NTRITIONAL QUALITY AND SAFETY OF COMPLEMENTARY FOODS DEVELOPED FROM ETHIOPIAN STAPLE GRAINS AND HONEY BEE *(APIS MELLIFERA)* LARVAE: AN *IN-VIVO* STUDY USING BALB/C MICE MODELS

SHEWANGZAW ADDISU MEKURIA

DOCTOR OF PHILOSOPHY

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Nutritional Quality and Safety of Complementary Foods Developed from Ethiopian Staple Grains and Honey Bee *(Apis Mellifera)* Larvae: An *In-Vivo* Study Using BALB/c Mice Models

Shewangzaw Addisu Mekuria

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DECLARATION

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

Signature: …… Date……...…….……

Shewangzaw Addisu Mekuira

This thesis has been submitted for examination with our approval as the university supervisors.

Signature: …… Date……...…….……

Dr. John Kinyuru, PhD

JKUAT, Kenya

Signature: …… Date……...…….……

Dr. Beatrice Nyanchama Kiage Mokua, PhD

JKUAT, Kenya

Signature: …… Date……...…….……

Dr. Mesfin Wogayehu Tenagashaw, PhD

Bahir Dar University, Ethiopia

DEDICATION

This work is dedicated to my beloved wife Kibrnesh Tegenaw, my mother Mebrat Alemayehu, my son Yofitahi Shewangzaw, my daughter Akotit Shewangzaw, and my new born Eureka Shewangzaw for giving me an easy moment and praying for my success during my studies.

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LIST OF ABBREVIATIONS

AIP	Atherogenic Indices of Plasma
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Collaboration
CFs	Complementary Foods
CFU	Colony Forming Unit
CHD	Coronary Heart Disease
CHO	Carbohydrate
CRI-I	Castelli's Risk Indices-I
CRI-II	Castelli's Risk Indices-II
DM	Dry Matter
EBF	Exclusive Breast Feeding
EDHS	Ethiopian Demographic and Health Survey
EDTA	Ethylene Di-Amine Tetra Acetic Acid
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
HCT	Haematocrit
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein Cholesterol
HGB	Haemoglobin
HPLC	High-Performance Liquid Chromatography

IYCF	Infants and Young Children Feeding
LDL	Low-Density Lipoprotein
LDL-C	Low-Density Lipoprotein Cholesterol
LSD	List Significance Difference
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
NIH	National Institutes of Health
PCM	Protein Calorie Malnutrition
PEM	Protein Energy Malnutrition
PLT	Platelet Count
PUFAs	Polyunsaturated Fatty Acids
RBC	Red Blood Cells
RDA	Recommended Dietary Allowance
RE	Retinol
RV	Recommended Value
SD	Standard Deviations
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan Africa
T	Treatment
TC	Total Cholesterol
TG	Total Glyceride

UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency of International Development
WBC	White Blood Cell
WFP	World Food Programme
WHO	World Health Organization

ABSTRACT

In sub-Saharan Africa, poor complementary feeding practices, mainly nutritionally inadequate, are an important factor contributing to infant and child malnutrition, associated with stunting, and high morbidity and mortality. Therefore, novel complementary foods need to be developed to alleviate malnutrition problems in infants and young children. This study aimed to evaluate the nutritional quality and safety of complementary foods developed from Ethiopian staple grains and honeybee larvae (*Apis Mellifera*) using mice models. Three Ethiopian staple grains constituted the composite flours that were formulated with two different ratios. Samples of teff, maize, soybean, and bee larvae were ground into flour and blended before extrusion as follows: $ComF_{01}$ (57 % maize, 29 % teff, and 14 % soybean) and $ComF_{02}$ (58 % maize, 29 % teff, and 13 % bee larvae). NutriSurvey software (version, 2007) was used for calculate the composite flours ratios. The nutritional, microbial, and sensory analysis of the developed foods was conducted with standard methods, and an *in-vivo* study was conducted for growth performance and biochemical assessment using mice. A complete randomized design was used, and a total of 75 BALB/c mice were assigned to each of the five treatments. The treatments were: T1 = Casein diet; T2 = 57 % Maize, 29 % Teff, 14 % Soybean; T3 = 58 % Maize, 29 % Teff, 13 % Bee larvae; T4 = Commercial wean mix; and T5 = Basal diet alone (corn starch (610 g/kg), wheat bran (50 g/kg), vegetable oil (100 g/kg), mineral and vitamin premix (50 g/kg), glucose (60 g/kg), oyster shell (20 g/kg), sucrose (88 g/kg), bone meal (20 g/kg), and NaCl (2 g/kg)). The *in-vivo* experiment trial was done for 28 days along with seven days of acclimatization. The data were analyzed using IBM SPSS version 23. The proximate composition of moisture, fiber, fat, carbohydrate, and energy was significantly different ($P<0.001$) between the developed foods. $ComF_{02}$ recorded the highest fat, energy, vitamin A, B3, and B9 content, which were 14.3 g/100g, 427.18 kcal/100g, 706 µg/100g, 8.2 mg/100g, and 86.7 mg/100g, respectively, while the highest protein content (12.56 g/100g) was in $ComF_{01}$. $ComF_{02}$ has the highest iron (40.94 mg/100g) and calcium (68.20 mg/100g) content. The highest tannins and phytate content were recorded on $ComF_{01}$. The microbial and sensory quality of the developed complementary foods were safe and acceptable. An *in-vivo* mice model study revealed that dietary intake was not significantly different ($P = 0.96$) between treatments; however, T2 (38.39 g) and T3 (38.52 g) had gained the highest final body weight. The highest spleen weight was recorded on T2 (0.53 g). Biochemical parameters (mg/dl) of T4 had the lowest serum protein (6.27) and globulin (3.61). T3 significantly ($P < 0.001$) increased WBC (4×10^6 mm^3), RBC (11.37×10^3 mm^3), haemoglobin (16.42 g/dl), and haematocrit (63.04 %) compared to others. HDL-C (67.18) and LDL-C (71.73) were lipid profiles (mg/dl) with the highest content in T3. T3 had low CRI-I, CRI-II (1.07), and AC (0.84). LDL-C was positively correlated with all atherogenic indices, while HDL-C levels were negatively correlated. The highest serum mineral (mg/dl) levels of zinc (0.55) and iron (2.08) were reported on T2, while the highest calcium content (10.64) was reported on T1. Overall, the bee larvae can be used to develop complementary foods that are nutritionally adequate, microbial safe, and sensory acceptable, meeting the dietary allowance of infants at an acceptable level. Also, can aid body growth, and prevent

malnutrition in infants and young children. However, research on the clinical and histopathological effects of newly developed complementary foods is needed.

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Malnutrition is a worldwide problem for infants and children, which affects the world population. According to the report of (UNICEF, 2021), children under five affected by stunting and wasting are 21.3 % and 2.1 %, respectively. People living in developing nations including Sub-Saharan African (SSA) or South Asian countries are frequently found to be seriously malnourished (Raza et al., 2020; Tao and Li, 2018). Malnutrition of varying degrees has been linked to infants being fed unwholesome and low-quality complementary foods (CFs) (Abiose et al., 2015). In addition, malnutrition due to inadequate complementary feeding is a serious problem in many low-income countries where CFs consist of starch-based cereals that may provide adequate energy but lack protein and micronutrients (Melese, 2013; Oladiran and Emmambux, 2020) which hinders an individual's health (Tian et al., 2016).

Protein Energy Malnutrition (PEM) is a common childhood disorder (Ikpeme-Emmanuel et al., 2009; Jee, 2021) and is primarily caused by a deficiency of energy, protein, and micronutrients (Batool et al., 2015), that frequently occurs during the critical transition period of weaning in infants, hindering their physical and mental development (Ahmed et al., 2020). This condition can be prevented to a large extent by introducing CFs of quality and quantity at the right time in the right proportions (Oladiran and Emmambux, 2020). Therefore, malnutrition can be prevented by feeding children adequate, nutritious, and safe CFs (FAO, 2017).

Ethiopia has the second-highest rate of undernutrition in SSA, a form of chronic undernutrition, at 38 % of stunting, (USAID, 2018). Stunting is largely irreversible due

to chronic undernutrition that contributes to weaker immune systems and decreased cognitive capacity. One in a tenth of under-five children in Ethiopia is wasted and suffers from acute malnutrition which is one of the leading causes (Tekile et al., 2019; USAID, 2018). Furthermore, in Ethiopia, 28 % of child deaths are associated with malnutrition (USAID, 2018). In addition to an increased risk of mortality, undernutrition in childhood has irreversible effects and serious health consequences, such as impaired cognitive function, which can lead to national economic and productivity losses (Chaturvedi et al., 2017). At the time of infancy, there will be rapid physical growth as well as physiological, immunological, and mental development. Breastfeeding alone will not meet the children's nutritional requirements due to the very rapid growth of infants in the first or second years (Elemo et al., 2011). However, studies have shown that 40 % of Ethiopian mothers exclusively breastfeed their infants at 6 months or less, which is against the WHO recommendation of 80 % (FAO, 2019). When breast milk alone is no longer enough to meet an infant's nutritional needs, complementary feeding involves providing additional foods and liquids in addition to breast milk (WHO, 2019). As a result, the infant abandons exclusive breastfeeding in favor of family foods. Breastfeeding generally lasts between 6 and 24 months, although it can last up to two years (WHO, 2019). This is a critical growth period, and nutritional deficiencies and illnesses cause higher rates of undernutrition among children under the age of five all around the world (WFP, 2018). Complementary feeding improvement should be the highest priority for Infants and Young Children Feeding (IYCF) nutrition because of its crucial role in preventing mortality and improving children's development. Therefore, adequate nutrient densities CFs are needed for optimal growth and development of infants.

In most SSA countries, CFs are plant-based and deficient in animal protein, essential minerals, and vitamins after suboptimal growth and increased premature deaths among children below five years of age (Fewtrell et al., 2017). This is because traditional CFs are based on starchy cereals such as maize, sorghum, finger millet, and rice, and non-

cereals such as cassava, round potato, sweet potato, yams, and plantains (Temesgen, 2013). These foods are generally related to nutritional deficiencies in preschool children.

In developing countries, starch-based foods are the main staples and have resulted in PEM, especially in infants, which has been one of the major nutritional problems worldwide. The high costs of animal proteins in most developing countries have resulted in inadequate protein intake for normal body growth and development (Obatolu et al., 2000). Therefore, in many developing countries, considerable attention should be paid to the formulation and development of nutritious CFs from locally available raw materials (Melese, 2013). In addition, nutritious, acceptable, and affordable CF can be formulated using locally available food items (Tufa et al., 2016). However, one of the greatest problems that affect millions of people in developing countries, particularly children, is the lack of adequate intake of animal protein (Adesogan et al., 2020). Although grains are normally low in protein, supplementing cereals with local high-protein legumes increases the protein level of cereal-legume blends (Abdulkadir and Danjuma, 2015). However, these plant diets are inadequate in terms of protein quality, hence the need to include animal proteins (Henchion et al., 2017).

Affordable animal proteins such as edible insects provide a strong protein source that is likely to be used in CFs (Adámková et al., 2017; Mmari et al., 2016). Insects are protein sources as a nutritious novel food (Kinyuru. et al., 2015). The use of edible insects in the production of CF has not been extensively studied. Only a few studies have used insects as CF components, such as grasshoppers (Mmari et al., 2016), palm weevil larvae (Agbemafle et al., 2020; Ayensu et al., 2020), termites (Adepoju and Ajayi, 2016b; Kinyuru et al., 2015), and crickets (Agbemafle et al., 2020) with other local foods ingredients. However, due to a lack of data and information on the supply and consumption of insects (Roos, 2018), culture, customs, taboos, and ethnic preferences have strongly influenced the consumption of edible insects (Kinyuru et al., 2018).

Bee larvae (*Apis mellifera*) are traditional foods in different African and Asian countries (Devillers and Pham-Delègue, 2002; Jensen et al., 2019). The world is consumed as a delicacy in many cultures (Jensen et al., 2019). Several studies have examined the nutritional value of honey bee brood as an alternative source of nutrients (Ghosh et al., 2016) found that bee larvae contain 35 % protein on a dry matter basis, similar to other protein sources, such as poultry, beef, and pork 54.7, 40.5, and 27.7 %, respectively. Hence, insect bee larvae will be one of the nutrients reach foods that have high nutrient content in quality but are not yet studied as a component of CFs and their growth and health effect. Therefore, CFs developed from locally available and acceptable food materials are possible and when well-formulated, are appropriate for resource-poor settings (Konyole et al., 2012). Typically, food materials will include staple cereal or starchy tubers (Abeshu et al., 2016).

1.2. Statement of the Problem

In Ethiopia, CFs given to infants by mothers or caregivers, are plant-based and therefore deficient in animal protein and essential micronutrients (minerals and vitamins) densely provided by animal source foods (Melese, 2013). Furthermore, the poor formulation of the ingredients and processing partly contribute to the poor quality of traditional CF. Therefore, adequate nutrient-density CFs are needed for optimal growth and development of infants.

According to the (Abeshu et al., 2016) study, a combination of cereals and legumes or tubers with fruit, vegetables, and animal source foods rather than a single diet can better support infants and children's growth and development. In Ethiopia during CF preparations, animal protein source foods are negligible and predominantly fed cereal-based for children (Abeshu et al., 2016). This was due to the high cost and less availability of animal products. Therefore, the use of locally available, nutritious, and affordable food items for children could be the best strategy to address macro and

micronutrient deficiencies. In Ethiopia, maize and red teff are two of the most important staple foods. Maize (*Zea mays L.)* is a major cereal crop that is widely cultivated and is regarded as a vital source of food and feed for humans and livestock (Tenaillon and Charcosset, 2011). For centuries Teff *(Eragrostis tef (Zucc.))* has been cultivated (Fitwi and Tadesse, 2013), and used for human consumption (Tekile et al., 2019). However, due to limited information on its composition of nutrients and processing challenges, the global use of teff for human consumption has been limited use and the production of teff-based foodstuffs has been partly restricted. Therefore, the strategic use of low-cost high-protein sources complements the protein quality of these staple foods to improve their nutritional value.

In addition, there is an increase in interest in the use of insects as sustainable diets, as many of them are important in terms of nutrition, economics, and the environment. However, less well-appreciated for insects especially, bees themselves rather than their products, are of considerable nutritional value as a food item (Ghosh et al., 2016). Numerous species of insect larvae are easier to grow, but of all the insects to eat, honeybees probably have the highest public demand and are probably more acceptable than others such as fly larvae or crickets (Krell, 1996). Therefore, the honey bee itself as a potential food source (good sources of protein, fat, and micronutrients, including minerals and vitamins) has received little attention (Finke, 2005; Ghosh et al., 2016).

1.3. Justification and Significance of the Study

Industrial infant food in a developing country like Ethiopia is very expensive and is not available for low-income families (Abeshu et al., 2016). Therefore, small-scale CF production could be cheaper and more affordable for the majority of the population (UNICEF, 2020). The development of easily digestible, nutritionally balanced, microbiologically safe foods with low dietary volumes and high-calorie density is, therefore, necessary (Shimelis and Rakshit, 2008). Traditional weaning foods could be

improved by combining local foods that complement each other and need to be changed or modified to improve their nutritional status (Okafor et al., 2008; Oladiran and Emmambux, 2020). Cereal-legume mixtures of weaning food production are a major contribution to alleviating PEM (Achidi et al., 2016). Hence, as a safe replacement for high-quality protein, legumes generally replace other sources of animal protein that are costly and not readily available (Melese, 2013).

Soybean (*Glycine max*), teff, and maize are readily available local food crops that can complement each other to meet the recommended daily food allowance for growing infants due to their nutritional composition (Mesfin et al., 2017). Also, future protein demand is expected to rise with global population growth (Haber et al., 2019; Henchion et al., 2017), and the deterioration of land. For soybean and bee larvae, their protein content is higher and less expensive than that of poultry. Cheap animal protein such as edible insects is a good source of CFs enrichment (Mmari et al., 2016). However, the use of edible insects as a CF production is not studied abundantly (Kinyuru. et al., 2015).

Insects provide good sources of protein, minerals, vitamins, and energy, and are less expensive than other animal proteins (Van Huis et al., 2013). The nutritional value of insects is close to that of other forms of animal protein, such as crustaceans, fish, and meat (Mézes, 2018; Van Huis et al., 2013). The high protein content, and the balance of the composition of saturated and monounsaturated fatty acids, in particular the high amounts of iron and zinc, could make honey bees (*A. mellifera*) ideal for foods throughout their development (Ghosh et al., 2016). The authors also postulated and compared that, honeybees can have the capacity to replace the protein contents that are gained from the plant (soybean) and animal sources (pork, beef, veal, and eggs). No records of food poisoning from bee brood or larvae currently exist (Jensen et al., 2019). However, there are no scientific studies using honeybees as a source or component of CFs. Therefore, the development of safe CF with low-cost and locally available food ingredients from honeybee insects and alternatively soybeans with staple foods (maize

and teff) should be necessary to satisfy future nutritional deficiency and IYC requirements in Ethiopia.

The biochemical assessment is used to assess the general nutritional status and to identify specific nutritional deficiencies. Protein-energy malnutrition results in widespread changes in physiological function. In the haematological system, PEM deficiency changes all blood cells (Khan et al., 2020). The most common haematologic change in children with PEM is anemia (Anticona and San Sebastian, 2014) and also the major cause of anemia is iron deficiency (Özkale et al., 2014). Micronutrient deficiencies, especially zinc deficiency, are associated with growth stunting and include anemia and increased susceptibility to infection (Bhutta et al., 2008; Ramesh et al., 2016). In addition, the development of Coronary Heart Disease (CHD) is a common disease caused by increased intake of cholesterol and saturated fat, decreased intake of polyunsaturated fatty acids (PUFAs), and increased obesity (Kuller, 2006). Therefore, animal feeding experiments are warranted, aiming at the identification of biomarkers suitable for human intervention studies (Weiskirchen et al., 2020).

1.4. Objectives

1.4.1. General Objective

- To evaluate the nutritional quality and safety of complementary foods developed from Ethiopian staple grains and honeybee (*Apis mellifera*) larvae.

1.4.2. Specific objectives

3. To evaluate the nutritional, microbial, and sensory quality of complementary foods developed from honeybee larvae, soybean, teff, and maize grain.
4. To assess the effect of developed complementary foods on the growth performance of experimental BALB/c mice.
5. To evaluate the effect of developed complementary foods on blood serum protein, haematological characteristics, and serum micronutrients of experimental BALB/c mice.

1.5. Research Questions

1. What is the nutritional, microbial and sensory quality of the developed complementary foods?
2. What is the effect of the developed complementary foods on the growth performance of BALB/c mice?
3. What is the effect of the developed complementary foods on the blood serum protein, haematological characteristics, and serum micronutrients of experimental BALB/c mice?

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Complementary foods must be safe and provide the appropriate energy, protein, and essential micronutrients (vitamins and minerals) necessary to meet the nutritional needs of growing infants (Oladiran and Emmambux, 2020). Good nutrition during the first twenty-four months of life is vital for healthy growth and development (UNICEF, 2021). In Ethiopia, appropriate CF is the main factor to ensure the healthy growth and survival of young children in their early years of life (Abdurahman et al., 2019). Therefore, ensuring adequate nutrition during the CF period is the main priority of global health (Dewey, 2013).

2.2. Malnutrition in Ethiopia

Child malnutrition remains high in Ethiopia, and public health concerns and inadequate complementary feeding were some of the contributing factors (Sako et al., 2018). In recent years, Ethiopia has only had limited success in reducing the prevalence of child malnutrition (Mekonnen et al., 2005; Tekile et al., 2019). According to the Ethiopian Demographic and Health Survey (EDHS, 2019), from 2005 to 2019, the prevalence of stunting, wasting, and underweight decreased significantly, falling from 51 % to 37 %, 13 % to 7 %, and 33 % to 21 %, respectively. In Ethiopia, according to the report (USAID, 2018), 53 % of child mortality (under 5 years of age) was attributed to malnutrition, and 38 % of children were classified as stunted. Factors for malnutrition in Ethiopia were improper feeding practices, limited micronutrient dietary selections, and lack of introduction of complementary nutritional foods at appropriate times (Sparkman, 2017).

According to the report of (Endris et al., 2017), the prevalence of malnutrition among rural children was 48.5 % and the factors associated with the nutritional status of the children in the rural area were the age of the children, the preceding birth interval, the education status of the mother, wealth status and region. In addition to this, according to the report (EDHS, 2016) children in rural areas were more likely to be malnourished than those in urban areas. Regionally the severity of stunting and wasting showed highest in Amhara (46 %), Benishangul-Gumuz (43 %), Afar (4 %), and Dire Dawa (41 %), while wasting is highest in Somali (22 %), Affar (18 %) and Gambela (14 %) (USAID, 2018). Differences in stunting levels can be seen according to maternal education and wealth levels. Generally, the cause of child malnutrition was indicated in Figure 2.1.

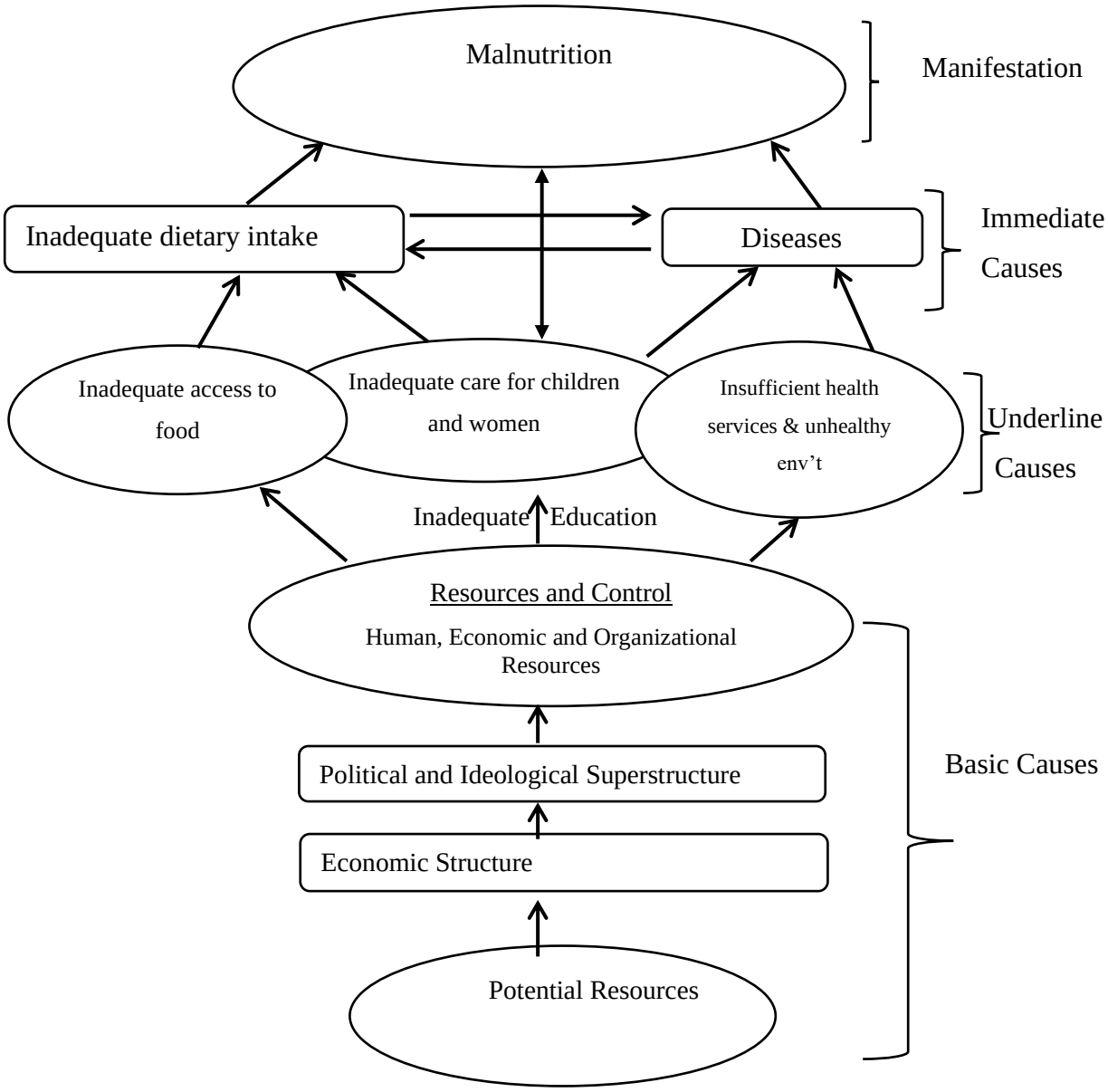

Figure 2.1: The causes of malnutrition (UNICEF Conceptual Framework)

Source:- (UNICEF, 2003)

2.2.1. Protein-energy malnutrition

Protein-energy malnutrition occurs most frequently in IYCF and was commonly associated with infection as a range of pathological conditions arising from a co-related lack in varying proportions of protein and calories (Gohain et al., 2016; Santos et al., 2017). Protein-energy malnutrition results in widespread changes in physiological function. In the haematological system, PEM changes all the blood cells (Khan et al., 2020). The most common haematologic change in children with PEM was anaemia and the major cause of anaemia was iron deficiency (Özkale and Sipahi, 2014). Protein Calorie Malnutrition (PCM) might be iron deficient (Saka et al., 2019). Lack of iron has been considered the main cause of anemia in malnutrition (Shubham et al., 2020).

Various clinical symptoms and syndromes can develop depending on the degree of PCM, which can make comparisons difficult. PCM can appear in several clinical forms, Kwashiorkor and Marasmus were the most characterized forms (Santos et al., 2017; Wilson et al., 2016). In addition to this severe acute malnutrition develops when the child does not get enough energy, protein, and other nutrients from food to meet their nutritional needs (WHO, 2011). Poor nutrition can cause anaemia (a reduction in red blood cell mass or blood haemoglobin concentration). According to the report of (Heard et al., 2007) research on high and low protein intake in rats, low protein intake specifically and consistently showed lower values for haemoglobin and plasma protein concentration than high protein intake. Dietary protein can also influence albumin metabolism and affect plasma albumin (Wada et al., 2018).

2.2.2. Micronutrients

Micronutrients were important for IYC growth, development, and disease prevention (Robert et al., 2021). Nowadays, micronutrient deficiencies affect billions of people in the world (Hailu and Addis, 2016). According to the estimation of the WHO, more than

2 billion people in the world were deficient in micronutrients mainly iron, vitamin A, iodine, and zinc, and of these, one-third were children below 5 years (Ramesh et al., 2016). Iron, zinc, and calcium were necessary to ensure optimal health, growth, and development of IYC. The WHO, on the other hand, considers iron, zinc, and calcium in CFs to be problem micronutrients since their concentrations in CFs fall below the estimated requirements for micronutrients, acquired through CFs for breastfed infants (Gibbs et al., 2011).

Micronutrient deficiencies were a major problem in Ethiopia (Sahile et al., 2020). In Ethiopia, the prevalence rate of anaemia showed that, 56 % of children aged 6 to 59 months and 40 % of children aged 48 - 59 months. According to the report (EDHS, 2016) mortality rate of under five due to anaemia in the country was 67 per 1,000 live births. Generally, stunting, anemia, and micronutrient deficiencies continue to affect millions of children worldwide (Santos et al., 2017). Therefore, the introduction of appropriate CF should include foods that provide sufficient energy and micronutrients to promote optimal growth and development, while being affordable and sustainable (Tzioumis et al., 2015).

2.3. Current Status of Complementary Feeding in Ethiopia

Typical CFs in developing countries often lack many micronutrients. In Ethiopia, inappropriate supplemental feeding practices are one of the leading causes of malnutrition among children under the age of two (USAID, 2011). One way of preventing micronutrient deficiency among IYC was to feed them a variety of nutrient-dense foods (Abizari et al., 2017). According to the report (UNICEF, 2020), the combined national prevalence of timely initiation of breastfeeding, Exclusive Breast Feeding (EBF), and timely additional feeding was 66.5 %, 60.1 %, and 62.5 %, respectively.

Optimal complementary feeding is recognized to be critical for the prevention of infectious morbidity and mortality and growth and development. Based on the dynamic composition of human milk and the physiology of infant nutritional requirements, the nutrients that become limiting in human milk after approximately 6 months of EBF were predictable. Complementary foods must be prepared under safe conditions and should contain optimal levels of specific nutrients such as vitamin A, iron, and zinc (Campoy et al., 2018). Iron and zinc are two micronutrients for which the concentrations in human milk are relatively independent of maternal intake and for which older infant is most dependent on CFs to meet requirements (Dewey, 2013). Traditional feeding practices, including the reliance on cereals and plant-based diets, do not complement these recognized gaps in human milk.

The nutritional content of CF given for under-five children was reviewed by (Melese, 2013). In Ethiopia, CF has been given to infants by mothers or caregivers and was deficient both in macronutrients (protein, carbohydrates, and fat) and micronutrients (minerals and vitamins). This leads to PEM and specifically micronutrient deficiency or both. The author also reviewed the common CFs and raw food for gruel ingredients including teff, sorghum, barley, maize, wheat, emmer wheat, and *enset*; porridge (Teff, sorghum, barley, maize, wheat, broad beans); *fetfet*; *kitta* (Teff, sorghum, barley, maize, wheat, enset, and chickpeas; *Dabo* (Teff, sorghum, barley, maize). Almost all ingredients are not meet the nutritional requirements for IYC. According to the report of (Molla et al., 2017) nearly half of the mothers did not follow appropriate complementary feeding methods, taking into account timely adoption, minimum dietary range, and duration. On the contrary, most mothers or caregivers did not use meat/fish/chicken or milk/milk products when feeding their children with CF such as porridge.

According to the report (GAIN, 2014) in Ethiopia, only 4–5 % of children between 6 and 23 months of young children transition directly from primarily breastfeeding to a cereal-based adult diet with poor nutrient density, and consume the minimum age-

appropriate frequency of meals and dietary diversity. Therefore, homemade CFs were still widely used. However, even when based on an improved recipe, plant-based unfortified CFs provide insufficient key micronutrients (especially iron, zinc, and calcium) at 6 to 23 months of age (Abeshu et al., 2016). The estimated total energy requirements at 6–8, 9–11, and 12–23 months of age for stable breastfed infants were 615, 686, and 894 kcal/day, respectively. The average intake of breast milk in developing countries raises the energy requirement of CF from 200 kcal/day at 6-8 months to 300 and 500 kcal/day at 9–11 and 12–23 months, respectively (Dewey, 2001). The author also suggests satisfying the daily nutritional requirement of protein required 9.1 g for 6–8 months, 9.6 g for 9–11 months, and 10.9 g for 12–23 months, respectively. The recommended dietary allowance for 6 months old indicated energy of 400 kcal, protein 9.1 g, vitamin A 400 µg, iron 9.0 mg, zinc 4.1 mg, and calcium 400 mg (Dewey, 2001; WFP, 2018).

2.4. Nutritional Value of Teff, Maize, and Soybean

Due to its very attractive diet and gluten-free grains, making it very delicious, interest in teff (*Eragrostis teff*) has increased significantly as a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease (Gebremariam et al., 2012). Teff contains high and unique nutritional values, which would meet the need of health-conscious consumers (Hyejin, 2018). Teff is composed of complex carbohydrates with slowly digestible starch and protein content like other more common cereals, such as wheat but is relatively richer than other cereals in the essential amino acid lysine (Tekile et al., 2019). Teff is also a good source of energy (357 kcal), starch (73 %), CP (11 %), essential amino acids (lysine 3.7 g/16g), crude fat (2.5 %) essential fatty acids such as oleic acid (32.4 %) and linoleic acids (23.8 %), crude fiber (3 %), minerals (especially calcium, iron and zinc which is 18 - 178, 11.6 - >150, and 2.3 - 6.7 mg/100g, respectively), and phytochemicals such as polyphenols and phytates (Akansha et al., 2018; Baye et al., 2018). Teff flour blending in all weaning and CF

enriches the products with iron and other essential minerals (Melese, 2013). Therefore, the high nutrient profile of teff suggests that it can be an excellent component of CF if combined with other common foods and staples (Tekile et al., 2019).

Legumes such as soybeans (*Glycine max*) cultivated in Africa and elsewhere are one of the richest and cheapest sources of plant protein that can be a good substitute for animal products (Tufa et al., 2016). Soybean has a high concentration of protein, fiber, minerals, and vitamins (Samtiya et al., 2020). However, most mothers never used soybean for CF preparation (Addisalem et al., 2015). The proximate composition of soybeans on a wet basis contains about 4.59 % ash, 17.46 % oil, 39.99 % protein, and 31.01 % carbohydrate (Massresha, 2011). Also, Tenagashaw and his colleagues (Tenagashaw et al., 2017) were reported the proximate composition of blanched soybean. The protein, fat, fiber and carbohydrate content of blanched soybean were 35.59, 10.22, 5.35, and 45.22 g/100g, respectively.

Maize (*Zea mays L.*) has a diverse forms of use for human food. Maize is another source of staple food grains that are mostly used as a component of CFs. The proximate composition (g/100g) of maize flour protein, fat, fiber, CHO, and energy (kcal/100 g) were 18.79, 12.86, 2.51, 58.81, and 426.14, respectively (Gemede, 2020). Also, (Gebrezgi, 2019) reported the nutritional value of processed maize flour, was 8.2 % protein, 4.58 % fat, 11.12 % fiber, 67 % carbohydrate, and 345.68 kcal of energy.

2.5. Edible Insects in Complementary Feeding

The increasing population worsens the serious problem of food security in developing countries. There was an urgent need to find alternative sources of protein due to the increased demand for meat and the decline in the availability of agricultural land. Edible insects can be part of the solution as a traditional and readily available food source (Adámková et al., 2017). Moreover, increased demand for animal-based protein was

expected to have a negative impact on the environment, generating greenhouse gas emissions and requiring more land and water (Henchion et al., 2017). From a nutritional point of view, insects had a significant nutrient content. Many edible insects have favorable characteristics, particularly in the context of a locally sourced traditional diet and nutritional composition has been tested, and many have favorable nutrient profiles (Payne et al., 2016). Insects' nutritional composition depends on the type and development stage. Also, the author reported the nutritional profile of insects on a DM basis had a significant protein content, which varies from 20 % to 76 % of DM, large variability of fat content (2 % to 50 % of DM), up to 70 % of total fatty acids, carbohydrates ranging primarily from 2.7 mg to 49.8 mg per kg of fresh material. Many edible insect species contain a reasonable amount of minerals (K, Na, Ca, Cu, Fe, Zn, Mn, and P) as well as vitamins such as B-vitamins, A, D, E, K, and C vitamins. However, their material was seasonal and depends on the feed (Kouřimská and Adámková, 2016). The most eaten groups of insects were beetles, caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leaf and planthoppers, scale insects and real bugs, termites, dragonflies, and flies. According to the (Huis, 2015) study, representatives from nearly all classes of insects were eaten, such as beetles (31 %), caterpillars (18 %), wasps, bees and ants (15 %), crickets, grasshoppers, and locusts (13 %), true bugs (11 %), and termites, dragonflies, flies, and others (12 %).

In developing countries, undernutrition, which is a critical problem using edible insects can radically improve food security and nutrition (Kinyuru et al., 2015). Edible insects can be produced with less environmental impact than livestock (Huis, 2015). Insects had adequate protein quantity and quality and a high content of unsaturated fatty acids and minerals like iron and zinc (Ghosh et al., 2016). However, in Africa, most of the insects consumed were still collected from wild environments. Therefore, utilization was hampered by regional and seasonal availability, sustainability issues, pathogenic risks, and high perishability (Imathiu, 2020). The idea was agreed with (Mézes, 2018) some

risks were occur associated with the consumption of insects, even if rearing in controlled systems.

In East Africa, local communities had developed the skills and techniques for harvesting, preparing, and preserving edible insects (Ng'ang'a et al., 2019). Insects, especially bee brood (larvae and pupae) in the tropical regions (Ghosh et al., 2016) of the world several ethnic populations were known to accept as a portion of food. The common countries' consumption of bee brood was Mexico, Ecuador, China, Thailand, Senegal, Zambia, and Australia (Jensen et al., 2019). In many African and Asian countries (Krell, 1996), brood combs were rich in protein and considered a delicacy, and consumed immediately. In Thailand (Chen et al., 1998) also reported that honey bees were an important part of the diets of much of the Thai population. Honeybees at all stages of development could be fifteen ideal foods due to their high protein content, balanced saturated and monounsaturated fatty acid composition, and in general their high iron and zinc content (Ghosh et al., 2016). However, there were only a few studies conducted on the nutritional quality of insects, particularly honeybees and there was no scientific research on using honeybee brood (*Apis Mellifera*) as a source of CFs for humans.

2.6. The Nutritional Component of Honeybees

In tropical regions of the world, bee broods (larvae and pupae) were known to be accepted by numerous ethnic populations (Ghosh et al., 2016) (Table 2.1). Bee brood serves as a food source for humans in many countries, although limited data exist regarding its nutrient composition (Finke, 2005). The nutritional potential of bee brood and adult honeybees that already exist was reported as good sources of protein, fat, and micronutrients, as well as minerals and vitamins (Chen et al., 1998; Ghosh et al., 2016; Payne et al., 2016; Rumpold and Schlüter, 2013). The proximate nutritional composition of bee larvae, pupae, and adult wings excluded was reported by (Ghosh et al., 2016).

Table 2.1: Proximate composition and energy of worker larvae, pupae, and adults (wings excluded) of *A. mellifera ligustica*

Bee Brood	Moisture	Protein	Fat	COH	Ash	Energy kcal/100g
Larvae	74.4 ± 0.33	35.3 ± 2.09	4.5 ± 0.15	46.1 ± 1.73	4.1 ± 0.16	455.8
Pupae	79.3 ± 0.19	45.9 ± 0.63	16.0 ± 0.24	34.3 ± 0.24	3.8 ± 0.06	465.0
Adults	65.6 ± 0.94	51.0 ± 0.01	6.9 ± 0.25	30.6 ± 0.38	11.5 ± 0.14	388.4

Were COH- Carbohydrate

Source:- (Ghosh et al., 2016)

There was also another report on the nutritional larvae of honey bees that were a richer source of protein than pork and similar in vitamin and mineral content to chicken and shrimp (Chen et al., 1998). The protein and lipid content (g/100g) of the bee larvae was 15.21 and 19.80, and the mineral content (mg/100g) of Fe, Zn, and Ca was 1.89, 1.05, and 0.50, respectively. According to the report (Krell, 1996), the chemical composition of mature and immature bees has not received as much attention as other insects. The author also reported the composition of mature and immature honeybees in Table 2.2. The protein levels of adult and immature bees were very similar. In adults, more than 40 % of the protein comes from the muscle tissue of the thoracic region, which was like egg white in protein.

Table 2.2: Composition of mature and immature bees compared to beef and soybeans, modified from Crane, 1990

Parameters	Honeybee			Beef	Soybeans
	Mature larvae	Pupae	Adult		
Water %	77.0	70.2	72.1	..74.1	70.0
Ash %	3.0	2.2	-	1.1	1.5
Protein %	15.4	18.2	17.9	17.7	12.9
Fat %	3.7	2.4	2.8	2.8	5.9
Glycogen %	0.4	0.8	1	0.1- 0.7	2.4
Vitamin A IU/g	107	51.3	-	0	-
Vitamin D IU/g	6863	5165	-	-	-
Chitin/Fiber %	-	-	4.1	-	1.7

Source:- Cited from (Krell, 1996)

In general, bees as food, feed, or nutritional supplements can improve the prospects for world food security, but they could also improve the economic situation of small and medium-scale beekeepers if they have been converted to an accepted beehive product, along with other beehive products (Ghosh et al., 2016). However, there was a gap in the nutritional analysis of honeybees compared to other insects.

2.7. Microbial Contamination of Complementary Foods

Children were prone to exposure to foodborne pathogens with the advent of CFs. The microbial quality of the diet was one of the most important standards in terms of consumers' CF requirements (Baskar and Aiswarya, 2016). As a result, safe and nutrient-dense CFs should be provided for child growth at an appropriate age and development (WHO, 2009). Complementary foods, as well as breast milk made with poor sanitation, were found to be severely contaminated with pathogenic microbes (Degaga et al., 2015).

In infants, foodborne microbial agents cause diarrheal diseases and ill health (Kirk et al., 2017; Kung'u et al., 2009). Diarrhea was one of the three leading causes of childhood mortality in Ethiopia. From 2011 to 2016, the prevalence rate decreased slightly from 13 % to 12 %; however, 8 % of children under five years of age were still responsible for the deaths in the country (EDHS, 2018). Foodborne microorganisms can cause spoilage of foods, infection, or intoxication disease in the consumer (Bintsis, 2017). Food safety, especially in weaning groups, was one of the biggest concerns that posed a threat to children's health. Every year, millions of children die due to diarrheal infections, and hundreds of millions more suffer from frequent attacks of diarrhea and nutritional deficiency. In the development of diarrheal diseases, contaminated food plays a significant role. In addition to food contamination, infection was spread through direct contact, which was highly favored by people's habits and customs (Uçar et al., 2016).

The introduction of CF in resource-poor areas can result in diets that were nutritionally inadequate and microbiologically unsafe, leading to possible multiple nutrient deficiencies and higher exposure to foodborne pathogens and following gastrointestinal illnesses (Islam et al., 2012). According to the report of (Islam et al., 2012) examine CF of the microbiological quality was indicated that correlate with diarrhea. There was a significantly high number of wasted diarrheal morbidity in rural children (6-24 months) who had CF with a high aerobic plate count. Therefore, food safety concerns should be considered when developing and preparation of CFs.

2.8. Extrusion Processing and its Effect on Nutrients

Extrusion cooking was one of the common techniques for the processing of CFs. The method has various beneficial effects such as starch gelatinization (Peng et al., 2017), decreased lipid oxidation (Bordoloi and Ganguly, 2014), reduction of antinutritional factors (Nikmaram et al., 2017; Omosebi et al., 2018), decreased microorganism contamination (Almendares et al., 2020), and increased soluble dietary fiber (Rashid et

al., 2015; Zhong et al., 2019) due to increased temperature and reduced moisture in the foods during extrusion (Baskar and Aiswarya, 2016).

Food extrusion was an emerging technology to process foods of varying sizes, shapes, textures, and tastes for the food industries. Extrusion cooking technology has contributed to the manufacture of various products such as pasta, breakfast cereals, bread crumbs, cookies, nuts, croutons, baby foods, snack foods, confectionery, chewing gum, texturized vegetable protein, altered starch, pet foods, dried soups, dry beverage mixes, etc (Alam et al., 2016). The effects of extrusion cooking on the nutritional quality of food were ambiguous. The protein and energy nutrition of foods can be positively or negatively affected by extrusion cooking (Muoki et al., 2012). Beneficial effects of extrusion cooking include the destruction of anti-nutritional factors, increased soluble dietary fiber, gelatinization of starch, and reduction of lipid oxidation (Singh et al., 2007; Yusuf et al., 2018). Vitamin stability varies with vitamin structure, extrusion conditions, and food matrix composition (M. E. Camire, 1998). Heat-sensitive vitamins might be lost to varying extents. Among vitamins, vitamins E and A and their related compounds tocopherols and carotenoids, respectively, were not stable in the presence of oxygen and heat however, vitamins D and K were fairly stable, (Tiwari and Jha, 2017).

The extrusion process also increases the bioavailability of mineral content in food. Yusuf and his colleagues (Yusuf et al., 2018), were reported Fe and Zn contents of extrusion food were higher than that of the non-extruded. The bioavailability of Fe appears adequate if excessive amounts of iron and related metals were not presented. The effects of extrusion on protein nutrition in animal feed and for human weaning foods showed that total protein changes very little during most extrusion operations (Tiwari and Jha, 2017). Extrusion cooking presents the highest starch digestibility of all listed processing methods, including gamma irradiation, autoclaving, roasting, and toasting (Leonard et al., 2020). Extrusion promotes higher starch digestibility by destructing the covalent hydrogen bonds and structure of starch granules, thus less

resistance to enzymatic digestion (Leonard et al., 2020). In general, changes in proteins and amino acid profile, dietary fiber, carbohydrates, vitamins, mineral content, and some antinutrient healthful components of food may be either beneficial or deleterious. Extrusion cooking results in the degradation of phytate antinutrients (Samtiya et al., 2020).

2.9. Effect of Antinutrients on Bioavailability of Minerals

Antinutrients were naturally present in animals, and many plant-based diets contain chemicals found in crop plants that interfere with nutrient absorption by the human body (Shigaki, 2016). The presence of toxic and antinutrient components, such as phytic acid, was a major limitation on mineral availability (Akond et al., 2011). Phytic acid and phytates were antinutrients found in all cereals (Price and Welch, 2013) that limit mineral availability and are poorly absorbed in the gut (Baruah et al., 2017). Phytate has increasingly been known as an anti-nutritional factor that affects the bioavailability of major minerals such as Ca, as well as trace minerals such as Fe, Cu, Zn, and Mn (Samtiya et al., 2020). Other antinutrients of importance in foods are saponins, tannins, polyphenols, lectins, oxalates, trypsin, gossypol, protease inhibitors, amylase inhibitor, and goitrogens (Ertop and Bektaş, 2018; Samtiya et al., 2020).

Legumes and grains were abundant in macro and micronutrients, but also include antinutritional factors such as trypsin inhibitors and phytates, which limit protein digestion and mineral absorption (Samtiya et al., 2020). Cereal grains were also considered rich sources of minerals, mainly zinc and iron, in addition to ample amounts of vitamins and calories (Temba et al., 2016). Compared to meat-based diets, plant-based diets are often limited in the content and bioavailability of essential minerals such as Zn (Binns et al., 2020), Fe, Ca, and Mn (Warrier et al., 1990). A major constraint to the availability of minerals is the presence of toxic and antinutrient constituents like phytic acid (Masum Akond et al., 2011).

Removing antinutrients, increasing the bioavailability of particular cations (Ca, Fe, and Zn), and increasing protein absorption all contribute to an improvement in the nutritional value of food (Ertop and Bektaş, 2018). Therefore, reducing antinutrients should be essential to increase the bioavailability of minerals. Pretreatment and processing techniques such as soaking, fermentation, germination, debranning, and autoclaving were even traditional methods that were generally used in the consumption of foods (Mitchodigni et al., 2018; Sathe and Venkatachalam, 2002).

2.10. Effect of Diets and Biochemical and Haematological Changes and Serum Lipid Profiles

The effects of newly developed CFs on IYC physiological changes and blood biochemical, haematological, and serum lipid profiles were not studied abundantly.

2.10.1. Biochemical and haematological changes

A protein-restricted diet did not alter biochemical and haematological parameters and appears to have no toxic effect on pregnant Wistar rats (Barros et al., 2018). Furthermore, protein consumption did not influence haematological measures such as Red Blood Cell (RBC), platelet and White Blood Cell (WBC) counts, haemoglobin levels, or monocyte and neutrophil concentrations ($P>0.10$). On the other hand, serum lymphocyte levels were substantially higher in the maize legume protein group ($P=0.03$), but haematocrit ($P=0.002$), mean cell volume (MCV) ($P=0.03$), and mean corpuscular haemoglobin concentration (MCHC) ($P=0.000$) were higher in the milk solids-fed monkeys (Johnson et al., 2001). A comparison of haematological and biochemical measurements between vegans and non-vegans showed that vegans had significantly lower leukocyte, lymphocyte, and platelet counts and lower concentrations of blood urea nitrogen but higher serum albumin concentrations (Haddad et al., 1999).

2.10.2. Lipid profile

The link between cardiovascular disease (CVD) and lipid profile was widely documented. A cardiac-healthy diet was a primary component that can alter this lipid-associated cardiovascular risk (Mielgo-Ayuso et al., 2013). Cardiovascular disease markers include elevated plasma triglycerides and low-density lipoprotein (LDL) cholesterol levels, as well as lower levels of high-density lipoprotein (HDL) cholesterol (Daoud et al., 2014).

The atherosclerotic process begins in childhood, and high blood lipid levels and obesity were the two key risk factors for CVD. In overweight and obese school children, saturated fat was associated with higher lipid levels (Rinaldi et al., 2012). Lifestyle changes remain the basis for the management of lipid and lipoprotein disorders and obesity and were warranted in primary and secondary prevention settings. Therefore, lifestyle changes recommended for those with high cholesterol levels include adopting a diet low in saturated and *trans*-fatty acids (Enkhmaa et al., 2018; Katcher et al., 2009). Saturated fat has been demonised as a dietary problem in heart disease due to its ability to raise LDL-C, while omega-6 PUFA has been regarded as heart-healthy due to its ability to lower total and LDL-C (DiNicolantonio and O'Keefe, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sample Collection and Preparation

3.1.1. Sample collection

The Bee larva *(A. mellifera)* were reared in modern beehives in a suitable hygienic environment on the University of Gondar apiary farm. Fresh larvae combs were collected from beehives and the larvae were immediately manually removed from each comb using swing and impact techniques. Soybean *(Glycine max) was collected from* Gondar Agricultural Research Center, *Ethiopia* and maize (*Zea mays*), and red teff *(Eragrostis tef (Zucc.))* were purchased from Gondar city local market and Gondar Agricultural Research Center, Ethiopia.

3.1.2. Sample preparation

The bee larvae were oven-dried for 24 hours at 60 °C, ground to powder, and stored in an airtight polyethylene bag until laboratory analysed (Ghosh et al., 2016; Jensen et al., 2019). Teff grains *(Eragrostis tef (Zucc.))* were cleaned, washed with tap water until all undesirable components were removed, and then sun-dried and ground to a fine flour using a local stone mill (Codex, 2017; Mesfin et al., 2017). Maize grain (*Zea mays*) was cleaned, soaked in potable water for 6 hours, drained, germinated at room temperature for 48 hours, sun-dried, dehulled, and milled in a local mill into particle sizes ranging from 0.6 to 1.0 mm in diameter (Abebe et al., 2006; Tona et al., 2015). Soybeans (*Glycine max*) were cleaned, boiled (30 minutes), dehulled, and dried at 60 °C for about 13 hours before being milled into flour and sieved (0.5 mm sieve) (Forsido et al., 2019;

Mesfin et al., 2017). Finally, the flour samples were sealed in ziplock polyethylene bags, labeled, and kept at room temperature until the extrusion process was performed.

3.2. Formulation of Complementary Foods

Figure 3.1 illustrates a flow chart of sample processing, formulation, and complementary foods developed products. Nutrisurvey software (version, 2007) was used to develop three composite flours with two different ratios, following complementary feeding guidelines for children aged 6 to 12 months (WFP, 2018). The first CF ($ComF_{01}$) was composed of 57:29:14 ratios of maize, teff, and soybean flours. Similarly, the second CF ($ComF_{02}$) was composed of 58:29:13 maize, teff, and bee larva flour. For comparison, commercially prepared infant food (Enriched Mama's Choice) was purchased from the local supermarket in Ethiopia.

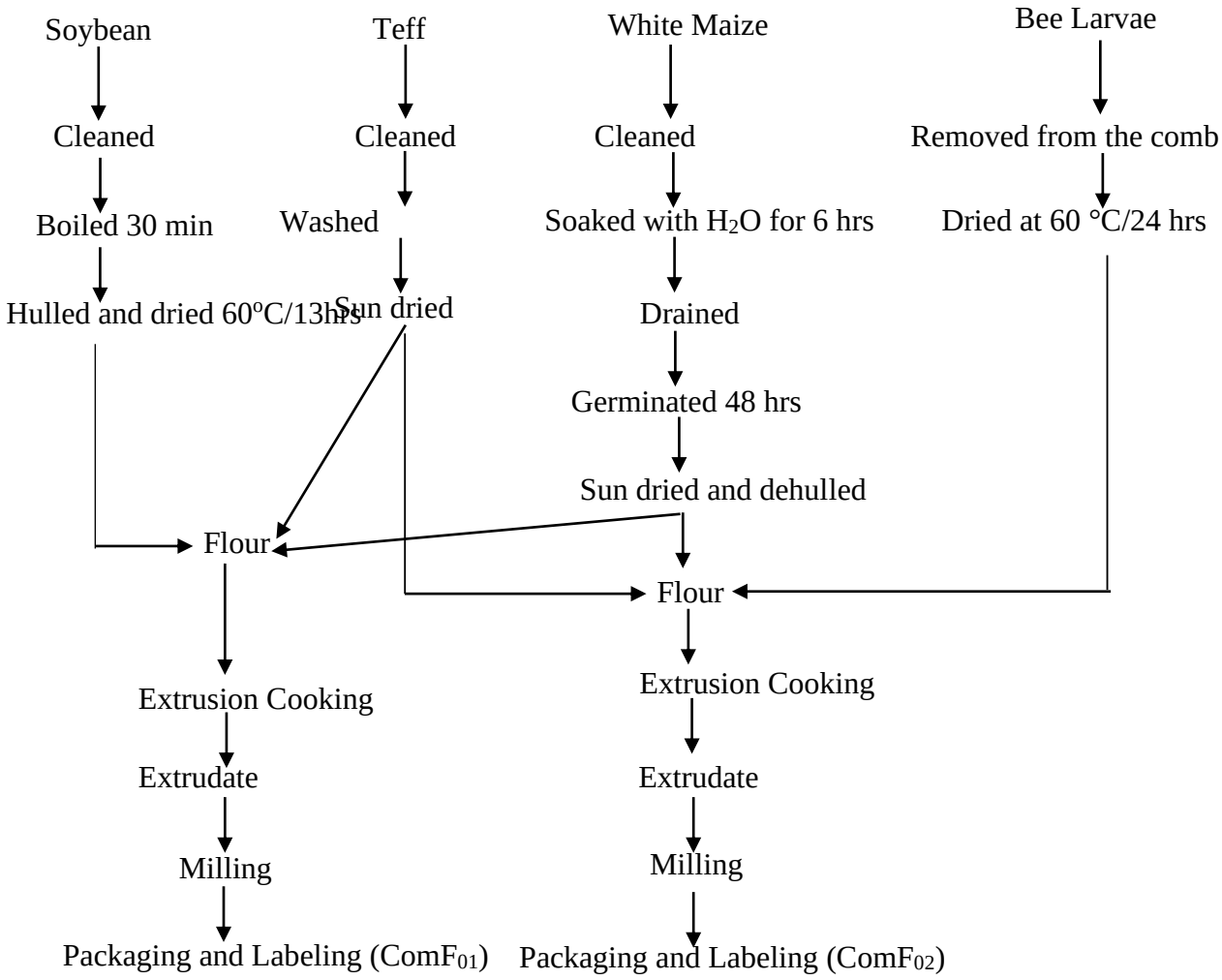

Figure 3.1: Flow chart of sample processing, formulation, and complementary food developed products.

3.3. Extrusion Processing

The following extrusion parameters were established for a blend formulation of composite flours, using a pilot-scale twin-screw extruder for ease of extrusion and best product quality (model Clextral, BC-21 No. 124, Clextral, Firminy, France). Moisture content (17 %), barrel temperature (150 °C), and screw speed with a 29 g/min feed rate

were all taken into consideration. A die plate with four circular holes, each measuring 10 mm in diameter, was used. The extrusion water pump was adjusted to give the amount of water required in the extruded flours to bring the moisture to 17 %, in the mixes at a constant material feed rate by using the hydration Equation based on the formula by (Golob et al., 2002) shown below Equation 1.

$$Wa = Sw\ x\ \frac{(M - Mo)}{100 - M} \quad \text{.....Equation 1}$$

Were

Wa = Weight of water (g)

Sw = Sample flour weight (g)

Mo = Original flour moisture content (%)

M = Required moisture content (%)

Finally, the extrudates were dried and stored at room temperature before being ground in a laboratory-scale mill (High-Speed Multifunctional Grinder Model-200) with a 0.5 mm sieve. The flours developed from CFs were packaged in high-density polyethylene bags and stored at room temperature until laboratory analysis was performed.

3.4. Nutrient Analysis

3.4.1. Proximate analysis of raw ingredient flours and extruded complementary foods

The proximate composition of flours made from raw ingredients, commercial wean-mix, and developed complementary foods was determined using the AOAC International standard methods (AOAC, 2005). The moisture content was determined using a circulating hot-air oven drying method (method #925.09). The ash content of a known weight sample was determined using a muffle furnace at 550 °C (method # 923.03). Micro-Kjeldahl was used to determine crude protein, which was calculated by

multiplying the total nitrogen content by 6.25 (method #979.09). The sample's crude fat content was analysed Using a Soxhlet extractor (method #930.09). The crude fiber content was determined using method #962.09. The total carbohydrate content was estimated as the difference, while energy was calculated using Atwater's calorie conversion factors of 4 kcal/g for available carbohydrate, 9 kcal/g for crude fat, and 4 kcal/g for crude protein (FAO, 2003).

3.4.2. Mineral and vitamins analysis

3.4.2.1. Mineral analysis

According to (AOAC, 2005) (method # 985.35), the mineral content of CFs, namely Fe, Ca, and Zn was determined using a Flame Atomic Absorption Spectrometry (AAS) (Shimadzu AA-6200; Shimadzu, Tokyo, Japan). Briefly, 2 g of the flour samples were weighed, and the samples were ashed at 550 ^{o}C, then boiled with 10 ml of 20 % hydrochloric acid in a beaker filtered into a 100 mL standard flask and topped up to the mark with deionized water. Fe, Ca, and Zn was also determined from the resulting solution using the AAS method. Respective hollow-cathode lamps were used for absorbance measurements. The wavelengths for the determination of the Zn, Ca, and Fe were 213.9, 422.7, and 248.3, respectively.

3.4.2.2. Vitamin analysis

3.4.2.2.1. *β*-carotene determination

The amount of *ß*-carotene in the sample was determined using a Shimadzu UV-Vis spectrophotometer and column chromatography (UV-1601PC, Japan) (Biswas et al., 2011). Food flour samples were ground separately until fine flour was obtained using a laboratory-scale mill (High-speed multifunctional grinder model-200). For extraction, a

representative portion of the flour sample (3 g) was accurately weighed in a glass test tube. Then 5 ml of chilled acetone was added to it, and the tube was held for 15 minutes with occasional shaking at 4 ±1 ^{o}C, vortexed at high speed for 10 min, and finally at 1370 x g for 10 min centrifuged. The supernatant was collected in a separate test tube, and the compound was reextracted with 5 mL of acetone, followed by centrifugation as before. Both supernatants were pooled together and then passed through the filter paper. The absorbance of the extract was determined at 449 nm wavelength in a UV-Vis spectrophotometer. Blank samples of 1.0 g were spiked with working standards to obtain final concentrations 16.0, 8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015 µg of β-carotene and extracted as previously described. Finally, calibration curves for the *β*-carotene content in the samples analyzed were plotted. According to (WHO/FAO, 2004), the results were converted to vitamin A values using a conversion factor of 6 g -carotene: 1 g RE.

3.4.2.2.2. Vitamin B-Complex analysis

Vitamin B-complex (B1, B2, B3, B6, and B9) was determined using High-Performance Liquid Chromatography (HPLC) (Shimadzu, RID-6A) (Oladejo and Kayode, 2016; Sami et al., 2014). Food flour samples were weighed 2 g of each sample in a 100 ml volumetric flask and added 50 ml of 0.1 M sulfuric acid and boiled in a boiling water bath (121 ^{o}C) with frequent shaking for 30 min approximately 5 ml of 2.5 M sodium acetate solution was added, and the flask was set in cold water to cool contents below 50 ^{o}C for 2 hours and then made up to the 100 ml mark. The preparation was stored at 35 ^{o}C overnight. The mixture was then filtered through a Whatman No. 41 filter and the filtrate was diluted with 50 ml of pure water and filtered again through a micropore filter (0.45 μm). A volume of 20 μl of the filtrate was injected into the HPLC system. The quantification of the vitamin B content was performed by comparison with the vitamin B standards. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, and folic acid were prepared as reported previously (Aslam et al., 2008). Chromatographic

separation was achieved on a reversed-phase HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 μm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023M H_3PO_4, pH = 3.54) at a flow rate of 0.5 ml/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature (Marzougui et al., 2009).

3.4.3. Antinutrients analysis

3.4.3.1. Determination of Phytates

The phytate content was determined according to (A. Camire and Clydesdale, 1982) using the HPLC. Approximately 0.5 g of flour food samples were extracted with 10 ml of 3 % sulfuric acid. Contents were filtered using Whatman filter paper No. 42 and the filtrate was transferred to a boiling water bath for 5 min followed by 3 ml of ferric chloride solution (6 mg ferric iron per ml in 3 % sulfuric acid) added. The contents were heated for 45 minutes to complete the precipitation of the ferric phytate complex and then centrifuged at 2500 rpm for 10 minutes and the supernatant was discarded. The precipitate was washed with 30 ml of distilled water, centrifuged, and the supernatant was discarded. A 3 ml of 1.5 N sodium hydroxide was added to the residues and the volume was brought to 30 ml with distilled water. The contents were heated for 30 minutes in a boiling water bath to precipitate the ferric hydroxide. The cool samples were centrifuged, and the supernatant was transferred to a 50 ml volumetric flask. The precipitate was rinsed with 10 ml distilled water, centrifuged, and the supernatant was added to the contents of the volumetric flask. Then the microfilter was filtered and kept awaiting HPLC analysis. HPLC analysis was performed using the Shimadzu Refractive Index Detector (RID- 6A). The mobile phase was 0.005 N sodium acetate in distilled water, at a flow rate of 0.5 µl/min.

3.4.3.2. Determination of Tannins

The tannin content was determined using a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) (Singleton et al., 1965). Before the commencement of laboratory analysis preliminary analysis was carried out either tannins were present or not in the food samples. About 0.5 g of dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. Several drops of 1.0 % ferric chloride were then added. A brownish-green or blue-black coloration indicated the presence of tannins. Then 5 g of food flour sample was dispersed in 50 ml distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28 ^{o}C before being filtered through Whatman filter paper No. 42. Different concentrations of standard tannic acid were made. Four ml of distilled water were placed in a different test tube for samples and standards. To this, 1 ml of flour sample extract/standard tannic acid was added to this. This was followed by the addition of 1 ml of flour sample extract/standard tannic acid. To each, 0.5 ml of Folin Ciocateau solution (10 ml of 2 N Folin Ciocateau solution in 100 ml distilled water) was added. Then 1.5 ml of 7.5 % sodium carbonate solution was added to each and then topped up with distilled water. Then 1.5 ml of 7.5 % Na_2Co_3 solution was added to each and then topped up with distilled water. Finally, the respective absorbance was measured on a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) at 720 nm using the reagent blank to calibrate the instrument at zero.

3.4.3.3. Determination of Saponins

The saponins were determined according to the procedures (Irigoyen and Giner, 2018). Extracts of saponins compounds were carried out by extraction of saponins, 2.5 g of sample was added to 25 ml of 50 % (v/v) ethanol and kept for 30 minutes at room temperature. Subsequently, the extracts were filtered through qualitative filter paper (grammage 80 g m^{-2}) into a 25 ml glass volumetric flask and topped to volume with 50

% ethanol. Determination of saponin content was carried out, by adding 1 ml of the diluted extract (1:20 dilution) to 3.5 ml of the Lieberman-Buchard reagent (16.7 % acetic anhydride in concentrated sulfuric acid). The solution was vortexed and stored in the dark for 30 minutes at room temperature before being placed on a 528 nm spectrophotometer. Oleanolic acid was used as a standard to prepare a calibration curve. All measurements were carried out in triplicate.

3.4.4. Contribution of complementary foods to Recommended Dietary Allowance (RDA)

The average contribution of CFs to the RDA (Equation 2) of each nutrient was calculated as a percent of the RDA (Agbemafle et al., 2020).

$$\% \, RDA = \frac{Amount\ of\ nutrient\ analysed}{RDA\ for\ a\ given\ nutrient} x\ 100 \ldots\ldots \quad Equation\ 2$$

3.4.5. Bioavailability of minerals

The molar ratios of phytate to zinc, calcium and iron were calculated as the millimoles of phytate intake per day divided by the millimoles of zinc, calcium, and iron intake per day, respectively (Castro-Alba et al., 2019).

3.5. Microbial Analysis

On day one, month three, and six of storage, microbial analysis was performed on each CF and commercial-wean mix. A serial dilution was done, using ten-fold and spread plating techniques. A 1 g sample of each CF was measured and dispensed separately aseptically, then mixed with 10 ml diluent saline solution. The food samples were then homogenized for 1 minute in diluent using a vortex shaker (Cat AC-H311 made in

India). Serial dilutions were made up of 10^{-10} dilution factors (Oluwole et al., 2013). Using a sterile pipette, 1 ml of the homogenized food sample was transferred to the first cleaned and sterile test tube containing 9 ml diluent to make serial 10^{-2} and 10^{-4} dilutions. For each food sample, each procedure was done in triplicate. For the microbial count, 1 ml of each dilution was transferred to a petri dish and spread on the specific types of solid media with a sterile bent glass rod. To determine the developed foods of total plate count, *Escherichia coli (E. coli), staphylococcus, salmonella, and shigella spp,* in the foods plate count agar, eosin methylene agar, mannitol salt agar, salmonella, and shigella agar, respectively were used and incubated for 24-36 h at 35 °C (Anigo et al., 2010; Getachew, 2017). To determine yeast and molds, the aliquot was spread on solidified potato dextrose agar supplemented with 0.1 g chloramphenicol and incubated at 28 ^{o}C for 5-7 days. The colonies were counted and the results were reported as $\log_{10}$ CFU/g (Spencer, 2001).

3.6. Sensory Analysis

A sensory evaluation of both the developed CF, and commercial wean-mix gruel acceptability was performed following the instructions of (Forsido et al., 2019; WFP, 2018). Gruel was made by mixing 50 g flour with 250 ml water, heating at 75 °C for 5 min, and stirring it with an aluminum ladle until it attained pasty consistency. Sensory analysis was carried out on 30 semi-trained mothers from Gondar, Ethiopia, who had a positive opinion toward eating the products. Each panelist was given instructions on how to code the appearance, aroma, taste, texture/mouthfeel, and overall acceptability of sample products. Before moving on to the next food test, the mothers were also informed to rinse their mouths with clean water. A five-point hedonic scale (5 = like very much, 4 = like moderately, 3 = neither like nor a dislike, 2 = dislike moderately, and 1 = dislike very much) was used (Forsido et al., 2019; Meilgaard et al., 2006).

3.7. *In-vivo* Experiments

3.7.1. Diet formulation and preparation

The ethical committee of the College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia, approved all aspects of animal care and experimentation in this study, which followed the National Institutes of Health's (NIH) Guide for Care and Use of Laboratory Animals and the EEC directive of 1986 (86/609/EEC). The basal diet was formulated, according to the methods by Adeoti and colleagues (Adeoti et al., 2018). The basal diet consisted of 610 g/kg corn starch, 50 g/kg wheat bran, 100 g/kg vegetable oil, 50 g/kg mineral and vitamin premix, 60 g/100kg glucose, 88 g/kg sucrose, 20 g/kg oyster shell, 2 g/kg NaCl, and 20 g/100kg bone meal. The commercial wean-mix was purchased from the supermarket in, Ethiopia while the two experimental formulated complementary diets were processed from locally available foodstuffs i.e., $ComF_{01}$ composed of in the ratio of 57:29:14 of maize, teff, and soybean, whereas $ComF_{02}$ was composed of in the ratio of 58:29:13 of maize, teff, and bee larvae, respectively and processed by extrusion technology. The experimental diets were formulated based on 10 % iso-nitrogenous protein levels and blended with the experimental diets into a basal (Adejuwon et al., 2021; Adeoti et al., 2018; Adepoju and Ajayi, 2016a; Osundahunsi and Aworh, 2003). The composition of the experimental food treatments was calculated using Equation 3.

$$IN = \frac{a}{100} x\, b = \frac{10}{100} x\, c \quad \text{.....Equation 3}$$

Where: a = original protein content of the sample as analyzed

b = Weight of sample required for the new feed mixture

c = Total weight of the mixture

IN= Isonitrogenous

The treatments were Treatment 1- Casein diet + basal diet; Treatment 2- $ComF_{01}$ (include soybean (14 %), teff (29 %), and maize (57 %) + basal diet; Treatment 3- $ComF_{02}$ (include bee larvae (13 %), teff (29 %), and maize (58 %) + basal diet; Treatment 4- Commercial wean mix (enriched mama's choice) + basal diet; Treatment 5- Basal diet alone. Finally, each group of BALB/c mice was randomly assigned to either of the treatments (Table 3.1).

Table 3.1: Experimental treatments formulation and preparation (g/kg)

Treatments	Protein Content %	Wt. of Basal Diet	Wt. of Food	Final Protein Level
Treatment 1	99	898.99	101.01	10
Treatment 2	12.56	203.82	796.18	10
Treatment 3	11.75	148.94	851.06	10
Treatment 4	10.78	72.36	927.64	10
Treatment 5	10	1000	_	10

Treatment 1=Casein diet; Treatment 2=soybean (14 %), teff (29 %), and maize (57 %); Treatment 3=bee larvae (13 %), teff (29 %), and maize (58 %); Treatment 4=Commercial wean mix (enriched mama's choice); Treatment Basal diet alone (610 g/kg corn starch, 50 g/kg wheat bran, 100 g/kg vegetable oil, 50 g/kg mineral, and vitamin premix, 60 g/100kg glucose, 88 g/kg sucrose, 20 g/kg oyster shell, 2 g/kg NaCl, and 20 g/100kg bone meal)

3.7.2. Experimental design

A total of seventy-five pathogen-free male, BALB/c mice, an average of 26–28 days old with an average body weight of 31.57 g, were obtained and maintained in the School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Ethiopia. Sample size calculation in animal studies was calculated according to (Arifin and Zahiruddin, 2017). The BALB/c mice were housed in polypropylene cages that measured 22.2 cm x 30.80 cm x 16.24 cm and included a wireframe-grade stainless steel feeder and a leak-proof water bottle fitting. The BALB/c mice were grouped into five

treatments, each with fifteen BALB/c mice. In a completely randomized design, the experimental treatment was done in triplicates with five BALB/c mice per cage. Each mouse and cage were given an identification mark. Each mouse was identified using a long-term marked felt-tip pen (skin marking) on their different parts of body. Each cage's bedding sawdust material was changed once a week. The experiment lasted 28 days, with seven days of acclimatization at the same environmental temperature (26 ± 0.42 °C) and relative humidity (55 ± 5 %), with a 12:12 hr. light-dark cycle. Throughout the experiment, clean tap water and diets were provided *ad libitum*. Water was changed every day for 28 days.

3.7.3. Feed intake and growth performance

The feeding and watering activities of all BALB/c mice were observed every day. Data of feed offered and leftovers from each group of BALB/c mice were recorded and calculated. The weekly body weight of each mouse was measured and recorded before the commencement of feeding. Growth performance was monitored and analyzed in terms of feed intake (Equation 4), weight gain (Equation 5), feed conversion ratio (Equation 6), and body weight gain (%) (Equation 7) which was calculated according to (Marwa et al., 2019) where:-

$$Feed\ Intake\ (FI) = (Feed\ offered - Feed\ leftover) \quad \text{Equation 4}$$

$$Weight\ Gain\ (WG) = (Final\ body\ weight - Initial\ body\ weight) \quad \text{Equation 5}$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Feed\ Intake}{Weight\ Gain} \quad \text{Equation 6}$$

The percentage of body weight gain (%) was calculated as follows (AL-Shinnawy, 2009):

$$Body\ Weight\ Gain\ (\%) = \frac{Mean\ Final\ Bodyweight - Mean\ Initial\ Bodyweight}{Mean\ Initial\ Bodyweight} \times 100 \quad \text{Equation 7}$$

3.7.4. Blood sample collection and analysis

After 12 hours of fast BALB/c mice were sacrificed on the 28 days of the experimental period. The BALB/c mice were anesthetized intraperitoneally with 0.25 ml/100 g body weight of ketamine-xylazine anaesthesia (mixed in a 4:1 ratio) before being sacrificed by cardiac puncture injection (Parasuraman et al., 2017; Wilson et al., 2016). Blood was drawn and immediately placed in the tube with anticoagulant Ethylene Di-Amine Tetra Acetic Acid (EDTA) bottles and serum containers, before being hand-mixed several times and placed on wet ice for further haematological analysis (Pierre et al., 2011). Plasma was isolated and frozen in aliquots at - 20 °C after centrifugation at 3000 rpm for 10 minutes at 4 °C in a digital laboratory centrifuge (TD4C dc brushless motor centrifuge, Hunan, China), while organ aliquots (liver, heart, kidney, and spleen) were removed and weighed immediately.

3.7.5. Haematological and biochemical analysis

Aliquots of blood samples were immediately subjected to a full blood count, which includes important haematological parameters namely, White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Platelet Count (PLT), Mean Cell Haemoglobin (MCH), Mean Cell Volume (MCV), and Mean Cell Haemoglobin Concentration (MCHC), using a fully automated ABX MICROS Pentra 60C+ Analyzer (Horiba ABX, Montpellier, France) (Mazzaccara et al., 2008). Briefly, 50-µl blood was aspired into a needle for the biochemical test, divided, and distributed to the various chambers for sample analysis. Total protein, serum albumin, and lipid profiles of Cholesterol (total) (TC), Triglyceride, High-Density Lipoprotein (HDL-C), and Low-Density Lipoprotein (LDL-C) evaluations, were done using automated pentra (C400, France).

3.7.6. Serum analysis for zinc, iron, and calcium

One ml of serum was mixed with 1 ml of concentrated nitric acid and 0.5 ml of hydrogen peroxide in propylene tubes. The mixture was maintained at 60 °C for 2 hr. to allow digestion of the samples until a clear colorless solution was achieved. The digest thus obtained was diluted by adding 2.5 ml of deionized water. The sample solutions were then centrifuged at 2000 rpm for 5 min and subsequently analysed (Luna et al., 2019). Different concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/l) stock solutions of trace elements were used for the calibration of standard graphs. Finally, the concentrations of Zn, Fe, and Ca were read using atomic absorption spectrophotometer (a Buck 210 VGP, U.S.A) with their hollow cathode lamp at their respective wavelengths of 213.9 nm, 248.3 nm, and 422.7 nm, respectively (AOAC, 2006).

3.7.7. Atherogenic and castelli's risk indices

To estimate the risk of CVD Castelli's Risk Indices (I and II) Atherogenic coefficient, and Atherogenic index of plasma were calculated (Akangbou et al., 2018; Ikewuchi, 2009; Olamoyegun et al., 2016) in Equations *7*, 8, 9, and 10.

$$Castelli's\ Risk\ Indices - I\ (CRI - I) = \frac{TC}{HDL - C} \quad \text{Equation 8}$$

$$Castelli's\ Risk\ Indices - II\ (CRI - II) = \frac{LDL - C}{HDL - C} \quad \text{Equation 9}$$

$$Atherogenic\ Coefficient\ (AC) = \frac{(TC - HDL - C)}{HDL - C} \quad \text{Equation 10}$$

$$Atherogenic\ index\ of\ Plasma\ (AIP) = Log\left(\frac{TG}{HDL - C}\right) \quad \text{Equation 11}$$

3.8. Statistical Analysis

The nutritional composition, microbiological, and sensory analyses of CFs, as well as growth performance, haematological, biochemical, and Castelli's risk, and, atherogenic indices, were reported as means and standard deviations. The above-mentioned data were subjected to a one-way analysis of variance, and the List Significant Difference test was used to test for similarities between all the experimental groups. The level of significant difference was set ($P<0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Nutritional Composition of Ingredients

For product development, regulatory purposes, or, quality control, the proximate composition of foods can be of interest in the food industry (Nielsen, 2016). Table 4.1 shows the proximate composition of different individual food ingredients. The moisture content of food ingredients ranged from 6.04 to 13.36 g/100g, with maize having the highest moisture content of 13.36 g/100g. Soybean (50.50 g/100g), had the highest protein content followed by bee larvae, maize, and teff which were 45.70, 10.10, and 9.79 g/100g, respectively. The protein content of soybean reported by (Heiru, 2017; Mesfin et al., 2017) was 27.00 and 35.59 g/100g, respectively, which was lower than the present study. There were high records of carbohydrate contents of teff 72.44 g/100g, followed by maize 69.55 g/100g, soybean 14.34 g/100g, and bee larvae 14.24 g/100g.

Table 4.1: Proximate composition of individual food ingredients (g/100g, dried weight basis)

Ingredients	Moisture %	Ash	Protein	Fat	Fiber	Carbohydrate
Bee larvae	8.68±0.17^{b}	3.66±0.19^{b}	45.70±0.85^{b}	24.98±0.12^{a}	2.74±0.10^{c}	14.24±0.59^{c}
Soybean	6.04±0.10^{c}	5.04±0.07^{a}	50.50±0.50^{a}	19.53±0.53^{b}	4.54±0.22^{a}	14.34±1.13^{c}
Red teff	8.45±0.05^{b}	3.44±0.04^{b}	9.79±0.07^{c}	2.65±0.06^{d}	3.23±0.10^{b}	72.44±0.06^{a}
White maize	13.36±0.21^{a}	0.61±0.19^{c}	10.10±0.13^{c}	4.88±0.21^{c}	1.50±0.06^{d}	69.55±0.12^{b}

The results are presented as the SD of the means. Means with different superscripts (alphabets) in the same column are significantly different, $P<0.05$.

The fat content of soybean was greater than the report of (Mesfin et al., 2017) and slightly lower than the values reported by (Heiru, 2017) which were 10.22 g/100g and 22.88 g/100g, respectively The protein (8.2 g/100g) and fat (4.58 g/100g) content of soaked and germinated maize grain flours were found to be similar, with the report (Gebrezgi, 2019). However, the carbohydrate content of teff flours was slightly lower than that reported by (Heiru, 2017; Mesfin et al., 2017). The variation of all these may be due to temperature, genotype, water availability, environmental conditions, soil fertility during grain development (Rooney, 1996), and the method of processing (Jean Mayer and Schweigert, 1975).

The bee larvae fat, and protein values of the present study were different from the reports of (Ghosh et al., 2016) who reported lower 14.5 g/100g fat and 35.3 g/100g protein. Moreover, (Hocking and Matsumura, 2015; Krell, 1996) reported lower ash, protein, and fat contents of insect bee larvae compared to our study. The variation may be due to season and climate (Ghosh et al., 2016), the type of insect feed (Kouřimská and Adámková, 2016), and the species of bee insect (Chapman et al., 2003).

4.2. Proximate Composition of Complementary Foods and Commercial Wean-Mix

To provide the right protein and calorie content for growing children, extruded complementary foods were developed from a blend of grains and legumes (Fellows, 2017). Food composition data is required for evaluating diet quality and developing and implementing food-based dietary recommendations, making it an important tool in the field of public health nutrition (Elmadfa and Meyer, 2010). Table 4.2 illustrates the proximate (g/100g) and mineral (mg/100g) composition, as well as the energy content (Kcal/100g) of extruded complementary foods and commercial wean-mix.

Table 4.2: Proximate (g/100g), energy content (kcal/100g), and mineral (mg/100g) composition of extruded complementary foods and commercial wean-mix

Nutrients	$ComF_{01}$	$ComF_{02}$	Commercial wean-mix	P-Value	RV^{+}
Moisture	4.41 ± 0.19^{b}	5.72 ± 0.17^{a}	2.46 ± 0.39^{c}	<0.001	10^{α}
Ash	2.09 ± 0.09^{a}	1.88 ± 0.04^{b}	2.01 ± 0.08^{ab}	0.037	$<4^{\alpha}$
Protein	12.56 ± 0.17^{a}	11.75 ± 0.15^{b}	10.78 ± 0.29^{c}	<0.01	15^{+}
Fat	12.4 ± 0.1^{b}	14.3 ± 0.1^{a}	2.82 ± 0.36^{c}	<0.001	$10\text{-}25^{+}$
Fiber	4.52 ± 0.04^{a}	3.47 ± 0.08^{b}	2.75 ± 0.17^{c}	<0.001	$<5^{+}$
Carbohydrate	64.02 ± 0.41^{b}	62.87 ± 0.23^{c}	79.19 ± 0.55^{a}	<0.001	$60\text{-}75^{\infty}$
Energy	417.93 ± 3.23^{b}	427.18 ± 2.42^{a}	385.25 ± 1.77^{c}	<0.001	$400\text{-}425^{+}$
Fe	40.17 ± 0.38^{b}	40.94 ± 0.29^{a}	5.79 ± 0.16^{c}	<0.001	9.3*
Zn	2.84 ± 0.18^{a}	2.92 ± 0.16^{a}	2.32 ± 0.11^{b}	0.006	4.1*
Ca	31.78 ± 0.11^{c}	44.34 ± 0.49^{b}	68.20 ± 0.12^{a}	<0.001	0.40*

The results are presented as SD of the means. $ComF_{01}$= Complementary Food 01 (white maize + Red teff + Soybean); $ComF_{02}$= Complementary Food 02 (white maize + Red teff + Insect bee larvae); Commercial wean-mix (Enriched Mama's Choice); means with different superscripts (alphabets) in the same row are significantly different (P<0.05); RV= Recommended Value; *sources: (Dewey and Brown, 2003; FAO/WHO, 2002); $^{+}$ source (Codex, 2017); $^{\alpha}$(WFP, 2013); $^{\infty}$Estimated from data given for protein and fat in the codex standard

The developed CFs and commercial wean-mix fulfilled the moisture, ash, protein, fat, fiber, carbohydrate, and energy (kcal) criteria set by the Codex Alimentarius Commission (Codex, 2010). Ash content was a statistically significant difference (P=0.037) between the foods and high records with $ComF_{01}$ (2.09 g/100g). There was a significant difference (P≤0.001) between the moisture, fat, fiber, carbohydrate, and energy contents of the foods. The moisture content of the foods ranged from 2.46 to 5.72 g/100g. The highest moisture content had in $ComF_{02}$ (5.72 g/100g) while the least (2.46 g/100g) was in the commercial wean-mix. Proteins were essential for a child's rapid

growth and development, both in terms of quantity and quality. The protein content of $ComF_{01}$, $ComF_{02}$, and commercial wean-mix was 12.56, 11.75, and 10.78 g/100g, respectively. $ComF_{01}$ has a considerably greater protein amount than bee larvae, which might be attributable to the soybean in the formulation. However, the values of $ComF_{01}$ and $ComF_{02}$ were significantly higher than the commercial wean-mix. There was higher fiber (4.52 g/100g) content on $ComF_{01}$ followed by $ComF_{02}$ (3.47 g/100g) and commercial wean-mix (2.75 g/100g). The highest fat (14.3 g/100g), and energy (427.18 g/100g) contents were observed in $ComF_{02}$, while the least 2.82 g/100g fat, and 385.25 g/100g energy were observed in the commercial wean-mix. The greater nutritional energy content of $ComF_{02}$ compared to other diets might be attributed to fat-rich bee larval mixes.

In developing countries, micronutrient deficiency is a serious public health concern, particularly among infants and children in their first two years of life (Eichler et al., 2012). Iron, zinc, and calcium were important minerals in the complementary feeding of IYCF. There was a significant difference ($P \leq 0.001$) in minerals iron, and calcium contents between the developed foods and commercial wean-mix (Table 4.2). High values of iron (40.94 mg/100g) and calcium (68.20 mg/100g) were recorded in $ComF_{02}$ and commercial wean-mix, respectively. Both developed CFs had higher mineral calcium and iron content than the recommended value (40 mg/100g) (Dewey and Brown, 2003; WFP, 2018). This could be due to mineral-rich insect bee larvae blending in complementary foods (Ghosh et al., 2016). However, the mineral content of zinc in all foods did not meet the recommended value. In contrast, commercial wean-mix zinc (2.32 mg/100g) was found to be significantly lower ($P=0.006$) than both $ComF_{01}$ and $ComF_{02}$ foods.

4.3. Vitamins Composition of Complementary Foods and Commercial Wean-Mix

Establishing accurate daily vitamin requirements was difficult, and there was a lot of individual differences; nevertheless, with a healthy balanced diet, meeting the reference nutrient consumption should be possible (Leaf, 2007). Table 4.3 shows the vitamin composition of the developed CFs and commercial wean-mix. The vitamin composition was a significant difference ($P \leq 0.001$) between the developed CFs and commercial wean-mix. Vitamin A results revealed that both $ComF_{02}$ and commercial wean-mix met the recommended value (FAO/WHO, 2002). The commercial wean-mix (2082.02 µg/100g) had a greater vitamin A composition followed $ComF_{02}$ (706.8 µg/100g) and $ComF_{01}$ (167.6 µg/100g). $ComF_{01}$ had the highest values of thiamine (0.81 mg/100g) and riboflavin (0.70 mg/100g), but lower records of pyridoxine (0.29 mg/100g) and folate (51 g/100g). Niacin (8.20 mg/100g) and folate (86.70 µg/100g) were also high in $ComF_{02}$, but riboflavin did not satisfy the recommended value.

Table 4.3: Vitamins composition of complementary foods and commercial wean-mix per 100g

Vitamins	$ComF_{01}$	$ComF_{02}$	Commercial wean-mix	P-Value	RV[α]
Vitamin A µg	167.3±5.84[c]	706.8±16.28[b]	2082.02±85.08[a]	<0.001	400
B1 (Thiamine) mg	0.81±0.09[a]	0.48±0.06[b]	0.24±0.36[c]	<0.001	0.36
B2 (Riboflavin) mg	0.70±0.07[a]	0.26±0.03[c]	0.44±0.20[b]	<0.001	0.36
B3 (Niacin) mg	5.23±0.41[bc]	8.20±0.32[a]	6.33±1.43[c]	<0.001	6.0
B6 (Pyridoxine) mg	0.29±0.019[c]	0.45±0.01[ab]	0.53±0.47[a]	<0.001	0.44
B9 (Folate) µg	51.0±4.9[c]	86.70±1.80[a]	77.76±16.31[ab]	<0.001	83

The results are presented as SD of the means. $ComF_{01}$= Complementary Food 01 (white maize + Red teff + Soybean); $ComF_{02}$= Complementary Food 02 (white maize + Red teff +Insect bee larvae); commercial

wean-mix (Mama's Choice); means with different superscripts (alphabets) in the same row are significantly different $P<0.05$; RV= Recommended Value; [α] sources (FAO/WHO, 2002).

4.4. Contribution to Recommended Dietary Allowance

The percentage contribution of macro and micronutrients from complementary foods and commercial wean-mix that met RDA for 6–12 months is summarized in Table 4.4. The highest contributions to the RDA of protein were made by $ComF_{01}$, $ComF_{02}$, and commercial wean-mix, at 114.18 %, 106.82 %, and 98 %, respectively. However, except for $ComF_{02}$ energy contribution, less than half of the RDA of fat and energy for IYC was met. All foods had the potential to contribute to the RDA of Fe (52.64–372.18 %) and Zn (73.33–97.33 %), however, the contribution of Ca (12.22–26.23 %) intake was very low. Apart from $ComF_{01}$ for vitamin A (33.34 %), the vitamins' potential contribution of CFs to the RDA was the highest.

Table 4.4: The percentage contribution of macro and micronutrients from complementary foods and commercial wean-mix that met RDA for 6-12 months

Nutrients	RDA$^{\beta}$	Percentage RDA met		
		ComF$_{01}$	**ComF$_{02}$**	**Commercial wean-mix**
Energy (kcal)	850	49.17	50.26	45.32
Protein (g/day)	11	114.18	106.82	98
Carbohydrate (g)	95	67.39	66.19	83.36
Fat (g)	30	41.33	47.67	9.40
Ca (mg/day)	260	12.22	17.05	26.23
Zn (mg/day)	3	94.67	97.33	73.33
Fe (mg/day)	11	365.18	372.18	52.64
Vitamin A (µg RE)	500	33.34	141.36	416
B1 (Thiamine) mg/day	0.3	270.67	160	80
B2 (Riboflavin) mg/day	0.4	175	65	110
B3 (Niacin) mg/day	4.0	130.75	205	158.25
B6 (Pyridoxine) mg/day	0.3	96.67	150	176.67
B9 (Folate) µg /day	80	63.75	108.38	97.2

ComF$_{01}$= Complementary Food 01 (white maize + Red teff + Soybean); ComF$_{02}$= Complementary Food 02 (white maize + Red teff +Insect bee larvae); commercial wean-mix (Mama's Choice); $^{\beta}$Source Dietary Reference Intake (National Academies of Sciences, 2019; WFP, 2018)

4.5. Antinutrients Composition of Complementary Foods

Antinutrients in children's CFs may have an adverse effect on their nutritional status (Roos et al., 2013). As a result, the antinutritional factors in the formulated diets were significantly reduced when extrusion was used (Omosebi et al., 2018). Table 4.5 shows the antinutrient profile of developed CFs mg/100g.

Table 4.5: Antinutrients composition of developed complementary foods (mg/100g)

CFs	Antinutrients		
	Tannins	**Phytates**	**Saponins**
$ComF_{01}$	208.93±0.04[a]	68.18±4.15[a]	Nd
$ComF_{02}$	119.37± 0.31[b]	13.13±0.63[b]	Nd
Commercial wean-mix	63.69 ± 0.34[c]	10.46 ± 0.5[b]	Nd
P-value	<0.001	<0.001	-

The results are presented as SD of the means, $ComF_{01}$= Complementary Foods (white maize + Red teff + Soybean); $ComF_{02}$= Complementary Foods (white maize + Red teff + Insect bee larvae); nd= not detected. Means with different superscripts (alphabets) in the same column are significantly different P<0.05.

$ComF_{01}$ had higher levels of tannins (208.93 mg/100g) and phytates (68.18 mg/100g) than $ComF_{02}$ and commercial wean-mix, which showed tannins (119.37 mg/100g and 63.60 mg/100g) and phytates (13.13 mg/100g and 10.46 mg/100g), respectively. Saponins, on the other hand, were not found in either CFs or commercial wean-mix. Phytates in cereal-based complementary diets reduce the bioavailability of the mineral including zinc, iron, calcium, and in certain instances proteins, all of which are essential for infant development (Amagloh, 2012). By associating protein substrates with ionizable iron, tannin-protein complexes can contribute to digestive enzyme deactivation and impair protein digestibility (Ogunkoya et al., 2006). Tannins in food can alter food quality, growth, iron absorption, the mucosal lining of the gastrointestinal system, excretion, and protein and essential amino acid excretion (Omosebi et al., 2018).

4.6. Bioavailability of Minerals

Both the phytate content and the mineral calcium, iron, and zinc molar ratio estimated by CF were within the prescribed ranges (Table 4.6).

Table 4.6: Minerals: phytate molar ratio in developed complementary foods and commercial wean-mix

Sample	Phytate: Calcium	Phytate: Iron	Phytate: Zinc
$ComF_{01}$	0.13^{a}	0.14^{c}	2.40^{a}
$ComF_{02}$	0.02^{b}	0.03^{b}	0.50^{bc}
Commercial wean-mix	0.01^{c}	0.02^{a}	0.45^{c}
P-value	<0.001	<0.001	<0.001
Limits	$<0.24^{*}$	$<1^{\alpha}$	$<15^{\beta}$

$ComF_{01}$= Complementary Foods 01 (white maize + Red teff + Soybean); $ComF_{02}$= Complementary Foods 02 (white maize + Red teff +Insect bee larvae); Commercial wean-mix (Mama's Choice); means with different superscripts (alphabets) in the same column are significantly different P<0.05 $^{\alpha}$sources: (Hallberg et al., 1989); $^{\beta}$source:(Sandberg et al., 1987); *(Bindra et al., 1986)

The recommended limits of phytate to calcium, iron and zinc were (phytate to calcium) < 0.24 for calcium (Bindra et al., 1986; Morris and Ellis, 1989), for (phytate to iron) < 1 for iron (Hallberg et al., 1989), and (phytate to zinc) <15 for zinc (Sandberg et al., 1987). Mineral (mg/100g) molar ration $ComF_{01}$, $ComF_{02}$, and commercial wean-mix of phytate/Calcium (0.13, 0.02 and 0.01), Zinc (2.40, 0.50 and 0.45), and Iron (0.14, 0.03 and 0.02), respectively showed good iron, zinc, and calcium bioavailability in both CFs and commercial wean-mix. This might be owing to the CFs' low levels of phytate. Adding legumes ($ComF_{01}$) to such diets can also greatly increase iron levels, even though non-heme iron has a lower bioavailability than heme iron (WHO/FAO, 2004). According to the report of (Anuonye et al., 2012) reduced phytic acid could improve the bioavailability of iron and zinc in extrudates, as phytic acid has been associated with their inaccessibility. Extrusion cooking degrades antinutrients in cereals and legumes by about 30 % (Sathe and Venkatachalam, 2002). With increasing phytate intake, the inhibitory effect of phytate on the bioavailability of minerals increases (Al Hasan et al., 2016). Phytic acid binds trace and macro elements including magnesium, iron, calcium,

and copper in the gastrointestinal system, making them unavailable for absorption and usage by the body (Ramakrishna et al., 2006).

4.7. Microbiological Loads of Complementary Foods

The microbiological load of any food item, on the other hand, is a valuable indicator of extrudate quality as well as exposing the possible safety status of extruded food products from the human consumption attitude and storage (Oluwole et al., 2013). The microbiological counts (log10 CFU/g) of the developed CFs on day one, and at three and six months after storage, as well as commercial wean-mix, are summarized in Table 4.7.

Table 4.7: Microbiological counts (log_{10} CFU/g) of the developed complementary foods after three and six months of storage and commercial wean-mix

Microorganisms	*Day 1*		*At 3 months*		*At 6 months*			Limit *
	ComF 01	**ComF 02**	**ComF 01**	**ComF 02**	**ComF 01**	**ComF 02**	**Commercial wean-mix**	
E. Coli	nd	nd	Nd	nd	nd	nd	Nd	<1
S. aureus	nd	nd	Nd	nd	nd	nd	Nd	< 1
Salmonella	nd	nd	Nd	nd	nd	nd	Nd	0 /25 g
Shigella	nd	nd	Nd	nd	nd	nd	Nd	0 /25 g
TPC	nd	nd	3.36	3.04	3.46	3.17	Nd	< 5
Yeast	nd	nd	2.00	2.00	2.30	2.18	Nd	< 3
Molds	nd	nd	2.17	2.30	2.60	2.40	Nd	< 3

Results are presented as means. CFU= Colony Forming Unite; TPC= Total Plate Count *****(ICMSF, 1978; WFP, 2013); nd= not detected

Microbiological results of both CFs were below the acceptable limit (<5 log_{10} CFU/g) (WFP, 2013), and not detected on commercial wean-mix. High moisture levels (over 10

%) increase spoilage by stimulating microbial activity and chemical reactions that shorten the food's shelf life (Amegovu et al., 2013). Thus, microbial counts of *E. coli, Salmonella*, and *Shigella spp, Staphylococcus*, total plate count, yeast, and molds of both CFs were below the acceptable limit. This might be because of the quality control measures used during manufacturing and techniques (Obi and Nwozor, 2012; Suleiman et al., 2013) and because of low moisture amount (Srivalli et al., 2017).

Microbial counts of both CFs were not observed on day one. Similarly, no *E. coli, Staphylococcus aureus, Salmonella,* or *Shigella spp* were found after six months of storage. The present findings of bacterial *Salmonella, Shigella,* and *Staphylococcus spp* were similar to (Cetinkaya et al., 2008), who reported no bacterial count observed in developed weaning food samples. This was most likely because microorganisms were either not persistent in the environment or were destroyed during cooking (Muleta and Ashenafi, 2001; Verma, 2017).

After three and six months of storage, there was a slight rise in microbial load. At three months, the mean total plate count of $ComF_{01}$ (3.36 log_{10} CFU/g) and $ComF_{02}$ (3.04 log_{10} CFU/g) was recorded, and at six months, the mean total plate count of $ComF_{01}$ (3.46 log_{10} CFU/g) and $ComF_{02}$ (3.17 log10 CFU/g) was reported. Cross-contamination by migration of substances from packaging into food (Gungor and Gokoglu, 2010), prolonged storage time (Oluwole et al., 2013), or contamination of packaging material (Tshipamba et al., 2018; Uzma Altaf, 2019) might all be factored in the detection of total plate counts in the CFs at an acceptable level.

$ComF_{01}$ yeast counts were recorded after three months (2.0 log_{10} CFU/g) and six months (2.30 log_{10} CFU/g) of storage, as well as mean mold counts after three and six months, were 2.17 log_{10} CFU/g, and 2.60 log_{10} CFU/g, respectively. Mold counts of $ComF_{02}$ at three (2.30 log_{10} CFU/g) and six months (2.40 log_{10} CFU/g), as well as yeast counts at three (2.00 log_{10} CFU/g) and six months (2.18 log_{10} CFU/g), were observed. In the

developed CFs the growth of mold and yeast after three and six months of storage might be associated with packaging or storage conditions. The majority of yeast and molds growth temperature ranges (10–35 °C) were broad and were obligatory aerobic (Valerie T et al., 2001). In comparison to yeast and mold, the developed CFs had greater mold counts (CFU/g) than yeast in both CFs, but not on commercial wean-mix. This might be because molds have minimal moisture needs, but yeasts require more water activity (Labuza and Altunakar, 2020). As a result, it appears that inhibition of microbial growth is preferred in high-temperature, low-moisture conditions (Leonard et al., 2020).

4.8. Sensory Analysis

Consumers provide data for sensory analysis, which is used to make decisions (Stone, 2018). Commercial wean-mix (Table 4.8) had better sensory attributes and overall acceptability than $ComF_{01}$ and $ComF_{02}$. The sensory test included a small number of participants, which raised the chances of achieving accurate and trustworthy findings (Svensson, 2012). The appearance and aroma were statistically significant differences ($P=0.003$) between the developed CFs and commercial wean-mix. Food acceptability and choice were heavily influenced by appearance (Muhimbula et al., 2011). The sensory evaluation revealed that the $ComF_{01}$ and $ComF_{02}$ samples appeared to be similar in appearance, however, the commercial wean-mix samples differed significantly. However, $ComF_{01}$ had a lower overall acceptability score than $ComF_{02}$. This might be related to the presence of more tannins in $ComF_{01}$. According to the report of (Anuonye et al., 2012), tannins also decrease palatability. There was a highly significant difference ($P\leq0.001$) between the foods of taste, texture, and overall acceptability. Panelists preferred $ComF_{02}$ over $ComF_{01}$ in terms of aroma, taste, and overall acceptability, although it was lower acceptable than the commercial wean-mix. The flavoring additives in the commercial wean-mix would give it the highest score rating than the developed CFs (Ikpeme-Emmanuel et al., 2009).

Table 4.8: Sensory analysis of the developed complementary foods compared to commercial wean-mix

Characteristic	$ComF_{01}$	$ComF_{02}$	Commercial wean-mix	P-Value
Appearance	3.77 ± 0.82^{c}	4.07 ± 0.69^{bc}	4.41 ± 0.74^{a}	0.003
Aroma	3.73 ± 0.91^{c}	4.23 ± 0.63^{ab}	4.40 ± 0.80^{a}	0.003
Taste	3.57 ± 0.73^{c}	4.43 ± 0.63^{ab}	4.50 ± 0.68^{a}	<0.001
Texture/Mouth Feel	3.77 ± 0.71^{c}	3.97 ± 0.61^{bc}	4.47 ± 0.51^{a}	<0.001
Overall Acceptability	3.63 ± 0.61^{c}	4.20 ± 0.55^{b}	4.63 ± 0.49^{a}	<0.001

The results are presented as SD of the means. $ComF_{01}$= Complementary Foods 01 (white maize + Red teff + Soybean); $ComF_{02}$= Complementary Foods 02 (white maize + Red teff +Insect bee larvae); Commercial wean-mix (Mama's Choice); means with different superscripts (alphabets) in the same row are significantly different ($P<0.05$).

Because of their inability to verbal communication, limited cognitive capabilities, and very low attention spans, IYC presents a challenge to sensory and consumer researchers (Lavin and Lawless, 1998). Furthermore, most investigations indicated that adults have higher sensitivity than young children (Guinard, 2000). Indirect techniques have been used often in IYC sensory testing. Therefore, mothers or caretakers performed the sensory evaluation for this study. The evaluation of parents' preferences is critical in evaluating whether a certain CF is appropriate for their children (Haro-Vicente et al., 2017; Madrelle et al., 2017). All sensory evaluations of the developed CFs and commercial wean-mix were above the minimum threshold, which was three neither likes nor dislikes on the hedonic scale (Agbemafle et al., 2020).

4.9. Feed Intake and Body Weight of Experimental BALB/c Mice

Infants the "first 1,000 days" of life is critical for child development, so complementary feeding practices should be optimized to maximize children's potential for growth and

development (Martorell, 2017). Inappropriate complementary feeding results in malnutrition due to a mismatch of nutritional requirements with intake (Keller Ulrich, 2019). The present finding showed that at the end of the feeding trial, significant differences in the final body weight (g), weight gain (g), body weight gain (%), and Feed Conversion Ratio (FCR) were observed among the dietary groups (Table 4.9). There was no statistically significant difference in feed intake (P=0.96) or initial body weight (P=0.99) between experimental BALB/c mice treatments, however, there was a significant difference (P=0.01) in final body weight. The nutritional status of the experimental BALB/c mice showed that mice fed with T2 (38.39 g) and T3 (38.52 g) had better growth performance, followed by T1 (37.15 g), T4 (35.02 g), and T5 (33.37 g). Similarly, the weight gain (g/day) of the BALB/c mice was statistically significantly different (P<0.01), and it ranged from 1.67 to 7.05, the highest being on T2 (7.05 g) and T3 (6.63 g). The difference in the final body weight of T2 and T3 could be due to the type of nutrients in the diets that were probably easily absorbed in the body (Obatolu et al., 2000).

Results of body weight gain (%) showed that there was a significant difference (P=0.03) between experimental treatments. A high percentage of body weight gain on T2 (22.61) and T3 (20.83), and low on T4 (11.79) and T5 (5.28) were recorded. The high percentage of body weight gain on T2 and T3 could be due to the high protein content inclusion of soybean (12.56 g/100g) and insect bee larvae (11.75 g/100g) within the ingredients of the developed diet. Indeed, according to (Adeoti et al., 2018), consuming the quality and quantity of a protein-containing diet could influence weight gain. A similar trend was observed in FCR. The FCR is a measure of how well an animal converts feed intake (feed usage) into live body weight (Adeyemi et al., 2015). The study showed a statistically significant difference (P=0.02) in FCR between treatments. BALB/c mice fed T2 (9.3) exhibited a higher FCR than T3, T1, T4, and T5, which were 10.24, 12.34, 15.91, and 36.25, respectively. From the observed results of the BALB/c

mice model, both T2 and T3 could be appropriate as CFs, support the growth of IYC and prevent from malnourished.

Table 4.9: Average feed intake (g), weight gain (g), body weight gain (%), and FCR of experimental BALB/c mice

	Experimental Groups					
Parameters	**T 1**	**T 2**	**T 3**	**T 4**	**T 5**	**P-Value**
Feed Intake	64.41±11.72	66.70±17.66	67.91±12.86	58.71±18.57	60.53±21.64	>0.05
Initial body weight	31.61±1.08	31.35±1.08	31.89±0.42	31.31±0.13	31.70±1.22	>0.05
Final Body Weight	$37.15±1.06^{a}$	$38.39±0.66^{a}$	$38.52±0.66^{a}$	$35.02±2.35^{b}$	$33.37±2.48^{b}$	0.01
Weight Gain	$5.53±2.23^{ab}$	$7.05±1.63^{a}$	$6.63±1.06^{a}$	$3.69±1.40^{b}$	$1.67±1.64^{b}$	0.01
Bodyweight gain	$17.53±4.23^{bc}$	$22.61±6.05^{a}$	$20.83±3.63^{a}$	$11.79±4.47^{c}$	$5.28±5.08^{c}$	0.03
FCR	$12.34±2.65^{c}$	$9.30±1.25^{e}$	$10.24±1.57^{d}$	$15.91±6.32^{b}$	$36.25±13.97^{a}$	0.02

The results are presented as SD of the means. Means with different superscripts (alphabets) in the same row are significantly different ($P<0.05$); T= Treatment, T1= Casein diet + Basal diet; T2=maize, teff with soybean + Basal diet; T3=maize, teff with bee larvae + Basal diet; T4=commercial wean mix (enriched mama's choice) + Basal diet; T5=Basal diet alone.

Trends of average body weight change of experimental animals over twenty-eight days were observed (Figure 4.1). From the initial experimental week to the end of the experimental period, there was an increment in body weight in all experimental treatments. T1, T2, and T3 had similar trends of body weight change over time; however, T4 and T5 increased steadily, especially T5, whose body weight change was very slight. The growth trends of BALB/c mice fed both T1 and T4 were significantly lower than T2 and T3 but had a greater increase than T5 (basal diets). The difference in growth trend between treatments would be due to the quality of proteins in the diets and their intake. According to the report of (Adejuwon et al., 2021) protein deficiency in young animals causes hypoproteinemia, stunting, muscular wasting, anemia, body protein depletion, emaciation, and, if severe enough, death.

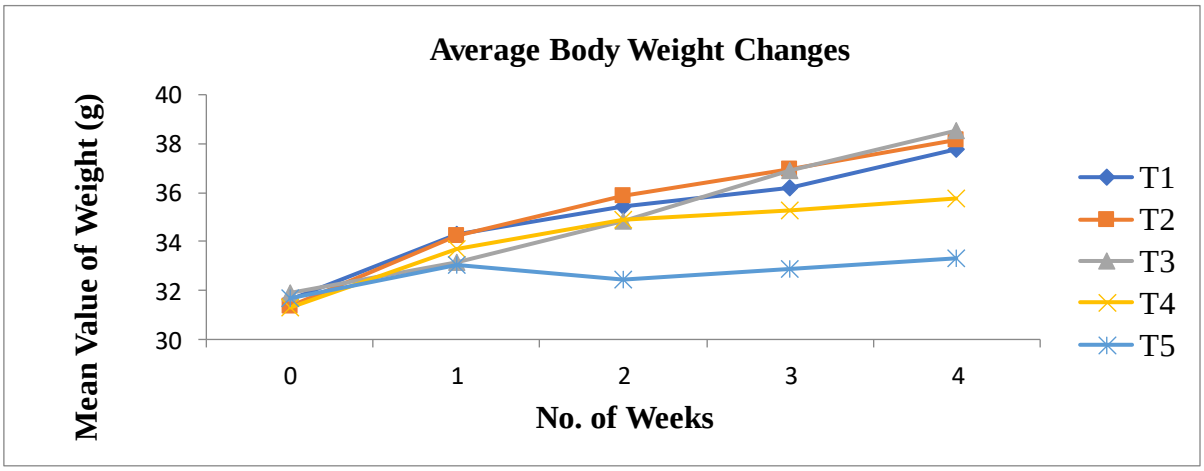

Figure 4.1: Trends of average body weight change of experimental BALB/c mice over twenty-eight days

Were T= Treatment, T1= Casein diet + basal diet, T2=Maize, teff with soybean + basal diet, T3=Maize, teff with bee larvae + basal diet, T4=Commercial wean mix (enriched mama's choice) + basal diet, T5=Basal diet alone.

4.10. Organ Weights

It's more difficult for mice to understand differences in organ weight. Internal organ weight variations between treatment groups are typically accompanied by body weight differences (Bailey et al., 2004). There were no statistically significant differences ($P>0.05$) in organ weights (kidneys, liver, and heart) between treatments (Table 4.10). The present finding was in line with the findings of (Osundahunsi and Aworh, 2003), the liver and kidney weights of mice given the complementary diets were not significantly different from those of BALB/c mice fed the basal and casein diets. The lower weight of organs in rats fed a basal diet, possibly indicated an abnormal development. However, the spleen weight (g) between experimental treatments was a statistically significant difference ($P<0.001$), which ranged between 0.29 and 0.53 g. T2 (0.53 g) had the highest spleen weight, followed by T1, T3, T4, and T5, which had 0.35, 0.29, 0.31, and 0.29 g, respectively. The difference in spleen weight between treatments would be due to the

positive correlation of body weight. Reports by (Bailey et al., 2004; Eisen, 1986; Simpson and Spears, 1973) indicated a positive genetic correlation between body weight and respective organ weights. In addition to this, there was an association between RBC size with enlargement of the spleen (Pivkin et al., 2016). However, the weight of the spleen change can be difficult to interpret (Resendez and Rehagen, 2017).

Table 4.10: Results of organ weight (g) of experimental BALB/c mice

Organs	T 1	T 2	T 3	T 4	T 5	P-Value
Kidneys	0.68±0.11	0.70±0.11	0.70±0.11	0.61±0.06	0.62±0.08	0.16
Liver	2.69±0.34	2.69±0.46	2.42±0.45	2.43±0.17	2.37±0.11	0.13
Heart	0.23±0.04	0.23±0.05	0.22±0.04	0.22±0.44	0.18±0.04	0.06
Spleen	0.35 ± 0.05^{b}	0.53 ± 0.05^{a}	0.29 ± 0.15^{b}	0.31 ± 0.13^{b}	0.29 ± 0.08^{b}	<0.001

The results are presented as SD of the means. Means with different superscripts (alphabets) in the same row are significantly different (P<0.05); T= Treatment, T1= Casein diet + basal diet, T2=Maize, teff with soybean + basal diet, T3=Maize, teff with bee larvae + basal diet, T4=Commercial wean mix (enriched mama's choice) + basal diet, T5=Basal diet alone

4.11. Biochemical and Haematological Parameters

The examination of blood allows for investigating the presence of several metabolites and other constituents in the body of animals and plays a vital role in the physiological, and nutritional status of an organism (Etim et al., 2014). To assess general nutritional status and identify specific nutritional deficiencies, the biochemical assessment of serum protein and serum micronutrients was used (Gilbert and Weiner, 2013). Also, haematological analysis was conducted in this study to further check the effects of different diets on blood parameters. Table 4.11 shows the effects of the experimental diets on the biochemical and haematological properties of BALB/c mice fed extrudate CF samples compared with casein, commercial wean mix, and basal diets.

Biochemical profiles of the mean serum albumin (mg/dl) were not significantly different (P=0.10) between the experimental treatments. In human serum albumin is the most abundant protein (Keller Ulrich, 2019). Serum protein and globulin, on the other hand, were significantly different (P<0.001) between the experimental treatments. Serum protein and globulin (mg/dl) showed that there were high amounts of serum protein (10.78), and globulin (7.66) on T5, and low amounts in T4, which was 6.27 serum protein, and 3.61 globulin. A high total serum protein level indicated dehydration and a low level indicated that protein is not being digested or absorbed properly and malnutrition (Wu, 2016). Therefore, based on this *in vivo* experiment when an infant was inadequately fed, there is the risk of stunted growth and a range of biochemical changes that can impair development to a large extent (Amankwah et al., 2010).

Table 4.11: Effects of nutritional intervention on biochemical and haematological parameters of white albino BALB/c mice

Parameters	T 1	T 2	T 3	T 4	T 5	P-Value
Serum protein						
Serum Protein (mg/dl)	8.37 ± 0.12^{bc}	8.80 ± 0.36^{b}	7.08 ± 0.72^{cd}	6.27 ± 0.84^{d}	10.78 ± 0.39^{a}	<0.001
Serum Albumin (mg/dl)	2.94±0.09	3.01±0.12	2.94±0.14	2.65±0.21	3.12±0.22	0.10
Serum Globulin (mg/dl)	5.43 ± 0.09^{bc}	5.79 ± 0.42^{b}	4.14 ± 0.80^{cd}	3.61 ± 0.79^{d}	7.66 ± 0.48^{a}	<0.001
Hematological characteristics						
WBC ($X10^6$ mm^3)	5.27 ± 0.95^{ab}	2.77 ± 0.67^{bc}	4.0 ± 0.30^{b}	1.97 ± 0.38^{c}	3.57 ± 0.21^{b}	<0.001
RBC ($X10^3$ mm^3)	9.72 ± 0.12^{b}	7.32 ± 0.41^{c}	11.37 ± 0.57^{a}	2.73 ± 0.44^{d}	9.09 ± 0.22^{b}	<0.001
HGB (g/dl)	13.27 ± 0.46^{b}	11.34 ± 0.60^{c}	16.42 ± 0.66^{a}	4.97 ± 0.80^{d}	12.4 ± 0.60^{bc}	<0.001
HCT (%)	48.80 ± 0.10^{bc}	41.55 ± 0.66^{d}	63.04 ± 0.55^{a}	13.40 ± 0.44^{e}	47.10 ± 0.26^{c}	<0.001
PLT ($X10^3$ mm^3)	740.10 ± 23.27^{a}	100.13 ± 1.02^{c}	165.37 ± 1.52^{c}	392.27 ± 4.31^{b}	187.07 ± 1.41^{c}	<0.001
MCV (μm^3)	53.00±1.00	53.67±2.08	54.67±0.58	54.67±1.53	52.67±1.98	0.69
MCH (pg)	14.67 ± 0.25^{b}	14.50 ± 0.20^{b}	14.37 ± 0.40^{b}	16.50 ± 0.30^{a}	13.53 ± 0.35^{c}	<0.001
MCHC (g/dl)	25.87 ± 2.25^{b}	27.67 ± 1.53^{b}	26.50 ± 2.17^{b}	32.20 ± 0.89^{a}	25.60 ± 0.98^{b}	<0.004
Red Cell Distribute (%)	16.17 ± 0.87^{d}	17.07 ± 0.25^{ad}	17.53 ± 0.67^{abcd}	17.97 ± 0.42^{abc}	18.97 ± 0.76^{a}	<0.004
MPV (μm^3)	7.73 ± 0.47^{a}	6.30 ± 0.26^{c}	6.53 ± 0.35^{c}	6.63 ± 0.38^{c}	9.53 ± 0.31^{b}	<0.001
PDW (%)	5.87 ± 1.53^{c}	1.97 ± 1.90^{cd}	2.33 ± 2.14^{cd}	12.93 ± 0.70^{b}	1.43 ± 1.24^{d}	<0.001

The results are presented as SD of the means. Means with different superscripts (alphabets) in the same rows are significantly different ($P<0.05$); T= Treatment, T1= Casein diet + basal diet, T2=Maize, teff with soybean + basal diet, T3=Maize, teff with bee larvae + basal diet, T4=Commercial wean mix (enriched mama's choice) + basal diet, T5=Basal diet alone; WBC=White Blood Cells; RBC=Red Blood Cells; HGB=Haemoglobin; HCT=Hematocrit; PLT=Platelet Count; MCV=Mean Cell Volume; MCH=Mean Cell Haemoglobin; MPV= Mean Platelet Volume; PDW= Platelet Distribution Width and MCHC=Mean Cell Haemoglobin Concentration.

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The nutritional status and infectiousness of IYC were determined by blood hematological parameters. Haematological profiles of WBC, RBC, HGB, HCT, PLT, MCH, MPV, and PDW were significantly different ($P<0.001$) between experimental treatments. There was a significant difference ($P<0.001$) in WBC ($X10^6$ mm^3) between experimental treatments. In the formation of disease barriers, WBC plays a crucial role and therefore, is involved in the formation of antibodies to protect the body against pathogens (Amara et al., 2020). As a result of the present blood laboratory findings, BALB/c mice intake T4 had low concentrations of WBC (1.97) whereas T1 animals had high concentrations (5.27). Therefore, intake of T1 increased the number of leukocytes and increased disease resistance. According to the report of (Soetan et al., 2013) animals with low WBC had a higher chance of disease infection, whereas those with high numbers could produce antibodies during phagocytosis and had a higher level of disease resistance.

A similar trend in RBC, HGB, and HCT was observed between experimental treatments. Results of RBC ($X10^3$ mm^3) and haemoglobin (gm/dl) were an indicator of anemia. The RBC was significantly different ($P<0.001$) between experimental treatments. The study showed that T4 (2.73) had low RBC amounts than that of T1, T2, T3, and T5, which were 9.72, 7.32, 11.37, and 9.09, respectively. The low RBC level can be an indicator of anemia, therefore BALB/c mice intake of T4 would be susceptible to anemic. According to the report of (da Silva Lopes et al., 2018) decreased RBC was seen in anemia of any cause. Likewise, HGB is another confirmatory parameter for anemia. The testing was the primary method of anemia diagnosis (Kariyeva et al., 2003; WHO, 2015). Results of HGB (g/dl) concentration ranged from 4.97 to 16.42 and therefore, T3 (16.42) had high amounts and low in T4 (4.97) were recorded. Results of HCT were significantly different ($P<0.001$) between experimental animals. A high HCT percentage was recorded on T3 (63.04 %) and low on T4 (13.40 %). The increased HCT percentage indicated either an increase in the number of RBCs or a reduction in circulating plasma volume (Chineke et al., 2006) and also better transportation of oxygen and absorption of

nutrients (Etim et al., 2014). According to the report of (Peters et al., 2011) HCT, HGB, and mean corpuscular HGB are major parameters for evaluating circulatory erythrocytes and are significant in the diagnosis of anaemia. Hence, the present study was agreed with the above reports.

Blood platelets were involved in blood clotting. The findings of PLT ($X10^3$ mm^3) revealed that there was a significant difference ($P<0.001$) between experimental treatments and ranged from 100.13 to 740.10. The highest PLT ($X10^3$ mm^3) counts were observed on T1 (740.10) and followed in T4 (392.27), T5 (187.07), T3 (165.37), and T2 (100.13). The low PLT concentration indicated that the process of clot formation (blood clotting) would be prolonged, resulting in excessive loss of blood in the case of injury (Etim et al., 2014). The MCV was not significantly different ($P=0.69$) between experimental treatment groups. Moreover, from the observed experiment T4 had high records of MCH (16.50 pg) and MCHC (32.20 g/dl), which was an indication of anaemia (Åsberg et al., 2014). Generally, biochemical and haematological profiles of experimental BALB/c mice varied due to several factors such as gender, age, genetic variation, diet, and environmental conditions (Wilson et al., 2016). Therefore, in this study, the variation might be due to diet differences.

4.12. Lipid Profile

The lipid profile and the development of atherosclerosis are influenced by nutrient diet throughout infancy (Thorsdottir et al., 2003). Multiple genetic and environmental factors, including obesity, trigger the atherosclerotic process, which begins in childhood and progresses rapidly (Guardamagna et al., 2012; Medina-Ruiz, 2011). A high-fat diet developed hyperlipidemia, which was characterized by a rise in TC, TG, LDL-C, and a decrease in HDL-C (Kazemi et al., 2018). The macro and micronutrient levels in the body were determined by dietary intake. The findings of the lipid profile (mg/dl) among experimental treatments are shown in Table 4.12. The lipid profiles of TC, TG, HDL-C,

and LDL-C were statistically significant differences (P<0.001) between experimental treatments. Serum TC, TG, and LDL-C elevation have been identified as major risk factors for atherosclerotic CVD (Erukainure et al., 2011). BALB/c mice intake of T4 had low records of TC (112.52 mg/dl), TG (97.83 mg/dl), and HDL-C (47.87 mg/dl). Also, BALB/c mice assigned on T5 had high records of serum TC and LDL-C which were 121.06 and 102.30 mg/dl, respectively, and low in HDL-C 50.12 mg/dl. The lower plasma HDL-C was a risk factor for CVD (Ikewuchi, 2009; Martirosyan et al., 2007). In addition, Chandrashekhar et al. (2020) reported, that LDL-C was proatherogenic and oxidation of LDL-C within the arterial wall may be an important early step in atherogenesis. Intake of T3 in BALB/c mice resulted in high (P<0.001) TG (167.79 mg/dl) and HDL-C (67.18 mg/dl) levels, but low LDL-C (71.73 mg/dl) levels. From the isolated laboratory findings, elevation in TG increased the risk for CVD however, according to the report of (Nordestgaard and Varbo, 2014) these effects can be balanced by cardioprotective lipoprotein of HDL cholesterol. Furthermore, if the other atherogenic risk parameters tend to be normal, atherogenic indices may be a viable diagnostic option (Nwagha and Igwe, 2005; Onoh et al., 2019). The increase in plasma HDL-C had been considered to reduce the risk of coronary heart disease. High HDL-C protects the body by boosting reverse cholesterol transport by scavenging excessive cholesterol from peripheral tissues, which it then esterifies with the aid of lecithin: cholesterol acyltransferase and transports to the liver and steroidogenic organs for the synthesis of bile acids and lipoproteins and ultimate removal from the body (Ademuyiwa et al., 2005; Ikewuchi, 2009).

Among the experimental groups T2, T3, and T5 had high TC (mg/dl) in the blood, which was 131.23, 123.34, and 121.06, respectively. Diets high in fat have a significant influence on serum lipids, (DiNicolantonio and O'Keefe, 2018), and have been related to metabolic and CVD (dos Santos Lacerda et al., 2018). Also, serum lipid levels especially serum cholesterol was a major risk factor for atherosclerosis and CVDs that contribute significantly to mortality (Avci et al., 2018). So, a decrease in serum lipids

leads to a good functioning of the heart muscle (Amara. et al., 2020). According to the report of (Billingsley & Carbone, 2018), a lower intake of PUFAs and a high intake of dietary cholesterol and saturated fats raise blood total cholesterol levels. Therefore, the lipid profile assay has found useful applications in the assessment of malnourished children (Rajesh et al., 2020). However, in addition to routine lipid investigations, the inclusion of atherogenic indices was better index for screening of early detection of atherogenic CVD (Sasikala and Goswami, 2020). Therefore, further atherogenic indices should be calculated to identify a predictive indicator of coronary artery diseases.

Table 4.12: Comparison of serum lipid profile (mg/dl) of experimental treatments

Lipid profile	T 1	T 2	T 3	T 4	T 5	P-Value
TC	113.07 ± 4.13^{b}	131.23 ± 1.19^{a}	123.34 ± 2.23^{a}	112.52 ± 5.25^{b}	121.06 ± 1.77^{a}	<0.001
TG	141.25 ± 0.79^{c}	123.04 ± 2.23^{d}	167.79 ± 3.85^{a}	97.83 ± 1.06^{e}	153.96 ± 1.52^{b}	<0.001
HDL-C	59.53 ± 2.63^{b}	59.28 ± 0.85^{b}	67.18 ± 0.67^{a}	47.87 ± 2.15^{c}	50.12 ± 1.15^{c}	<0.001
LDL-C	86.66 ± 2.11^{c}	92.12 ± 1.09^{b}	71.73 ± 0.60^{d}	94.74 ± 1.40^{b}	102.30 ± 2.12^{a}	<0.001

The results are presented as SD of the means. Means with different superscripts (alphabets) in the same row are significantly different (P<0.05); T= Treatment, T1= Casein diet, T2=maize, teff with soybean, T3=maize, teff with bee larvae, T4=commercial wean mix (enriched mama's choice), T5=Basal diet; TC=Total Cholesterol; TG=Triglyceride; HDL-C=High-Density Lipoprotein Cholesterol; LDL-C=Low-Density Lipoprotein Cholesterol

4.12.1. Atherogenic and Castelli's Risk Indices

Beyond the standard lipid parameters, Atherogenic Indices (AI), Castelli's Risk Indices (CRI) (Olamoyegun et al., 2016), Atherogenic Coefficient (AC), and Atherogenic Indices of Plasma (AIP) have recently been proposed as a powerful predictor of CVD risk assessment (Akangbou et al., 2018; Kamoru et al., 2017). There was a significant difference (P=0.000) between experimental treatments for AIP, CRI-I, CRI-II, and AC (Table 4.13). There was high AIP (0.49), CRI-I (2.50), CRI-II (2.04), and AC (1.50),

recorded on T5. Also, T4 was recorded high CRI-I, CRI-II, and AC which were 2.35, 1.98, and 1.35, respectively. However, T3 had low recoded of CRI-I (1.84), CRI-II (1.07), and AC (0.84). Based on the estimated data, both T4 and T5 might be predicted for CVD. Furthermore, as compared to standard LDL-C or HDL-C fractions, CRI-II had a better predictive value (Sasikala and Goswami, 2020). Both, AIP (Niroumand et al., 2015; Ranjit et al., 2015; Sasikala and Goswami, 2020) and AC (Dobiasˇova, 2004) were also useful diagnostic tools to predict the risk of atherosclerosis and CHD the higher the value, the higher the risk of developing CVD and *vice versa.*

Table 4.13: The distribution of Atherogenic Indices of Plasma, Castelli's Risk Indices, and Atherogenic Coefficient among the experimental treatments

Indices	T 1	T 2	T 3	T 4	T 5	P-Value
AIP	0.38 ± 0.03^{b}	0.32 ± 0.01^{c}	0.40 ± 0.01^{b}	0.31 ± 0.02^{c}	0.49 ± 0.01^{a}	0.000
CRI-I	1.90 ± 0.08^{c}	2.21 ± 0.04^{b}	1.84 ± 0.04^{c}	2.35 ± 0.04^{ab}	2.50 ± 0.08^{a}	0.000
CRI-II	1.46 ± 0.08^{b}	1.55 ± 0.02^{b}	1.07 ± 0.00^{c}	1.98 ± 0.12^{a}	2.04 ± 0.04^{a}	0.000
AC	0.90 ± 0.08^{c}	1.21 ± 0.04^{b}	0.84 ± 0.04^{c}	1.35 ± 0.04^{ab}	1.50 ± 0.08^{a}	0.000

The results are presented as SD of the means. ^{abc}: means with different superscripts (alphabets) in the same row are significantly different (P<0.05); T= Treatment, T1= Casein diet, T2=maize, teff with soybean, T3=maize, teff with bee larvae, T4=commercial wean mix (enriched mama's choice), T5=Basal diet; AIP=Atherogenic indices of plasma; CRI=Castelli's risk indices; AC=Atherogenic coefficient

Table 4.14 presented the results of the correlation between the atherogenic indices AIP, CRI-I, CRI-II, and AC and the risk factors TC, TG, HDL-C, and LDL-C. A positive correlation of AIP with TC (P>0.05; r = 0.115) and LDL-C (P>0.05; r = 0.151) was observed, as well as a strong positive correlation with TG (P<0.01; r = 0.737). Nevertheless, AIP was negatively correlated (P>0.05; r = -0.096) with HDL-C. Thus, the present finding was agreed with the study of (Bo et al., 2018; Niroumand et al., 2015), AIP was a positive correlation with TC, LDL-C, and TG and a negative correlation with HDL-C. TC had no significant difference (P>0.05) but, a positive correlation between

AIP (r = 0.115), CRI-I and AC (r = 0.161), and negatively correlation with CRI-II (r = -0.190). The present findings were agreed with the report of (Luka et al., 2014), no significant association (P>0.05) was observed between TC and any indices of atherogenicity. On the other hand, plasma TG had a negative correlation (P>0.05; r = -0.429) with CRI-I and AC indices. Also, the HDL-C level showed a significant (P<0.001) negative correlation between CRI-I (r = -0.872), CRI-II (r = -0.971), and AC (r = -0.872). However, LDL-C levels was a significant (P<0.001) positive correlation between CRI-I (r = 0.875), CRI-II (r = 0.931), and AC (r = 0.875). Individual atherogenicity indices revealed significant positive correlation (P<0.001) with one another; CRI-I vs CRI-II (P<0.001; r = 0.919), CRI-I vs AC (P<0.001; r = 1), CRI-II vs AC (P<0.001; r = 0.919). Furthermore, AIP indices were not significantly (P>0.05) correlated with CRI-I (P=0.466; r = 0.204), CRI-II (P=0.569; r=0.160), and AC (P=0.466; r=0.204).

Table 4.14: Person correlation between Atherogenic Indices and lipid profiles of experimental treatments

Parameters	**AIP**		***CRI-I***		***CRI-II***		***AC***	
	R	*p*-Value	*R*	*p*-Value	R	*p*-Value	r	*p*-Value
TC	0.115	0.684	0.161	0.567	-0.190	0.499	0.161	0.567
TG	0.737	0.002**	-0.429	0.111	-0.535	0.04*	-0.429	0.083
HDL-C	-0.096	0.732	-0.872	0.000**	-0.971	0.000**	-0.872	0.000**
LDL-C	0.151	0.592	0.875	0.000**	0.931	0.000**	0.875	0.000**
AIP		1	0.204	0.466	0.160	0.569	0.204	0.466
CRI-I				1	0.919	0.000**	1	0.000**
CRI-II						1	0.919	0.000**
AC								1

TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AIP: Atherogenic indices of plasma; CRI: Castelli‟s risk indices; AC:

Atherogenic coefficient *= Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

4.13. Serum Mineral Bioavailability

Table 4.15 shows the effect of the experimental diets on serum minerals (Zn, Fe, and Ca) content. There were significantly different ($P<0.001$) of serum Zn and Ca between experimental treatments. Also, serum Fe was significantly different ($P=0.02$) between experimental treatments. High concentration of serum Zn was observed on T2 (0.55 mg/dl) and T3 (0.62 mg/dl). The reason might be associated with the protein quality in the foods. According to the findings of (Willoughby and Bowen, 2014), Zn intake was closely related to dietary protein intake. The bioavailability of serum Fe has been observed ranged from 0.83 to 2.08 mg/dl to the experimental animals. The presence of soybean legumes might explain the high amount of serum Fe on T2 (2.08 mg/dl) (Çakir et al., 2019) and red teff (Baye, 2014), which might have contributed iron to the CFs. The necessity of appropriate Fe and Zn intakes for older infants was recognized to avoid iron deficiency anemia and growth failures (Krebs, 2001). Also, (Lee and Yang, 2017) reported, that during early childhood, trace elements like Fe and Zn were found to be linked with appetite, brain development, growth, and function in growing children.

Deficiencies in certain micronutrients, such as iron, can have irreversible effects on brain development and other undesirable psychological consequences (Lozoff et al., 2006). However, serum Zn (0.09 mg/dl) and Ca (0.26 mg/dl) levels in BALB/c mice were found to be low upon T4 intake. Inadequate Zn intake or absorption from the food was the major cause of low Zn deficiency (Tian et al., 2016). According to the findings of (Keller Ulrich, 2019) deficiency of zinc, would cause anemia, anorexia, skin lesions, decreased lymphocyte function, diarrhea, mental retardation, and impaired visual function. Furthermore, compared to non-stunted children, stunted children had lower blood serum zinc levels and lower dietary intakes, which were linked to anorexia caused by zinc deficiency (Gibson et al., 2007).

Table 4.15: Bioavailability of serum minerals (Zn, Fe, and Ca) in experimental BALB/c mice (mg/dl)

Minerals	T 1	T 2	T 3	T 4	T 5	P-Value
Zn	0.26 ± 0.04^{b}	0.55 ± 0.02^{a}	0.62 ± 0.03^{a}	0.09 ± 0.04^{c}	0.22 ± 0.04^{b}	<0.001
Fe	1.25 ± 0.42^{abc}	2.08 ± 0.42^{a}	1.17 ± 0.13^{abc}	1.67 ± 0.42^{abc}	0.83 ± 0.42^{c}	0.02
Ca	10.64 ± 1.03^{a}	5.51 ± 0.13^{b}	2.18 ± 0.26^{c}	0.26 ± 0.13^{d}	0.31 ± 0.04^{d}	<0.001

The results are presented as SD of the means. Means with different superscripts (alphabets) in the same row are significantly different (P<0.05); Zn=Zinc; Fe=Iron; Ca=Calcium; T= Treatment, T1= Casein diet + basal diet, T2=maize, teff with soybean + basal diet, T3=maize, teff with bee larvae + basal diet, T4=commercial wean mix (enriched mama's choice) + basal diet, T5=Basal diet

BALB/C mice intake of T5 was recorded low serum Fe (0.83 mg/dl) and Ca (0.31 mg/dl) amount. Low intakes of Fe were consistent with a high prevalence of anemia seen in IYC (Lutter and Rivera, 2003). The highest serum Ca (10.64 mg/dl) observed on T1 could be due to the intake of the high content of calcium in the casein diet. According to the report of (Bennett et al., 2000), due to increased dietary casein consumption, the efficiency of calcium absorption was improved. Generally, micronutrient deficiencies are common during the weaning period, owing to infants' higher nutrient demands compared to their increased energy requirements (Qasem et al., 2015). Therefore, CFs that contain optimal levels of specific nutrients such as iron and zinc (Campoy et al., 2018) should be provided for IYCF.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The outcomes of the present study showed that the development of CFs using extrusion cooking makes the product desirable in nutritional quality, microbial safety, and sensory acceptability. The nutritional composition of the developed CFs meets the Codex Alimentarius Standard of the recommended dietary allowance for infants. The potential of using bee larvae as a novel ingredient in the development of CFs has been an alternative protein gained from plant-based protein sources. Using bee larvae with staple grains as a component of CFs is appropriate for macro- and micronutrient improvement of CF development for infant and young children. The bee larvae based extruded complementary foods are microbially safe and acceptable similar to cereal-based complementary foods and have the potential to contribute to the recommended dietary allowance. An *in-vivo* study using a BALB/c mice model showed that the newly formulated CFs would have the potential to increase body weight and be capable of preventing IYC from anemia and malnutrition. Compared to the soybean-based and commercial CFs, the bee larvae-based CFs improved body weight and enhanced haematological parameters such as WBC, RBC, HGB, HCT, MCH, MCHC, and serum Zn bioavailability. The results of atherogenic indices showed that intake of insect bee larvae-based CFs can prevent the risk of atherosclerotic cardiovascular disease. Therefore, bee larvae-based CF is appropriate for infant food, supports infant growth and development, and prevents malnutrition using the mouse model.

5.2. Recommendations

However, based on the above information further study should be done on:

1. Effects of the developed complementary foods on clinical and histopathological changes using BALB/c mice model.
2. The intake of newly developed complementary foods on physiological and biological effects on infants and young children.
3. Adverse effects of the developed complementary foods on clinical and allergies in infants and young children.
4. Economic analysis and willingness to pay for insect bee larvae-based complementary foods over other complementary foods.
5. The Ethiopian government should give attention to insect honey bee larvae as a source of complementary food ingredients as well as use for infants and young children and incorporate them with national nutrition policies.
6. Food processing industries should involve and processed this insect based complementary foods to reach for infants and children.

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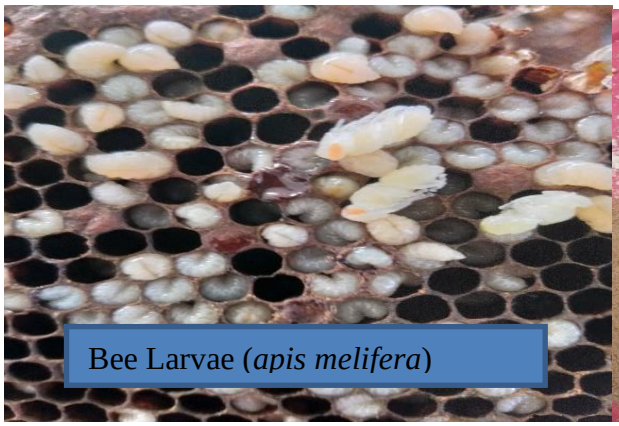
APPENDICES

Appendix 1: Images of food ingredients, formulation, and sensory evaluation of the developed CFs.



Bee Larvae (*apis melifera*)

A mixture of flours

Ingredient formulation for extrusion

Extruded complementary foods

Sensory evaluation for acceptability

Experimental BALB/c mice feeding trial

Appendix 2: Development of formulations for $ComF_{01}$, and $ComF_{02}$ using Nutrisurvey Software

1. A formulation for $ComF_{01}$ (Values per 100g)

Ingredients (%)		**57.00**	**29.00**	**14.00**	**Formulated product**	**RDA**
Nutrients	Units	Maize	Teff	Soybean		
Water	G	13.36	8.45	6.04	99.8	100
Energy	Kcal	362.52	352.77	435.14	413.2	400-425
Protein	G	10.10	9.79	50.50	17.7	15
Fat	G	4.88	2.65	19.53	7.1	10-25
Carbohydrate	G	69.55	72.44	14.34	70.0	60-75
Dietary Fiber	G	1.50	3.23	4.54	2.7	
Calcium	Mg	48.3	165.2	300.36	48.6	400
Iron	Mg	4.8	15.7	16.4	38.42	9.3
Zinc	Mg	4.6	4.8	2.7	2.67	4.1

RDA=Recommended Dietary Allowance

2. A formulation for ComF$_{02}$ (Values per 100g)

Ingredients (%)		**58.00**	**29.00**	**13.00**	**Formulated product**	**RDA**
Nutrients	Units	Maize	Teff	Bee larvae		
Water	G	13.36	8.45	8.68	98.4	100
Energy	Kcal	362.52	352.77	464.58	413.2	400-425
Protein	G	10.10	9.79	45.70	16.5	15
Fat	G	4.88	2.65	24.98	7.7	10-25
Carbohydrate	G	69.55	72.44	14.24	69.8	60-75
Dietary Fiber	G	1.50	3.23	2.74	2.4	
Calcium	Mg	48.3	165.2	84.9	53.28	400
Iron	Mg	4.8	15.7	13.3	39.76	9.3
Zinc	Mg	4.6	4.8	11.6	2.72	4.1

RDA= Recommended Dietary Allowance

Appendix 3: Sensory evaluation of five points hedonic rate scale record sheet for the development of complementary foods acceptability

Hedonic Test

Name……. Date……..….

You are provided with three (3) coded samples of complementary gruel. Please rate the samples (1-5) according to the scale provided below by filling in the table against each sample and attribute with

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

Sample	Appearance	Aroma	Taste	Mouth feel	Overall Acceptability
201					
402					
803					

Appendix 4: Summary table form of sensory evaluation of individual participants

Record Sheet-Hedonic Rating Scale Name of the Judge:_____ Date_____								
Food Characteristics- Appearance, Aroma, Taste, Mouth Feel, And Overall Acceptability								
5 = like very much, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately, and 1 = dislike very much								
	Test						Total Score	Average Score (Total score / No. of Tests)
	1	2	3	4	5	30		
Appearance								
Aroma								
Taste								
Texture/Mouth Feel								
Overall Acceptability								

Appendix 5: Ethical clearance for *in-vivo* experimental feeding trial on BALB/c mice

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University of Gondar
College of Veterinary Medicine & Animal Science
Gondar, Ethiopia

Ref. No.: CVMAS/13/.8064../2020
Date: 15/07 /2020

To: Shewangzaw Addisu Mekuria
University of Gondar

Subject: **Letter of Research Ethical Clearance**

This refers your request for research ethical clearance for your research proposal entitled *"Nutritional Quality and Safety of Complementray Foods Developed from Ethiopian Staple Grains and Honey Bee (Apis mellifera) Larvae: An In-Vivo Study Using Mice Models"*. The research ethical review committee of the College of Veterinary Medicine and Animal Sciences has reviewed your proposal as per the guidelines of the research ethical review process of the University of Gondar and approved it on July 09, 2020. Hence, this letter of ethical clearance is offered for you to implement your research project.

Sincerely,

ፀጋው ፈንቴ ካሣ (ዶ/ር)
Tsegaw Fentie Kassa (DVM)
የእንስሳት ሀክምናና ሳይንስ ኮሌጅ ዲን
College of Veterinary Medicine & Animal Science: Dean

የፖ.ሣ.ቁ P.O.Box 196
ጎንደር ኢትዮጵያ Gondar Ethiopia
ቴሌግራም ሕጡ.ኮ Cable A.A.U.P.H.
Fax- 251-058-114 1240
251-058 114 1233
251- 058 114 1235
URL Address: WWW.uog.edu.et
Fax – 251-058- 114 2145
Telephone PBX 058 1110174
Presidn's Offic 058114 1231
V/P/for Academic &Resarch 058 1141236
V/President for Admin 058114 1238
Human Resource Mange Dep't 0581142134
Faculty of Veterinary Medicine 058114 2145
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Appendix 6: Publications from the thesis research outputs

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Research Article

Nutritional Quality and Safety of Complementary Foods Developed from Blends of Staple Grains and Honey Bee Larvae (*Apis mellifera*)

Shewangzaw Addisu Mekuria,[1,2] **John N. Kinyuru**,[1] **Beatrice Kiage Mokua**,[1] **and Mesfin Wogayehu Tenagashaw**[3]

[1]*Department of Food Science and Nutrition, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya*
[2]*University of Gondar, P.O. Box 196, Gondar, Ethiopia*
[3]*Department of Applied Human Nutrition, Bahir Dar University, P.O. Box 26 Bahir Dar, Ethiopia*

Correspondence should be addressed to Shewangzaw Addisu Mekuria; shewakibr12@gmail.com

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Complementary foods must be adequate to satisfy the nutritional needs of the growing child together with breastfeeding. This study was aimed at evaluating the nutritional composition, microbial safety, and sensory quality of extruded complementary foods developed from blends of staple grains and insect bee larva (*Apis mellifera*). Teff, maize, soybean, and bee larva samples were milled to flour and blended before extrusion as follows: $ComF_{01}$ (57% maize, 29% teff, and 14% soybean) and $ComF_{02}$ (58% maize, 29% teff, and 13% bee larvae) using NutriSurvey software (version, 2007). Nutrient composition, microbial, and sensory analyses of developed flour blends were conducted using standard methods. The proximate composition of moisture, fat, fiber, carbohydrate, and energy was significantly different between the developed and commercial wean-mix foods. $ComF_{02}$ recorded the highest fat content (14.3 g/100 g), energy (427.18 kcal/100 g), and vitamins A (706 μg/100 g), B3 (8.2 mg/100 g), and B9 (86.7 mg/100 g) while $ComF_{01}$ had the highest protein content (12.56 g/100 g). Iron (40.94 mg/100 g) and calcium (68.20 mg/100 g) were the minerals with the highest content in $ComF_{02}$. Both $ComF_{01}$ and $ComF_{02}$ met the recommended dietary allowance of nutrients for infants aged 6-12 months. Overall, the present study showed that bee larvae can be used to develop complementary foods that are nutritionally adequate, microbiologically safe, and sensory acceptable meeting the dietary allowance of infants at an acceptable level compared to conventional cereal-based foods.

1. Introduction

Complementary feeding is the process of providing alternative foods 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, the infant transitions from exclusive breastfeeding to family foods. This period is typically from 6 to 24 months of age, even though breastfeeding may continue to two years of age and beyond [1]. This is a critical period of growth during which nutrient deficiencies and illnesses contribute globally to higher rates of undernutrition among children under five years of age [2].

While cereals are typically low in protein, cereal supplementation with local legumes that are high in protein improves the protein content of cereal-legume blends [3]. However, these plant diets are inadequate in terms of protein quality hence the need to include animal proteins [4]. Also, due to the increasing cost of animal proteins, food insecurity, population growth, and increasing need for protein-rich food [5], there should find another alternative.

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Research article

Growth performance, biochemical and haematological parameters of BALB/c mice fed on staple grains and bee larvae (*Apis Mellifera*) blended complementary foods

Shewangzaw Addisu Mekuria [a,b,*], John N. Kinyuru [a], Beatrice Kiage Mokua [a], Mesfin Wogayehu Tenagashaw [c]

[a] Department of Food Science and Nutrition, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya
[b] University of Gondar, P.O. Box 196, Gondar, Ethiopia
[c] Applied Human Nutrition Chair, Bahir Dar University, P.O. Box 26, Bahir Dar, Ethiopia

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ABSTRACT

In Sub-Saharan Africa, inadequate complementary feeding practices and being nutritionally inadequate are primary factors in infant and young child malnutrition, growth failure, and high morbidity and mortality. Therefore, novel complementary foods need to be developed to alleviate malnutrition problems in IYC. Therefore, this experimental study aimed to assess the effects of newly developed grain-bee larvae blended complementary foods on the growth performance, haematological, and biochemical parameters of BALB/c mice. A complete randomized design was used and a total of 75 BALB/c mice were assigned to each of the five treatments. The treatments were: T1 = Casein diet; T2 = 57 % Maize, 29 % Teff, 14 % Soybean; T3 = 58 % Maize, 29 % Teff, 13 % Bee larvae; T4 = Commercial wean mix; and T5 = Basal diet alone. The *in vivo* experiment trial was done for 28 days along with seven days of adaptation. Dietary intake was not significantly different ($P = 0.96$) between treatments, but it was noted that T3 had gained the highest final body weight (38.52 g). The examined biochemical parameters showed T4 had the lowest serum protein (6.27 mg/dl) and globulin (3.61 mg/dl). Compared to others, T3 significantly ($P < 0.001$) increased WBC (4×10^6 mm^3), RBC (11.37×10^3 mm^3), Haemoglobin (16.42 g/dl), and Hematocrit (63.04 %). The highest serum levels of zinc (0.55 mg/dl) and iron (2.08 mg/dl) were reported on T2, while the highest serum calcium content (10.64 mg/dl) was reported on T1. The results indicated that T3 can aid body growth, health, and prevent malnutrition in infants and young children.

1. Introduction

Malnutrition is a global problem in infants and children, that affects the world population. According to the reports of, UNICEF (2021), children under five affected by stunting and wasting are 21.3 and 2.1%, respectively. People living in those developing nations, including in Sub-Saharan African (SSA) or South Asian countries, are frequently considered seriously malnourished (Raza et al., 2020; Tao and Li, 2018). Therefore, undernutrition remains a significant problem in developing countries. Malnourished children have a greater risk of infection, ill-health, and mortality, and early growth retardation is associated with distinct variants of negative functional outcomes, including reduced cognitive function, delayed motor development, and poor school performance. According to recent studies, undernutrition accounts for around half of all mortality in children below the age of five (UNICEF, 2021).

In many developing countries, including SSA, poor complementary infant and young children (IYC) feeding practices and nutritional deficiency are key contributors to malnutrition, growth failure, and high morbidity and mortality (Mutisya et al., 2021). Malnutrition of varying degrees is associated with feeding infants unhealthy and low-quality Complementary Foods (CFs) (Abiose et al., 2015; Jeelani et al., 2020). Malnutrition due to poor complementary feeding practices is a serious concern in many low-income countries when CFs are composed of starch-based grains (Oladiran and Emmambux, 2020) that provide insufficient protein and micronutrients but adequate energy (Dewey, 2003), which reflect on hinders an individual's health (Tian et al., 2016). Protein - Energy Malnutrition (PEM) is a prevalent childhood disorder

* Corresponding author.
E-mail address: shewakibr12@gmail.com (S.A. Mekuria).

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