \times 100}{Ni - (Nf - Neu)}$$

Net protein utilization (NPU) (= BV × TD)

$$NPU = \frac{Ni - (Nf - Nef) - (Nu - Neu) \times 100}{Ni}$$

True protein digestibility (TD)

$$TD = \frac{Ni - (Nf - Nef) \times 100}{Ni}$$

Protein efficiency ratio (PER)

$$PER = \frac{Weight\ gain}{Protein\ intake}$$

Food efficiency (FER):

$$FER = \frac{Weight\ gained}{Food\ intake}$$

Where:

Ni, nitrogen intake; Nf, fecal nitrogen; Nef, endogenous fecal nitrogen; Nu, urinary nitrogen; Neu, endogenous urinary nitrogen

3.7.2 Evaluation of haematological and serum biochemical parameters

At the end of the feeding experiment, each animal was sacrificed by decapitation. About 5 ml blood samples were collected from three randomly selected rats into EDTA test tubes for haematological parameters and plain vacuum tubes for biochemical parameters on the 28th day of the experiment. After 10 min, the blood samples for biochemical parameters were allowed to clot for 20 minutes, before being centrifuged for 15 min at 3000rpmto obtain serum. The serum samples were then gently transferred with pipettes into clean, dry labeled light-shielded tubes and stored at freezing temperature until required. The blood samples for hematological and biochemical parameters were analyzed at the University of Gondar referral hospital, Gondar, Ethiopia.

For haematological parameters, full blood count was carried out using Automated Haematologic Analyzer (Sysmex, KX-21N, Sysmex Corporation, Kobe, Japan). Blood samples were centrifuged at 4500 rpm for 15 min and the plasma was removed and assayed for the determination of serum parameters. Biochemical analyses including total protein, glucose, albumin, total bilirubin, triglyceride, HDL and alkaline phosphatase (ALP), and blood urea were determined using Mindray diagnostic kits supplied by Shenzhen Mindray Bio-Medical, China. Total protein was determined using the biuret method (Henry and Stobel, 1957). The glucose oxidase-peroxidase (GOD-POD) method was employed to determine serum glucose. The Bromocresol Green method was used to determine serum albumin concentration (Doumas and Biggs, 1972).Total bilirubin was done using the Diazotized Sulfanilic Acid (DSA) method (Spencer and Price, 1977). While the difference between total protein and albumin was taken to determined globulin concentration (Turnwald and Barta 1989). Triglyceride was determined using the GPO-POD method (Gidez *et al*., 1982). Serum urea was determined by the urease-GLDH, UV method. While alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined by using the Modified IFCC method with commercial kits (DiaSys Diagnostic Systems GmbH, Germany). Creatinine was determined using CREATININE liquicolor complete kit (Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany). The principle of the

method is creatinine forms in alkaline solution an orange-red colored complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Total cholesterol determination: Cholesterol concentration in serum of rats fed the experimental diets was determined using the CHOD-POD method of Allain *et al*. (1974). The method involves the enzymatic hydrolysis and oxidation of cholesterol esters and cholesterol respectively. The concentration of low density lipoprotein (LDL) cholesterol was calculated by a modification of the Friedelwald formula. Low-density lipoproteins (LDL) = Total cholesterol –high density lipoprotein (HDL)-TAG/5 (Allain *et al*., 1974).

3.8 Statistical analysis

At the beginning the data was filtered and checked for any errors before treating statistically. Then the lab. Analytical data was tested for their normality or goodness of fit before running the actual analysis. All data obtained from the experiment on food intake, body weight, protein quality, hematological and biochemical parameters were subjected to One-way ANOVA using SPSS Version 20 and expressed as mean±SD. The means were separated and compared using Least Significance Difference (LSD) test and Duncan Multiple Range test at $P<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate composition of the ingredients

The proximate composition of the raw materials used for the formulation of the blended diets is summarized in **Table 4.1**. The protein content was in the range of 8.46-37.21%. Processed soybeans, beans, pea and chickpea exhibited higher value of protein content. Processed soybean in the present study had higher protein value (37.21%) compared to literature value of the raw soybean (34.57%) reported by Tenagashaw *et al*. (2017). This is due to the roasting and germination process which improves the nutritional quality of foods (Msheliza *et al*., 2018). Germination increases the nutritional value of legumes and cereals by increasing nutrient digestibility and enhancing amino acid contents. Whereas, roasting reduces the antinutrients by lowering the bulk viscosity and reducing the fiber content, thereby increases the nutrient density (Msheliza *et al*., 2018). However, our result is very close to soaked soybean value (37%) reported by Agume *et al*. (2017). The moisture and ash values ranged from 2.25-11.81% and 1.31-6.04%, respectively. Ash values indicate the total mineral content of a particular food sample (Kavitha and Parimalavalli, 2014). The samples were significantly different in ash content. Ash content of pea, chickpeas, soybean and beans were found to be low compared to literature values of the respective unprocessed raw materials (Naret, 2019; Desalegn, 2015; Agume *et al*., 2017). This could be because some inorganic minerals may be lost and leached out of the grains during processing (soaking, dehulling, and roasting) (Ukwo *et al*., 2013). Ash content was significantly higher ($p=0.00$) in spinach and sesame in comparison to the rest of the samples. Fat content in sesame and soybean (50.39 percent and 20.80 percent, respectively) was found to be significantly higher ($p<0.05$) than in the other sample. The crude fiber content ranged between 1.99-10.35%. Amount of crude fiber was significantly higher ($p<0.05$) in spinach with the lowest value in maize.

Table 4.1: Proximate composition (g/100g) of the ingredients used in the formulation of the blends on dry weight basis.

Ingredients	Moisture	Ash	Crude protein	Crude fat	Available CHO	Crude fiber
Teff	6.47±0.29^{bcd}	2.60±0.03^{cd}	9.33±0.39^{a}	3.45±1.45^{bc}	73.91 ±1.81^{f}	4.25±0.77^{ab}
Maize	7.58±0.41^{d}	1.51±0.06^{ab}	9.85±1.48^{a}	3.83±0.11^{bc}	75.23±1.51^{f}	1.99±0.11^{a}
Wheat	5.36±0.78^{bc}	1.31±0.18^{a}	8.46±1.01^{a}	2.03±0.15^{a}	81.03±1.14^{g}	2.16±0.08^{a}
Barley	5.49±0.25^{bcd}	1.71±0.04^{ab}	9.41±0.95^{a}	1.91±0.37^{a}	77.95±1.21^{g}	3.52±1.21^{ab}
Oat	7.39±1.92^{cd}	2.17±0.09^{bc}	9.80±1.64^{a}	2.77±1.00^{ab}	74.92±4.35^{f}	2.95±0.36^{a}
Beans	6.42±0.94^{bcd}	3.03±0.16^{d}	31.30±0.98^{d}	1.73±0.02^{a}	53.22.±6.13^{d}	6.12±0.51^{b}
Chickpea	4.93±0.52^{b}	2.27±0.15^{bc}	22.24±0.85^{b}	4.56± 0.74^{c}	63.45±2.80^{e}	2.98±0.50^{a}
Peas	7.34±2.77^{cd}	2.73±0.13^{cd}	27.18±0.80^{c}	1.53 ±0.62^{a}	58.36±1.82^{e}	4.03±0.64^{ab}
Soybeans	4.72±0.30^{b}	2.70±0.02^{cd}	37.21±0.74^{e}	20.80±1.57^{d}	29.48±1.27^{b}	5.76±0.94^{b}
Sesame	2.25±0.01^{a}	5.96±0.87^{g}	20.73±0.06^{b}	50.39±0.17^{e}	16.97±0.88^{a}	3.71±0.31^{ab}
Spinach	11.81±0.58^{e}	6.04±0.79^{e}	17.34±2.32^{b}	5.87±0.36^{bc}	45.53±5.68^{c}	10.35±4.44^{c}

Each value is mean ± standard deviation of three replications on dry matter basis. The different superscript letters in the same column indicates significantly different ($p<0.05$).

4.2 Macronutrient composition of the complementary foods

The proximate composition of the formulated diets and proprietary formula (Cerifam) is presented in **Table 4.2.** The crude protein values of cereal-legume based diets were in the range of 12.20-17.14%. Mean separation using LSD showed protein content was significantly different ($p= 0.01$) among the different composite mixtures. Initial differences in protein composition among legumes contributed to the variations observed. Nevertheless, the crude protein values of all blended foods were statistically higher compared to the control diet and met the recommended dietary allowance for complementary foods (Gropper *et al.*, 2009). The inclusion of legumes during formulation might be attributed to the increased protein composition of the developed products. In addition, Fasasi (2007) reported that ingredient processing methods such as

soaking and germination improves the protein content and the quality of the food products. Increase in the protein content during soaking and germination of the seeds may be attributed to the net synthesis of enzymatic protein by the germinating seeds (Nkhata *et al*., 2018). Protein content of Diet 4 was significantly higher (p=0.00) than all other diets (17.14%). This is likely to be due to the presence of soybean in Diet 4, which has significantly higher protein content than the other foods (Etiosa *et al*., 2017). Recent studies indicated that the protein quality of a cereal-legume combination is better compared to that of a cereal or legume alone (Wakil and Onilude, 2009). The protein content is comparable to 15.27-17.00% reported by Adeoti and Osundahunsi (2017) for maize-based complementary food enriched with fermented and germinated moringa oleifera seed flour. The calories of an infant diet are provided by the carbohydrate, fat and protein, which are main components of complementary foods that help to fulfill growing infants' energy needs and lack of either of these can lead to malnutrition (Asma *et al*., 2006). Calculated energy values provided by the blends ranged from 377.36-408.33 kcal/100g dry matter. Energy values in all the formulated mixtures were significantly (p=0.02) higher than that of the control diet (Cerifam) and met the specific minimum requirement of 380-425 kcal as recommended by FAO (2004). The energy content of the formulated diets was also similar to the values of 372 -397 kcal/100g previously reported for complementary diets based on cereal-legume combination (Baskaran *et al*, 2004).

The crude fat content ranged between 2.44 and 14.94%. A significant (p=0.00) difference was observed in fat content among the diets. Crude fat content was significantly higher in all the formulated diets in comparison to the control diet (Cerifam). However, the highest fat content was recorded in Diet 4 (14.94%). This could be due to the inclusion of oil-dense soybean during diet formulation. This is also in line with the recommendations of FAO/WHO (1998) that oil seeds and vegetable oils be included in food meant for infants and children, which will increase the energy density. Though high fat content is nutritionally beneficial as it can increase the calorie level of the diet, it could reduce the shelf life and stability of the food product during storage

since unsaturated oils are prone to oxidative rancidity (Adebayo-Oyetoro et al., 2012). The content of fat was very low in other diets this is attributed to the lower fat levels of cereals. Dietary fat is vital to facilitate absorption of fat-soluble vitamins, to supply energy in the body and to provide essential fatty acids that are required for normal brain development (Aranceta and Perez-Rodrigo, 2012). Among all the composite diets, Diet 3 and Diet 4 were able to meet the minimum requirement of 10–25% fat recommended by WHO (2001) for infant food. In this study, fat content of the formulated diet was higher than that reported (4.68-6.22%) by Tenagashaw *et al*. (2017) for teff-based complementary blends.

Total carbohydrate was found to be significantly (p=0.00) higher in the control diet (81.78%), compared to the formulated diets (35.29 -73.41%). The total carbohydrate content of the sample diets is in agreement with the values reported for maize-based complementary food enriched with fermented and germinated moringa oleifera seed flour (59.26-61.75%) as reported by Adeoti and Osundahunsi (2017). The protein and fat content to the blend were mainly provided by chickpea, pea, beans, soybean and sesame while the main source of carbohydrate were cereals. The high protein and energy contents of these formulated diets revealed that they are suitable to support growth and development of infants.

The crude fiber values in the complementary food ranged from 4.69 -8.36%. These values are higher when compared to the control diets. A significant (p=0.01) difference was observed in crude fiber composition among all the blended mixtures. The highest crude fiber values were observed in Diet 4 (8.36%), while the least value was recorded in Diet 3 (4.69%). In this study, fiber content was higher than that reported by (Fikiru *et al*. (2016)**,** who reported fiber values of 3.1-4.1% in complementary food blended from maize, roasted pea, and malted barley. The crude fiber content is slightly higher compared to the recommended daily allowance of 5% fiber in complementary food (Gibson *et al*., 2010). A low level of fiber in infant formulations has been recommended so as not to adversely affect digestibility and absorption of foods by infants (Adepeju *et al*., 2016). Infants' gastrointestinal systems are not well developed to handle high-fiber

diets, which results in impaired protein and mineral digestion and absorption (Asma *et al*., 2006).

Moisture content is critical for predicting the shelf life of food products. In this study, the moisture content ranged from 2.29- 5.53%. The highest moisture content was observed in Diet 4 (5.53%), while the least value was observed in the control diet (2.29%). The moisture content of all the blended diets were significantly (p=0.03) higher than that of the control sample. Nevertheless, all of the figures were within the acceptable range of 5% moisture (CODEX, 1991). The low moisture content of the formulated diets are an important attribute, as it could improve the storage quality of the food due to the low water activity for microbial growth. The lower moisture content could be due to longer soaking and germination time which decreases moisture content significantly as a result of utilization of water for metabolic activities initiated by soaking (Nwosu, 2013; Desalegn, 2015). Our finding is similar to that of Mensa-Wilmot *et al*. (2003) who reported moisture level of 2.52–4.89% in formulated cereal/legume-based food supplements. It is also comparable to that of Tenagashaw *et al*. (2017) who reported moisture levels of 2.51 to 7.36% in Teff-based complementary foods fortified with soybean and orange-fleshed sweet potato. However, the moisture content in our study is lower than that reported by Ijarotimi and Keshinro (2012), who observed moisture levels of 5.7-10.2% in infant formulations from germinated popcorn, Bambara groundnut and African locust bean flour. Ash content ranged from 2.33 to 3.21% in the various diets. The ash content of Diet 2 and control sample was significantly lower than that of the blended diets. Among all the formulated diets, Diet 3 and Diet 4 were found to be most suitable as complementary foods in terms of proximate composition when compared with the other formulations. They also found to be better than the control (Cerifam) sold in Ethiopia for specific age range of 6 to 12 months. The results showed that the energy and most of the nutrient values were higher in Diet 3 and Diet 4 than in Cerifam product.

Table 4.2: Proximate composition (g/100g) of the different formulated diets on dry weight basis

Diets	Moisture	Ash	Crude protein	Crude fat	Crude fiber	Total CHO	Energy/kcal
Diet 1	4.91±0.10^{c}	3.21±0.27^{b}	12.20±0.08^{b}	6.28±0.20^{b}	5.40±0.22^{c}	68.01±0.40^{c}	377.36^{a}
Diet 2	4.46±0.13^{c}	2.63±0.49^{a}	14.97±0.58c	8.14±0.06^{c}	7.74±0.12^{d}	62.05±0.20^{b}	381.34^{c}
Diet 3	3.38±0.46^{b}	3.12±0.06^{b}	15.34±0.61^{c}	10.61±0.37^{d}	4.69±0.23^{b}	62.87±1.56^{b}	408.33^{d}
Diet 4	5.53±0.49^{d}	3.17±0.04^{b}	17.14±0.21^{d}	14.94±0.27^{e}	8.36±0.17^{e}	50.86±0.75^{a}	406.46^{d}
Diet 5	2.29±0.18^{a}	2.33±0.36^{a}	11.15±0.26^{a}	2.44±0.15^{a}	3.47±0.05^{a}	78.33±0.69^{d}	379.88^{b}

Each value is mean ± standard deviation of three replications on dry matter basis. Where the diets are described in (table 3.1) and diet 5 is the control (Cerifam). The different superscript letters in the same column indicates significantly different ($p<0.05$).

Figure 4.1: Packed composite flours

4.3 The mineral composition of the formulated mixture

The mineral composition of the compounded diets, as well as the daily estimated intake from 65 g of the diet by infants compared with RDA, is presented in **Table 4.3 and Table 4.4**. There were significant differences in iron, calcium and magnesium content among the diets. Iron content was significantly (p=0.00) higher in Diet 4 (21.57mg/100g) in comparison to other diets, with the lowest value the control diet (6.57 mg/100g). Diet 3 (187.61 mg/100g) and Diet 2 (185 mg/100g) had significantly higher calcium content than the other diets, with the lowest value of 38.97 mg/100g in Diet1. Magnesium was significantly higher in Diet 2 (111.91 mg/100g) in comparison to the other diets, while it was significantly lower in the control diet (21.86 mg/100g). Magnesium plays a significant role in the structure and the function of the human body.

No significant (p=0.12, p=0.23) difference was observed in zinc content between Diet1 (4.97 mg/100g), Diet 4 (4.96 mg/100g) and the control diet (4.98 mg/100g). There was no significant difference in copper content among the diets, which ranged from 0.19 to 0.23 mg/100g.

The mineral composition of some of the developed diets was better than the mineral content of the commercial diet (cerifam). Furthermore, the daily intake of 65g of Diet 1 and Diet 4 were able to meet RDAs for the minerals zinc and iron for infants of age 6 to 12 months. However, none of the formulated diets were able to meet the daily requirement of calcium, magnesium, and copper. This observation is different from that of Achidi *et al*. (2016) who reported higher mineral levels in nine instant complementary foods formulated from cereal, legume, tuber, vegetable and crayfish. It was also reported that mineral elements such as iron and zinc are very low in cereals but iron content can be improved by the addition of legumes (FAO/WHO, 2001). Since complementary foods are intended to complement breast milk, it is believed that continuing breastfeeding along with the consumption of these local formulations would go a long way in meeting most of the requirement for infants. Zinc and iron are critical trace minerals essential for young children and infants to facilitate their normal growth and development (Makori *et al*., 2017). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Ojinnaka et al., 2016).The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Ojinnaka *et al*., 2016). Relatively a high iron, copper and zinc content of the developed diets could be attributed to the significant removal of iron and zinc inhibitors by the household processing approaches of ingredients.

Table 4.3: The mineral profiles (mg/100 g) of the developed diets on dry matter basis

Element	Diet 1	Diet 2	Diet 3	Diet 4	Diet5 (control)
Calcium	38.97±0.15^{a}	185.00±0.46^{d}	187.61±0.01^{e}	49.78±0.21^{b}	97.64±0.03^{c}
Magnesium	73.41±0.02^{c}	111.91±0.02^{e}	85.49±0.03^{d}	38.60±0.14^{b}	21.86±1.59^{a}
Zinc	4.97±0.01^{b}	3.68±0.01^{a}	3.62±0.01^{a}	4.96±0.01^{b}	4.98±0.01^{b}
Iron	18.55±0.06^{d}	12.65±0.01^{c}	10.95±0.01^{b}	21.57±0.07^{e}	6.57±0.07^{a}
Copper	0.21±0.01^{a}	0.23±0.01^{a}	0.19±0.00^{a}	0.20±0.00^{a}	0.21±0.01^{a}

Each value is mean±standard deviation of three replications on dry matter basis. The different superscript letters in the same horizontal row indicates significantly different ($p<0.05$).

Table 4.4: Amount (mg) of minerals that can be provided in 65g of the diets

Mineral	Diet 1	Diet 2	Diet 3	Diet 4	control	RDA (mg/day)
Cal	25.3	120.3	122	32.4	63.5	**270**
Mg	47.7	72.7	55.6	25.1	14.2	**75**
Zn	3.2	2.4	2.4	3.2	3.2	**3**
Fe	12.1	8.2	7.1	14	14	**11**
Cu	0.14	0.15	0.12	0.13	0.14	**0.22**

Source: Recommended Dietary Allowance from Gropper *et al*. (2009)

4.4 Vitamin content of the compounded diets

The vitamin content, as well as the estimated amount in 65 g of the formulated diets and the control compared with RDA, are presented **Tables 4.5 and 4.6**, respectively. A significant difference was observed in content of B-vitamins between the formulated and

the control diets except pyridoxine (B6). Furthermore, thiamine (3.93 mg/100g), riboflavin (3.81 mg/100g) and nicotinic acid (8.48 mg/100g) were significantly ($p=0.02$) higher in Diet 2 compared to the other diets. No significant ($p=0.63$) difference was observed in pyridoxine (B6) values among all the formulated and control diets. In general, the values of all water-soluble vitamins in the formulated and control diets would meet the RDA value for 6 to 12 months infants (Gropper *et al*., 2009). Beta carotene is the main provitamin A carotenoid in many green leafy vegetables. Higher levels of beta carotene (5300- µg/100g) have been reported in Spinach leaves. In this study, β-carotene content ranged from 0.31 to 2.25 mg/100g. Among the diets evaluated, Diet 1 and Diet 2 had significantly higher β-carotene content compared to other diets. The β-carotene value of the control diet (0.31 mg/100g) was statistically lower than the remaining diets. The β-carotene content in all the formulated diets would meet the RDA value of 0.5 mg/day for 6-12-month infants (Gropper et al., 2009). However, β-carotene content in the control diet (0.31 mg/100g) was below the RDA level. The incorporation of spinach in the diets during formulation could be a reason for the high β-carotene content observed. Ascorbic acid content ranged between 0.00 to 20.21 mg/100g. The content of total vitamin C was very low in all diets, may be due to ascorbic acid being sensitive to light and may have been lost during sample processing or storage. None of the diets was able to meet the recommended dietary allowance (RDA) value (50mg/day) of vitamin C needs of 6-12 month infants. Vitamin C protects the body from oxidation reaction, and helps in the formation of connective tissue. Fruits and vegetables should therefore be included in complementary diets, as they are good sources of vitamins.

Table 4.5: Vitamin content (mg/100g) of the compounded diets in dry mater basis

Vitamins	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5 (control
β-carotene	2.25 ± 0.07^{d}	2.23 ± 0.17^{d}	1.11 ± 0.15^{b}	1.53 ± 0.13^{c}	0.31 ± 0.01^{a}
Ascorbic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.86 ± 1.48^{a}	1.54 ± 0.42^{a}	20.21 ± 7.44^{b}
Thiamine (B1)	3.45 ± 0.36^{c}	3.93 ± 0.26^{d}	2.94 ± 0.11^{b}	2.41 ± 0.05^{a}	3.26 ± 0.02^{bc}
Riboflavin (B2)	2.95 ± 0.37^{b}	3.81 ± 0.29^{c}	3.20 ± 0.12^{b}	2.15 ± 0.41^{a}	1.84 ± 0.22^{a}
Niacin (B3)	7.75 ± 0.53^{c}	8.48 ± 0.55^{d}	7.46 ± 0.57^{c}	6.53 ± 0.03^{b}	4.25 ± 0.03^{a}
Pyridoxine (B6)	3.84 ± 0.49^{a}	3.09 ± 2.55^{a}	3.14 ± 0.13^{a}	2.43 ± 0.07^{a}	3.57 ± 0.02^{a}

Each value is mean ± standard deviation of three replications on dry matter basis. The different superscript letters in the same horizontal row indicates significantly different ($P<0.05$).

Table 4.6: Estimated amount of vitamins (mg) in a daily intake of 65g of composite flours compared with RDAs

Vitamins	Diet 1	Diet 2	Diet 3	Diet 4	Diet5 (control)	**RDA*0.5 to 1 year**
β-carotene	1.46	1.45	0.72	1.00	0.20	**0.5 mg**
Ascorbic acid	0.00	0.00	0.56	1.00	13.14	**50mg**
Thiamine (B1)	2.24	2.56	1.91	1.57	2.12	**0.3mg**
Riboflavin (B2)	1.92	2.48	2.08	1.41	1.21	**0.4mg**
Niacin (B3)	5.04	5.51	4.85	4.24	2.76	**4mg**
Pyridoxine (B6)	2.51	2.01	2.04	1.58	2.32	**0.3mg**

Source: Recommended dietary allowance from (Gropper *et al*., 2009).

4.5 Assay of antinutritional factors

Table 4.7 shows the antinutritional content of the formulated diets. The result showed that tannin content was in the range of 0.77-3.03 mg/100g, while oxalate value was between 0.62-11.03 mg/100g. Phytate was detected in only Diet 1 (14.41mg/100g) and Diet 3 (9.62 mg/100g). Tannin content was significantly (p=0.01) higher in Diet 4 (3.03

mg/100g) than the rest of the diets. The antinutrient content of the control sample was significantly lower than those of the formulated diets. Plants are naturally high in antinutrients such as phytates, tannins and oxalates which limit the bioavailability and accessibility of essential nutrients (Abdel-Gawad *et al*., 2013). Antinutrients can also negatively affect protein and carbohydrate digestibility and bioavailability in infant foods. Despite high mineral content in infant foods, their bioavailability in the body might be compromised by the presence of antinutrients (Lazarte *et al*. 2015). This effect may be worsened when diets are marginal for nutritional adequacy. Previous reports from Sandberg (2002) indicate that cereals and legumes in particular have high content of minerals, but their bioavailability is deprived due to the presence of phytic acid, which is a main inhibitor for Fe and Zn absorption. One of the important approaches to increase mineral bio-accessibility is to reduce the amount of antinutrients like phytate, tannin and oxalate in the foods. Oxalate forms complexes with calcium, magnesium and iron leading to the formation of insoluble salts that reduce absorption and bioavailability of the respective minerals. High concentration of oxalate in the food can reduce calcium absorption to such an extent that there is a risk of developing kidney stones. The oxalate content of the cereal-legume diets in this study ranged from 0.62-11.03 mg/100g. These values were below the range (16.4-36.0 mg/100g) reported by Suma and Urooj, (2011). The oxalate content obtained in this study was <50 mg/100 g, falling in the range of low oxalate foods. The tannin content in this study was found to be very low compared to that reported by Desalegn (2015) (35.61-162.82 mg/100g) in soaked and germinated chickpea flour. In our study, the phytate content in all diets was found to be within the acceptable level for human consumption (Onomi *et al*., 2004). The phytate content in our study was lower than that reported by Gibson *et al*. (2010) who observed content of 600 mg/100 g in unprocessed cereals and legumes-based complementary foods.

Generally, all the antinutrients were found to be low in all the formulated diets. This is in line with the previous findings that household processing strategies such as soaking, dehulling, germination, roasting and milling processes of ingredients are effective in reducing the content of antinutrients in foods (Gibson *et al*., 2010; Kalpanadevi and

Mohan, 2013; Desalegn, 2015). During germination phytate ions are leached out in to the soaking water and increase phytase activity. Enzymatic hydrolysis of phytic acid due to increased phytase activity during sprouting decreases phytic acid content and also releases soluble proteins and minerals (Shah *et al*., 2011). In addition, since most the tannins are located in the testa of legumes, its dehulling process reduced tannin content significantly.

Table 4.7: Mean antinutritional content (mg/ 100 g) of the formulated and control diets

Antinutrients	Diet 1	Diet 2	Diet 3	Diet 4	Control diet
Phytate	14.41 ± 12.49^{c}	0.00 ± 0.00^{a}	9.62 ± 10.35^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Total oxalate	9.95 ± 2.31^{c}	11.03 ± 2.83^{c}	7.85 ± 0.43^{b}	7.84 ± 0.82^{b}	0.62 ± 1.07^{a}
Tannin	0.77 ± 0.80^{a}	1.81 ± 0.61^{a}	1.11 ± 0.53^{a}	3.03 ± 0.81^{b}	0.91 ± 0.31^{a}

Each value is mean±standard deviation of three replications on dry matter basis. The different superscript letters in the same horizontal row indicates significantly different ($p<0.05$).

4.5.1 Bioavailability of minerals

The bioavailability of minerals in the formulated diets is presented in **Table 4.8**. The bioavailability of minerals depends on the amount of antinutrients and the antinutrients/minerals molar ratio (Suma and Urooj, 2011; Makori *et al*., 2017). As the molar ratio increases, the inhibitory effect of mineral absorption increases. The molar ratios for [phytate]/ [iron], [Phytate]/ [Zinc], [Phytate]/ [calcium], [tannin]/ [iron] and [oxalate]/ [calcium] were calculated to determine the effect of antinutrients on mineral bio-accessibility. Accordingly, all sample diets had a [phytate]/[iron] and [phytate]/ [zinc] molar ratio below the cutoff value of 1 and 15, respectively, which is within the acceptable range (Ojinnaka *et al*., 2016). The [phytate]/[calcium] and [oxalate]/[calcium] molar ratios of the formulated and control samples were below the recommended values of 0.16 and 1.0 respectively (Davies, 1979; Umeta *et al*., 2005).

This indicates that mineral absorption and bioavailability was not adversely affected in the formulated diets. The molar ratios of Phytate: Calcium and Oxalate: Calcium are of significance when they are greater than the recommended critical values. In such cases, oxalate and phytate have a potential to complex calcium thus impairing its absorption (Davies, 1979).

Table 4.8: Antinutrient/mineral molar ratios of the formulated diets

ANF:Mineral	Diet 1	Diet 1	Diet 3	Diet 4	Control diet
Phytate:Iron	0.11	0.00	0.10	0.00	0.00
Phytate:Zinc	0.31	0.00	0.11	0.00	0.00
Phytate:Calcium	0.02	0.00	0.003	0.00	0.00
Tannin:Iron	0.001	0.0002	0.0002	0.002	0.0002
Oxalate:Calcium	0.12	0.03	0.02	0.07	0.003

Whereby: ANF-antinutritional factors

4.6 Protein quality and growth response of rats to experimental diets

Results of the cumulated food and water intake of animals placed on experimental and control diets are presented in figures **4.2** and **4.3**, respectively. In both cases, the experimental animals placed on Diet 3, Diet 4, casein, and cerifam consumed significantly higher quantities of water and food. This was also reflected in the weight change of the animals in which rats in this group of diets showed a higher mean growth rate than the rest of the diets. Rat groups placed on the protein-free diet had significantly lower food and water intake than the rest of the diets. Food intake is determined by the ability of the foods to satisfy the needs, as well as the body's growth and development requirement of the animal (Adeoti et *al*., 2018). The higher food intake in the protein-

containing diets might be attributed to the improved flavor of the foods due to the processing of the ingredients, the presence of aromatic amino acids, and its palatability.

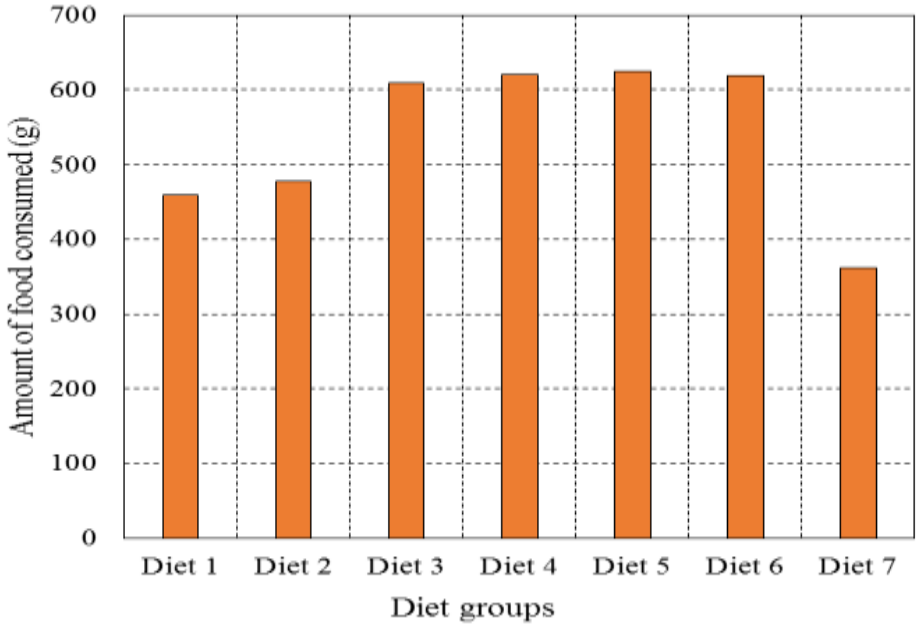


Figure 4.2: **Foods consumed by animals over the experimental period of 28 days. Where diets 1-4 described in table 3.1and diet5= Cerifam (commercial control), diet 6=Casein control and diet 7= protein-free diet (Starch))**

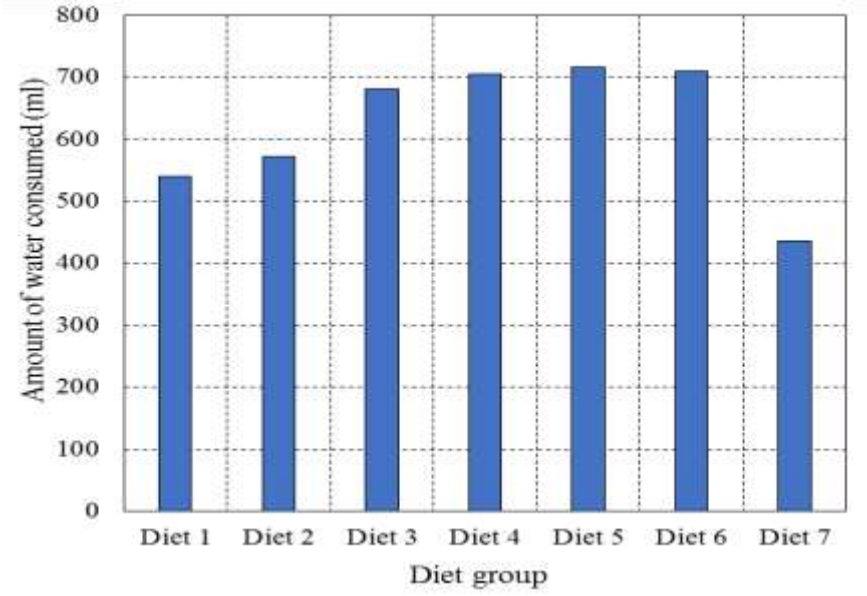


Figure 4.3: Water consumed by animals over the experimental period of 28 days.

The growth response and weight change of the experimental animal is depicted in **Table 4.9**. There was an increase in the average body weight gain in all the treatment groups showing that the rats were utilizing the diets during the experimental period. The mean weight gain of the experimental rats ranged from 12.08 g to 53.00 g. Weight gain was influenced by the protein quality and the amount of food consumed (Abiose *et al*., 2015). It was also observed that the mean growth rate of animals fed on Diet 3 and Diet 4 was significantly higher than those fed on Diet 1, Diet 2, and diet 7 (protein-free diet). Mean body weight gain of animals fed on Diet 3 and Diet 4 was as good as gain in body weight of animals fed on the commercial diet (cerifam) and casein. Better weight gain attained by rats fed on Diet 3 and Diet 4 may be attributed to the inclusion of chickpea and soybeans that contains high quality protein that is not found in cereals. Protein is required for fast growth, healthy life, and better tissue and body cell production and maintenance. The experiment revealed that the least body weight gain of the growing animals was recorded in the protein-free diet (12.08 g), and this might be because of less food and protein consumption. This is in line with the previous reports that cereals are low in some essential amino acids like lysine and tryptophan (Dayiya and Kapoor, 1993; Milan-Crrillo *et al*., 2007). There was no mortality records in all groups of animals

fed on the formulated and control diets. Besides, no side effects, such as diarrhea or emesis, were observed in animals consuming these formulations. These observations suggest that diets 3 and 4 can provide adequate nutrients to enhance the higher growth rate/growth pattern in rats without causing any harmful effects, as indicated in **Figure 4.4**.

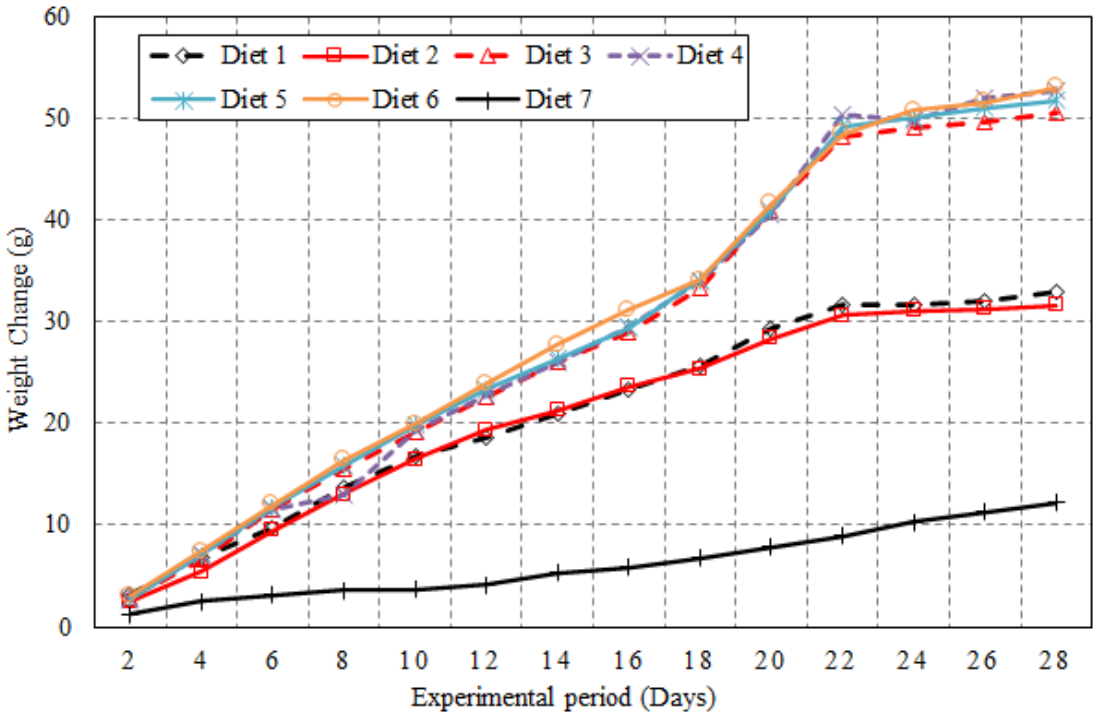


Figure 4.4: Growth response of weanling albino rats fed with the formulated and control diets (mean values of 6 animals per group). Where diet 5= Cerifam (commercial control), diet 6=Casein control and diet 7= protein-free diet (Starch)

The results of the protein efficiency ratio (PER) and feed efficiency ratio (FER) of the experimental animals are shown in **Table 4.9**. The PER and FER of the formulated and control diets ranged from 1.20 to 2.43 and 0.02 to 0.087, respectively. It was observed that the PER and FER values of rats fed on Diet 3, Diet 4, cerifam (commercial diet), and casein were significantly higher than those fed with Diet1, Diet 2, and Diet7

(protein-free diet). Incorporating chickpea and soybean flour into the diets may have significantly improved the PER of Diet 3 and Diet 4, which is favorably similar with the PER of casein and cerifam. No significant difference (p=0.57) was observed in PER values among Diet 3, Diet 4, cerifam, and casein. The protein efficiency ratio is a widely used method for evaluating the quality of protein in a food (FAO, 2013). A food with a higher protein efficiency ratio is believed to be superior to a food with lower PER. The feed efficiency ratio (FER) of the diets showed similar trend with the PER. In this study, the PER value obtained was higher than the values reported previously by Folorunso *et al*. (2019). However, these values were lower than those reported by Ajibola *et al*. (2016) for complementary foods formulated from maize gruel 'ogi' and crayfish using combined traditional processing technology. The U.S Department of Agriculture and the Protein Advisory Group (PAG) guidelines recommend a PER of not less than 2.1 and preferably greater than 2.3 for corn-based blends and complementary foods (Abiose *et al*., 2015; Ahmad *et al*., 2013; PAG, 1971).

The results of the biological value (BV), net protein utilization (NPU), and true digestibility of the formulated and control diets ranged from 54.53 to 69.48%, 65.62 to 70.21%, and 59.01 to 64.01% respectively. Diet 3, Diet 4, cerifam and casein had significantly higher biological value than Diet 1 and Diet 2. Moreover, Diet 3 and Diet 4, which contains chickpea and soybean, had biological value comparable with the commercial diet and casein. The biological value assesses the ability of the protein to support growth by holding the protein in the body. In other words, BV is directly related to the efficiency of the protein utilization. The mean NPU, and TD values of Diets 3 and Diet 4 were not significantly different (p=0.62) from the control group, but were significantly (p=0.02) higher when compared to Diet 1 and Diet 2. The values reported for BV, NPU, and TD in this study were lower than the values reported by Ajibola *et al*. (2016). The net protein utilization is defined as the percentage of nitrogen uptake, and allows the effectiveness of the protein to be assessed for the normal growth and development. Based on the results of this study, the values of NPU were directly proportional to the products of biological value and true digestibility. In general, all the

formulated diets showed better BV, NPU, and TD values. This may have been due to the higher protein consumption of the rats, the higher content of lysine and tryptophan (PAG, 1971), and reduction of antinutritional factors though the combined traditional processes.

Table 4.9: The protein quality and growth response of rats fed on the control and blended diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Food intake (g)	459.40	478	609.80	620.40	625.33	618.57	361.67
Water intake(ml)	540.80	571.80	681.50	705.60	716.30	710	435.60
Feacal nitrogen	1.07±0.06^{b}	1.10±0.10^{b}	1.41±0.01^{c}	1.43±0.01^{c}	1.46±0.01^{c}	1.45±0.01^{c}	0.15±0.01^{a}
Urinary Nitrogen	0.20±0.01^{b}	0.21±0.01^{b}	0.23±0.01^{c}	0.24±0.01^{c}	0.24±0.01^{c}	0.23±0.01^{c}	0.01±0.00^{a}
Initial weight	35.60±2.34^{a}	35.52±2.09^{a}	36.00±1.87^{a}	36.00±2.39^{a}	36.00±2.21^{a}	35.95±2.37^{a}	35.58±2.06^{a}
Final weight	68.52±2.54^{b}	67.13±2.24^{b}	86.45±3.41^{c}	88.87±2.78^{c}	87.93±4.22^{c}	88.95±3.01^{c}	47.67±2.99^{a}
Weight change	32.92±1.53^{b}	31.62±1.81^{b}	50.45±2.84^{c}	52.87±2.99^{c}	51.88±3.28^{c}	53.00±3.58^{c}	12.08±2.08^{a}
PER	1.77±1.53^{b}	1.64±1.81^{b}	2.31±2.84^{c}	2.37±3.07^{c}	2.32±3.28^{c}	2.43±3.58^{c}	1.20±1.83^{a}
FER	0.06±1.53^{b}	0.06±1.81^{b}	0.08±2.84^{c}	0.08±3.07^{c}	0.08±3.28^{c}	0.087±3.58^{c}	0.02±1.83^{a}
BV (%)	54.53±3.97^{a}	54.55±7.44^{a}	66.48±1.35^{b}	65.62±2.68^{b}	68.04±0.31^{b}	69.48±2.4^{b}	Nil
NPU (%)	65.62±1.93^{a}	66.02±3.66^{a}	70.21±0.91^{b}	70.01±0.33^{b}	69.78±0.32^{b}	69.06±0.44^{b}	Nil
TD (%)	59.01±1.85^{a}	59.22±3.47^{a}	63.86±0.65^{b}	64.01±0.76^{b}	63.34±0.06^{b}	62.74±0.72^{b}	Nil

Values are means ± SD of 6 animals per group for assay period of 28 days. The different superscripts in the same horizontal rows with different letters are significantly different ($p<0.05$). Diet 5= cerifam, diet6= casein, diet7= protein-free die (Starch)

4.7 Influence of the experimental and control diets on biochemical indices of rats

Table 4.10 present serum biochemical profiles of rats fed on the formulated and control diets. The total serum protein ranged from 4.10 to 7.11g/dL, albumin concentration 2.37 to 4.73 g/dL, globulin 0.6 to 2.63 g/dL, total bilirubin 0.04 to 0.19 mg/dL and total glucose 94.33 to 99.67 mg/dL. Mean separation using LSD showed significant differences in the concentrations of albumin, total protein, globulin, and total bilirubin in rats fed with the experimental diets. This observation may be attributed to the variation

in the quantity and quality of protein in the experimental food samples. However, no significant difference was observed in glucose concentration in the blood of rats fed on different diets. Serum albumin and total protein levels of experimental rats fed on Diet 3, Diet 4, cerifam, and casein were significantly higher than those fed on Diets 1, Diet 2, and protein-free diet. The higher total protein, albumin, and globulin obtained for rats fed on diets 3, 4, cerifam and casein might be due to increased protein content of the diets as well as higher crude protein intake. The reference range of total protein and albumin in human serum range from 6-8 g/dL and 4.4-5.3 g/dL, respectively (Giannini *et al*., 1999; Bender, 1965). Consequently, among the test diets, the values of Diet 3, Diet 4, Diet 5 (control), and Diet 6 (casein) were within the normal range for total protein and albumin. Therefore, it could be further inferred that the protein content of Diet 3 and Diet 4 in the present study are of quality, and are suitable to meet protein requirement of its consumers. Nevertheless, rats fed with Diet1, Diet 2, and Diet 7 recorded serum protein and albumin levels below the reference range. This implies that these diets were unable to maintain normal circulating levels of albumin and protein. This might be due to the lower protein nature of the plant source. It is expected that the bioavailability of plant protein is generally lower compared to proteins from animal-source, and the concentration of plasma proteins, especially albumin, depends on the amount of protein intake and its quality (Fujita *et al*., 1978). The decrease in serum protein may be an indication of the impaired synthetic function of the liver. The serum globulin content of rats fed on Diet 3 (2.33g/dl), Diet 4 (2.27 g/dl) and the control diet (2.63 g/dl) was significantly ($p<0.05$) higher compared to rats fed on Diet 1(1.73 g/dl), Diet 2 (1.97 g/dl), casein (1.47 g/dl) and Diet 7 (0.6 g/dl).

Table 4.10: Serum biochemical parameters of rats fed on the diets

Serum parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Total protein (g/dL)	4.1±0.20^{a}	4.21±0.15^{a}	7.11±1.17^{b}	6.93±0.97^{b}	7.03±2.50^{b}	6.90±0.53^{b}	4.10± 0.60^{a}
Glucose (mg/dL)	98.67±058^{a}	98.00±1.00^{a}	96.4±4.76^{a}	95.07±4.47^{a}	96.33±4.93^{a}	94.33±2.88^{a}	99.67±0.58^{a}
Albumin (g/dL)	2.37±0.38^{a}	2.51±0.21^{a}	4.73±0.93^{b}	4.50±0.10^{b}	4.4±1.55^{b}	4.67±0.57^{b}	2.11±0.21^{a}
Globulin (g/dL)	1.73±0.25^{ab}	1.97±0.21^{ab}	2.33±0.38^{b}	2.27±1.16^{b}	2.63±0.96^{b}	1.47±1.27^{ab}	0.6±0.52^{a}
T Bilirubin (mg/dL)	0.06±0.01^{a}	0.07±0.01^{ab}	0.12±0.05^{ab}	0.19±0.11^{b}	0.14±0.02^{ab}	0.16±0.12^{ab}	0.04±0.01^{a}
Creatinine (mg/dL)	0.23±0.02^{a}	0.35±0.1^{ab}	0.48±0.13^{b}	0.49±0.08^{b}	0.36±0.11^{ab}	0.34±0.06^{ab}	0.31±0.12^{ab}
Urea (mg/dL)	6.27±0.05^{a}	6.75±0.17^{a}	9.64±0.1^{b}	9.94±0.14^{b}	6.14±0.07^{a}	10.12±0.07^{b}	5.84±0.03^{a}

Values are means ± SD of three determinations. The different superscripts in the same horizontal rows with different letters are significantly different ($p<0.05$). Diet 5= cerifam, diet6= casein, diet 7= protein-free diet (starch)

Creatinine and urea are nitrogenous end products that are transported through the bloodstream and excreted by the kidneys. Healthy kidneys remove creatinine and urea from the blood. However, creatinine and urea blood level rises with kidney failure that is the higher the creatinine and urea values the less effective the kidney filtration (RusulArif and Haider, 2014). Creatinine levels of rats fed on the developed diets ranged from 0.23 mg/dL in Diet 1 to 0.49 mg/dL in Diet 4, while for the controls cerifam and casein was 0.36 mg/dL and 0.34 mg/dL respectively. The concentration of urea in the serum of rats fed on the control and test diets ranged from 5.84 mg/dL to 10.12 mg/dl. Serum urea level of rats was significantly ($p<0.05$) higher in Diet 3, Diet 4, and casein than the rest of the test diets. Among all the treatments evaluated, the creatinine level of rats fed on Diet 3 and Diet 4 was found to be higher than others. In this study, the creatinine level was lower than that reported earlier (Hasan *et al*., 2018). Comparatively, the creatinine and urea levels of rats fed on the control and formulated diets were within the normal range and consistent with the findings reported in another study (Giannini *et al*., 1999). This suggests that both the control and test diets had no toxic chemicals that

may have negative side effects on the consumers. Hence, the diets may be suitable for human consumption.

4.8 Haematological indices of rats fed on the formulated and control diets

The Haematological properties of Albino Wistar rats fed on formulated and control diets are presented in **Table 4.11**. The blood indices varied in terms of hemoglobin concentration (Hbc) 9.97-15.37 g/dL, packed cell volume (PCV) 42.2-52.2%, white blood cells (WBC) 5.40-10.9x10^9/L, red blood cells (RBC) 8.18-9.87x10^{12}/L, and platelets 513.67- 643x10^9/L. Lymphocytes and neutrophils ranged between 42.40-64.43% and 22.27-25.97%, respectively. The values of the mean cell hemoglobin concentration (MCHC), mean concentration hemoglobin (MCH), and mean cell volume (MCV) for the control and formulated diets ranged from 20.97-31.17 g/dl, 13.07-16.50 pg, and 40-54.33 FL, respectively. Haematological properties provide important information on the health and nutritional status of an animal. The haematocrit percentage (PCV) and haemoglobin concentration are believed to be a convenient and rapid measure of the degree of anemia (Abiose *et al*., 2015).

Progressive stages of iron deficiency in humans are linked to a significant reduction in blood haemoglobin levels. There was no significant difference in the haemoglobin content of rats fed on different diets. While, the protein-free-diet exhibited significantly lower haemoglobin level as compared to the controls and developed diets. This is in line with the reports of rats fed a maize-based complementary diet enriched with fermented and germinated moringa seed flour (Adeoti *et al*., 2018). For human subjects, the cut-off levels for infants and pregnant women are 11 g/dL (Hercberg *et al*., 1991). The recommended levels of haemoglobin for healthy rats range from 10-15 g/dL (Baskaran *et al*., 2001). Therefore, all the complementary diets except for Diet7 exhibited haemoglobin levels in rats fed on them above the cut-off point for children. Comparatively, the PCV values for rats fed on commercial formula and Diet 3 were significantly ($p<0.05$) higher than in those fed on other diets. The PCV values of all diets

were comparable with the values reported in a study that included rats fed with millet and maize-based complementary foods (Umar *et al*., 2010).

Packed cell volume is an indicator of the capacity for transportation of oxygen and absorbed nutrients, thus increased PCV shows better transportation capacity (Isaac *et al*., 2013). It was observed that rats fed on the control and developed diets showed higher PCV values above the cut-off point. This implies that there a better oxygen transportation in rats fed on those diets. The cut-off levels in humans range from 32% in children aged 0.5 to 4 years to 40% in men above 15 years (Hercberg *et al*., 1991). In this study, the PCV and haemoglobin values of the control and formulated diets were higher than those reported by Ajibola *et al*. (2016). The high concentration of PCV, Hbc and RBC in this study further established the protein quality of the formulated diets. Previous findings indicate that diets containing quality protein and iron usually boost production of haemoglobin and immunity in animals, while low quality protein food lead to poor production of red blood cells and haemoglobin, hence, leading to anaemia (Ijarotimi and Keshinro, 2012; Oluwajuyitan and Ijarotimi, 2019).

Table 4.11: Haematological indices of rats fed with the formulated and control diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Hb (g/dl)	13.40±2.14^{b}	13.83±0.49^{b}	15.20±0.1^{b}	13.9±3.14^{b}	15.37±0.55^{b}	14.7±1.45^{b}	9.97±1.28^{a}
PCV (%)	42.65±4.05^{a}	46.47±1.50^{ab}	52.20±0.7^{b}	42.2±7.85^{a}	50.35±2.25^{b}	45.40±1.10^{ab}	49.40±3.5^{ab}
WBC (x10$^{9/L}$)	8.43±1.50^{b}	8.36±1.62^{b}	10.93±5.12^{b}	9.03±0.50^{b}	9.87±1.55^{b}	9.50±1.47^{b}	5.40±1.01^{a}
RBC (X10$^{12/L}$)	8.18±0.58^{a}	9.09±0.12^{ab}	9.87±0.72^{b}	8.28±1.46^{a}	9.20±0.83^{ab}	8.33±0.25^{a}	8.94±0.73^{ab}
Platelet (x10$^{9/L}$)	544±98.00^{ab}	513.67±61.32^{a}	557±10.44^{abc}	611.67±2.08^{bc}	559.67±5.85^{abc}	585±40.93^{abc}	643±3.00^{c}
MCHC (g/dl)	30.60±2.34^{b}	29.77±0.23^{b}	29.07±0.60^{b}	30.67±0.55^{b}	30.97±0.49^{b}	31.17±1.07^{b}	20.97±1.21^{a}
MCH (pg)	15.07±0.90^{b}	15.23±0.32^{bc}	15.50±1.2 2^{bc}	16.33±0.64^{bc}	16.40±0.52^{bc}	16.50±0.50^{c}	13.07±0.51^{a}
MCV (FL)	53.23±2.25^{b}	51.1±0.98^{b}	52.57±2.74^{b}	51.97±2.45^{b}	52.90±0.82^{b}	54.33±1.07^{b}	40.00±5.16^{a}
LYM (%)	42.40±1.31^{a}	57.73±1.43^{b}	61.43±6.96^{b}	64.50±0.20^{b}	62.3±5.74^{b}	64.43±0.58^{b}	52.63±11.94^{ab}
NEU (%)	22.27±0.75^{a}	24.13±1.00^{bc}	24.47±0.47^{cd}	25.50±0.92^{cd}	25.97±0.25^{d}	25.97±0.15^{d}	22.67±1.53^{ab}

Values are means ± SD of three determinations. The different superscripts in the same horizontal rows with different letters ar significantly different ($p<0.05$). Diet 5= cerifam, diet6= casein, diet7= protein-free diet

The white blood cells and lymphocytes are important indices of infection, toxicity and immunity status. There was no significant difference in the WBC count of casein, commercial formula, and formulated diets. However, the WBC of the protein-free diet was lower than the WBC of all other diets. White blood cells are important in defending the body against infections. Several factors are involved in the reduction of WBC count, which includes protein-energy malnutrition, viral infections, and drugs that destroy white blood cells. Normal white cell counts range from 5 to 9x10^{9}/L (Skala *et al*., 1981). The rats fed on all the diets had white blood cell and lymphocyte counts within the normal range. This indicates that the diets were free of toxic chemicals and infectious microorganisms; hence, it is suitable for consumption. This is lower than the findings of an earlier report (Umar *et al*., 2010). The mean MCHC, MCH, and MCV values of the experimental rats fed on casein, commercial, and formulated diets were significantly higher than those fed on protein- free diet. The MCHC, MCH, and MCV levels are major indicators of the occurrence of anemia and a low level of these indicators is a sign of anemia. The values obtained in this study showed that the rats fed on these diets were not at risk of anaemia.

4.9 Serum lipid and liver enzyme profiles of rats fed on the diets

The transaminases (ALT and AST) are usually used as specific markers of active hepatic injury and hepatocellular necrosis (Simon-giavarotti *et al*., 2002; Oluwajuyitan and Ijarotimi, 2019). When body tissues are injured due to any reason, extra AST, ALT, and ALP are released in the bloodstream, thereby raising the respective serum enzyme level (Hasan *et al*., 2018; Aliyu et al., 2007). Consequently, the level of AST, ALT, and ALP in the blood is directly related to the amount of tissue toxicity or damage. The values of serum AST, ALT and ALP in rats fed on the control and formulated diets are shown in **Table 4.12**. It was observed that the ALT values of rats fed on the control and formulated diets ranged from 22.03 IU/L in Diet 2 to 37.76 IU/L in Diet 4 and were not significantly different from each other. Moreover, the ALT values of all the treatments were within the normal range (10 to 40 IU/L) for Human subjects (Sharp and Regina, 1998). The AST values ranged between 20.03 IU/L in Diet 2 to 49.57 IU/L in Diet 6. The AST values of rats fed on different diets were significantly ($p=0.01$) different. The AST values of rats fed on Diet 1, Diet 2, and Diet 7 were below the cut-off point whereas the values were within the reference range for Diet 3, Diet 4, and the control diets. Patterns in which enzymes are normal or abnormal can offer meaningful clues about an underlying problem. Since enzyme levels in the blood can rise when cells are damaged, very high levels of AST often reflect short-term liver damage while smaller but persistent elevations in AST over time can be tied to chronic conditions like cirrhosis or hepatitis (Hasan *et al*., 2018). The low activities of ALT and AST in serum of rats observed seem to indicate that the formulated diets may not exert adverse effect on the liver during the feeding trial. ALP is used as the marker of obstructive jaundice and intrahepatic cholestasis and it is also a marker of kidney, bones and placenta. The ALP enzyme levels were in the range of 24.10 IU/L in Diet 1 to 130 IU/L in Diet 4. Comparatively, a significant ($p<0.05$) difference was observed in the levels of ALP enzyme in rats fed on different diets. Nevertheless, all the values were within the reference limits for human beings (Giannini *et al*., 1999). This may suggest that the test diets are suitable for consumption and will not affect liver cells, as none of the values for

ALT and AST in the serum were higher than the reference range for human beings, which could be an indication of liver damage (Oluwajuyitan and Ijarotimi, 2019; Aliyu et al., 2007).

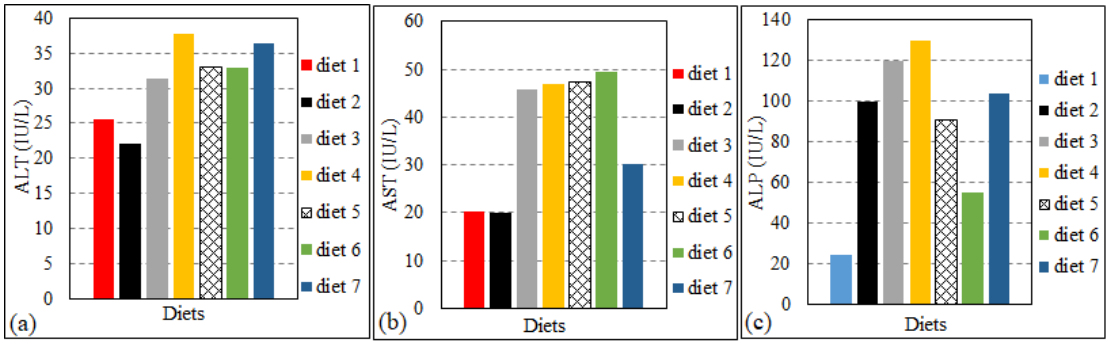


Figure 4.5: Serum ALT (a), AST (b), and ALP (c) levels in weanling albino rats fed on the developed and control diets. Values are expressed in mean ± SD. n=3, *p<0.05, where Diet 5= commercial control (Cerifam), Diet 6=casein control, Diet 7= protein-free die (Starch)

The lipid profile of rats fed on the test diets is presented in **Table 4.12**. There was no significant (p=0.66) difference in total cholesterol values in rats fed on the control and experimental diets. However, Diet 7 (negative control) was found to have significantly lower total cholesterol content ranging between 30 mg/dL to 59 mg/dL compared to the rest of the diets. Triglyceride concentrations ranged from 46.33 mg/dL to 75.33 mg/dL. No significant (p=0.58) difference was observed in triglyceride concentrations of rats fed with the control and formulated diets. The overall mean serum HDL ranged between 18.67 mg/dL to 35.33 mg/dL. The mean serum HDL differed significantly (p=0.02) between rats fed with the protein-free Diet 7 (18.67 mg/dL) and Diet 3 (35.33 mg/dL), while diets 1, 2, and 4 did not differ significantly from each other and the control. The mean serum LDL ranged from 1.67 mg/dL to 16.53 mg/dL. However, there was no significant (p=0.65) difference in LDL levels among the treatments. For the total

cholesterol: HDL ratios, there were no statistical differences between the control and the formulated diets. For adults and children, the reference ranges for TC, TG, HDL and LDL are 122.7-187.1, 62.0-115.9, 32.2-52.8 and 69.8-122.5 mg/dL respectively (Durgawale *et al*., 2009). All the lipid profiles of rats fed on the control and formulated diets were below the reference ranges. High cholesterol, triglyceride and LDL levels above the reference range can increase the risk of heart diseases due to fat deposits that make blood flow difficult along the arteries (Durgawale *et al*., 2009. However, the metabolism of lipids by itself could be affected by age. During ageing a significant increase in lipoprotein levels usually occurs, mostly, in LDL levels. However, in this study the lipid profile of the weanling rats were very low, may be due to their young age.

Table 4.12: Serum lipid and liver enzyme profiles of rats fed on the control and formulated diets

Biomolecules	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Cholesterol (mg/dL)	53.33±2.08^{b}	57.33±8.14^{b}	50.33±11.55^{b}	57.33±10.68^{b}	43±2.00^{b}	59±20.07^{b}	30±10.44^{a}
Triglyceride (mg/dL)	66.33±10.69^{b}	75.33±0.58^{b}	65.67±8.39^{b}	67±8.50^{b}	61.67±10.41^{b}	64.00±9.16^{b}	46.33±1.53^{a}
HDL (mg/dL)	29.67±4.04^{ab}	26.67±5.13^{ab}	35.33±5.13^{b}	29.33±4.60^{ab}	29±1.73^{ab}	29.67±9.81^{ab}	18.67±10.78^{a}
LDL (mg/dL)	10.50±4.05^{a}	15.60±11.11^{a}	1.87±14.87^{a}	14.47±13.07^{a}	1.67±4.16^{a}	16.53±10.10^{a}	11.80±19.73^{a}
TC: HDL ratio	1.80±0.31^{a}	2.20±0.70^{a}	0.83±0.76^{a}	2.53±1.36^{a}	1.43±0.15^{a}	1.93±0.35^{a}	2.68±1.80^{a}
ALT (IU/L)	25.50±10.96^{a}	22.03±5.71^{a}	31.33±8.96^{a}	37.76±7.88^{a}	32.93±11.81^{a}	33.00±12.16^{a}	36.33±4.16^{a}
AST (IU/L)	20.23±1.50^{a}	20.03±1.00^{a}	45.83±1.89^{c}	46.87±1.27^{cd}	47.43±1.51^{cd}	49.57±1.4^{d}	30.20±2.40^{b}
ALP (IU/L)	24.10±0.95^{a}	99.8±18.47^{bc}	119.67±18.93^{c}	130±3.61^{c}	90.67±51.43^{bc}	54.67±50.64^{ab}	103.67±30.89^{c}

Values are means ± SD of three determinations. The different superscripts (a-d) in the same horizontal rows with different letters are significantly different ($p<0.05$) as assessed by Least significant difference and Duncan multiple range test. Diet 5= cerifam, diet 6= casein, diet 7= protein-free diet

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study found that ready-to-eat complementary food products made from locally available food commodities can meet the recommended dietary allowance for infants and children aged 6 to 12 months. The crude protein and energy content of all blended diets were statistically higher compared to the control diet and met or exceed the recommended dietary allowance for complementary foods. Among the composite diets, Diet 3 and Diet 4 were able to meet the minimum requirement of 10–25 percent fat for infant food. The mineral composition of the developed diets was better than the control diet and met RDAs for the minerals zinc and iron.

According to the findings of this study, adequate processing and formulation of locally available cereals and legumes, as well as modification with some vegetables, can provide nutritious foods that can be used as home-based complementary foods to combat protein-energy malnutrition. The formulated diets can be used to substitute the more expensive commercial formula products, and have a huge positive impact to use as complementary foods in poor rural and urban mothers.

The antinutritional factors detected in all the formulated diets were within tolerable levels, suggesting that they may not pose a significant problem to nutrient bioavailability and utilization.

The results of the diets' growth response and protein quality (PER, BV, NPU, TD) evaluation revealed that these diets were nutritious enough and comparable to standard casein and commercially available baby food (Cerifam).

Absence of toxic substances in these diets, as evidenced by liver function tests, biochemical and haematological findings, could also reveal that the diets could provide

adequate nutrients and support higher growth rates in infants without causing any harmful effects. Furthermore, in terms of nutrient quality and quantity, as well as haematological and biochemical indices and lipid profiles, diet 3 and diet 4 were better than the rest of the formulated diets and comparable with that of rats fed on standard casein and Cerifam.

5.2 Recommendations

a) It is recommended to exploit cheap and accessible cereal-legume sources accompanied with simple processing methods for the formulation of nutrient dense supplements to attain the need of those lower class families especially in rural areas.

b) The findings of this study recommends the use of various processing techniques (combined strategies) as the most appropriate method for reduction of anti-nutrients as these processing techniques are cheap, therefore can be administered in poor resource settings

c) Training on the adoption and processing of the blends is recommended for mothers and caregivers.

d) Mothers should be encouraged to complement their children with diets 3 and 4, which were found to be adequate in macronutrients and micronutrients. These diets may need to be fortified or supplemented in order to meet the recommended dietary allowances for calcium and magnesium.

e) More study is needed to determine the organoleptic and sensory quality, as well as the microbial analysis.

f) We have not addressed the in-vitro protein digestibility and availability, amino acid profile, and fatty acid content due to financial and time constraints; therefore, additional research is required to determine the in-vitro protein digestibility and availability, amino acid profile, and fatty acid content.

g) The effect of cooking on nutrients, as well as shelf-life studies of formulations, should be investigated.

REFERENCES

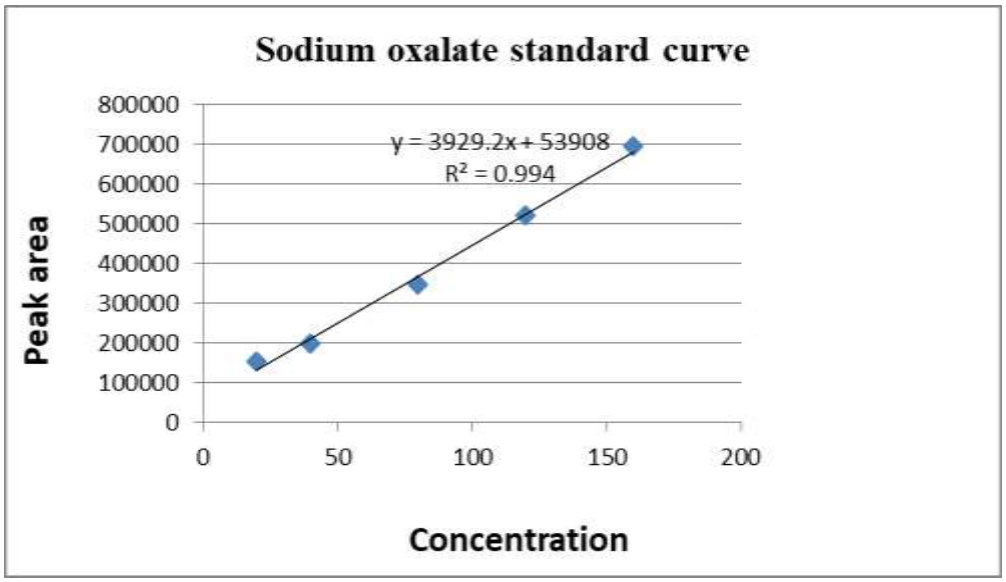
APPENDICES

Appendix I: Image taken during crude fiber analysis

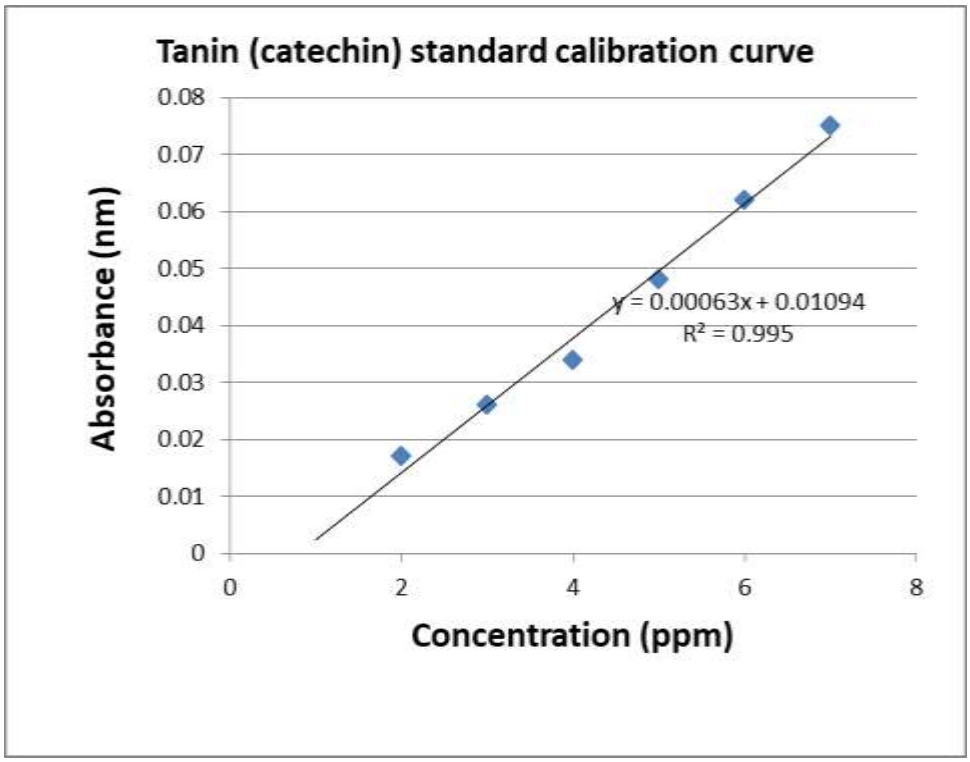
Appendix II: Images of Faeces and urine collection using metabolic cages for protein quality analysis

Appendix III: White albino rats during the feeding experiment

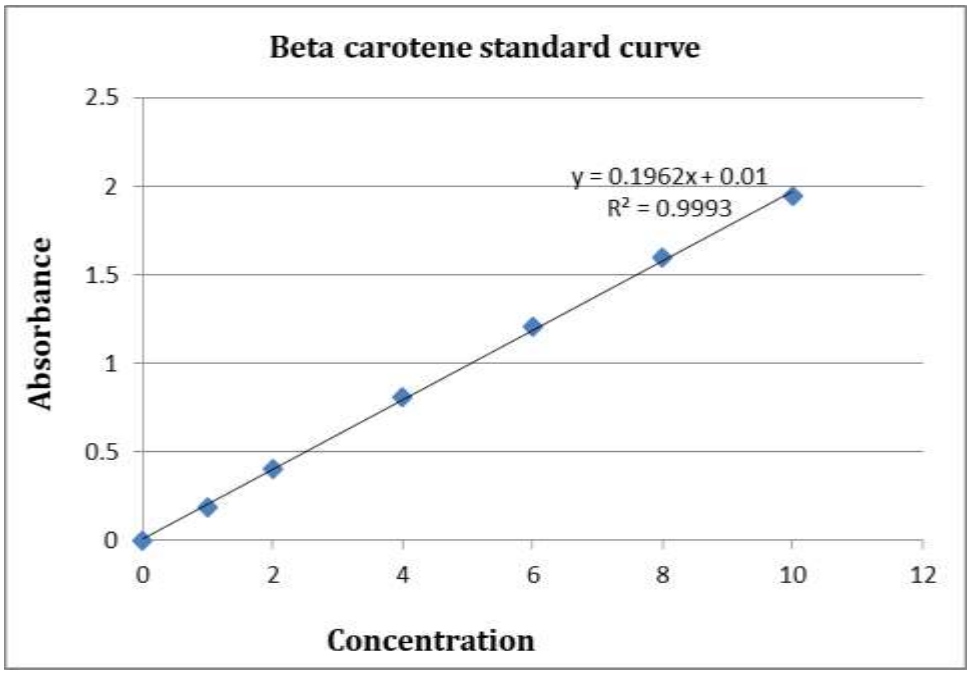
Appendix IV: Standard curves for oxalate antinutrient



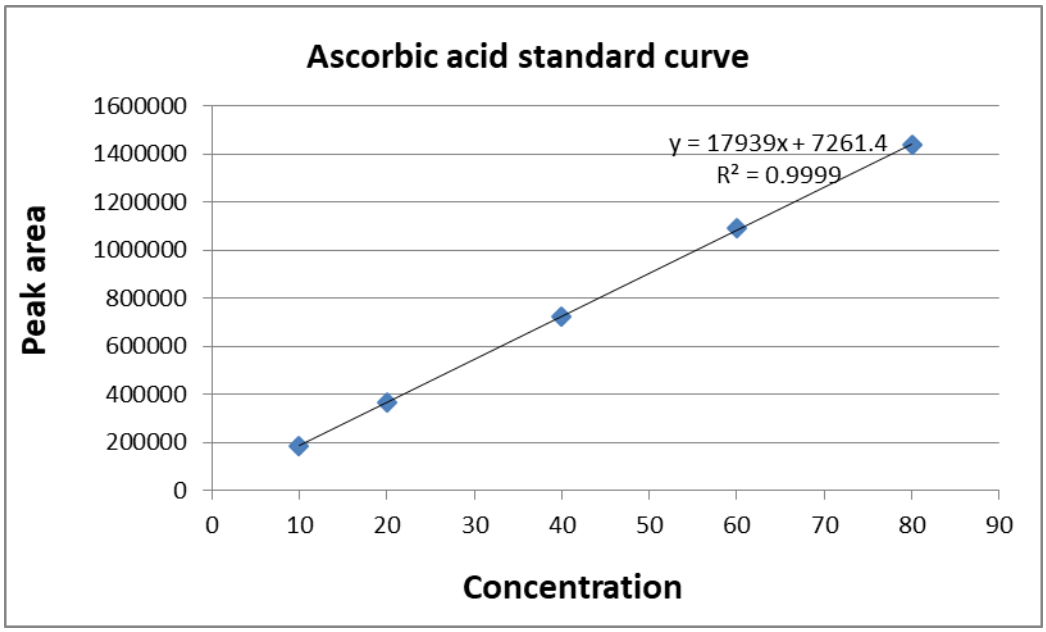
Appendix V: Tannin (catechin) standard calibration curves



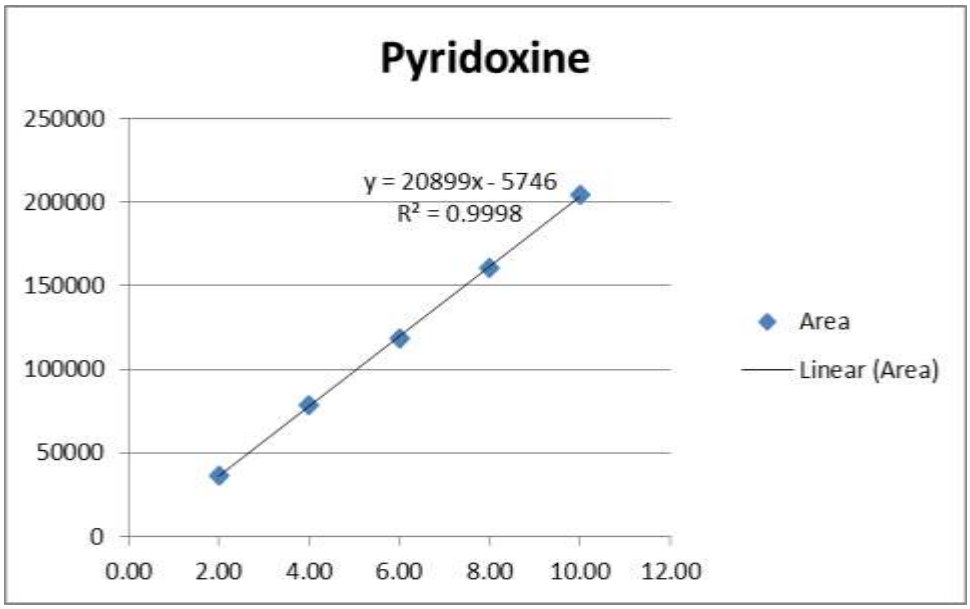
Appendix VI: Beta carotene standard curves



Appendix VII: Ascorbic acid standard curves



Appendix VIII: Vitamin B6 (pyridoxine) standard curve



Appendix IX: Publication

1. Yohannes, T. G., Makokha, A. O., Okoth, J. K., & Tenagashaw, M. W. (2020). Developing and nutritional quality evaluation of complementary diets produced from selected cereals and legumes cultivated in Gondar province, Ethiopia. *Current Research in Nutrition and Food Science Journal*, *8*(1), 291-302.
2. Yohannes, T. G., Makokha, A. O., Okoth, J. K., & Tenagashaw, M. W. (2021). Nutritional, Biochemical and Haematological Indices of White Albino Rats Fed Complementary Diets Developed from Selected Cereals and Legumes. *Current Nutrition & Food Science*, *17*(5), 523-531.