NUTRIENT PROFILE, PREBIOTIC POTENTIAL OF EDIBLE CRICKET, AND EFFECT OF CRICKET-BASED PORRIDGE ON GROWTH, HAEMOGLOBIN AND FATTY ACID LEVELS OF SCHOOL CHILDREN

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JOMO KENYATTA UNIVERSITY OF

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2019

Nutrient profile, prebiotic potential of edible cricket, and effect of cricket-based porridge on growth, haemoglobin and fatty acid levels of school children

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A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in Food Science and Nutrition in the Jomo Kenyatta University of Agriculture and Technology, Kenya.

2019

DECLARATION

This thesis is my original work and has not been presented for a Degree award in any University.

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DEDICATION

I dedicate this work to my husband John Kosgei, Sons: Victor Kimutai and Will Kipruto and baby Winner Chemutai. They give me the reason to work hard.

ACKNOWLEDGMENT

I wish to thank the almighty God for the strength and health during my study period. Special thanks to GREEiNSECT project for funding this work. I am very grateful to my supervisors who had undivided attention in supporting me, Dr. John Kinyuru, Dr. Samuel Imathiu, and Prof. Nanna Roos.

Special thanks to my parents Irene and Jonathan, my family, John, Victor, Will, and Winner, for understanding me when I was away for studies, sisters; Lydia, Sharon, Miriam and Grace, brothers; Isaiah, Anderson, Kosgei, and Kipchumba. I wish to sincerely appreciate Cheptigit primary school community, through the leadership of Mr. Patrick Amalemba, the BOM chair Madam Edna Magut. The nursery school teachers; Mercy, Helen, Caro, Prisca and Ann, the cooks, Gladys and mama Khayeri, all the parents, pupils and Cheptigit community at large are acknowledged for allowing the use of their school during the field study.

I am grateful to Uasin Gishu County government through the executive committee members of education and health, Uasin Gishu County early childhood education leadership and staff, for their time in reading through the study protocol and permission to carry out research within their area of governance. To the local administration, Mr. Salil, Chief Kaptagat location and all Cheptigit community heads, I thank you sincerely.

Special thanks to my friends; Grace, Sally, Wanja, Mary, Isaac, Anderson, Charles, and John, for the constant reminder that I should work hard, and reminding me to eat when I was deeply overtaken by my research work.

I sincerely thank the Food Science and Technology department laboratory staff: Dr. Karanja, Jennifer, David, and Jessica. University of Copenhagen laboratory staff, Inge and Rasmus and all other staff for their support during the lab analysis. Thank you, staff and colleagues, in the College of Agriculture and Natural Resources at JKUAT for the moral support you gave me. God bless you all.

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LIST OF ABBREVIATION

AAS	Atomic Absorption Spectrophotometry
AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Unit
CHO	Carbohydrates
EED	Environmental Enteric Dysfunction
ERC	Ethical Research Council
FAO	Food and Agriculture Organization
HPLC	High-Pressure Liquid Chromatography
IREC	Institutional Research and Ethics Committee
JIF	Jomo Kenyatta University Insect Farm
KDHS	Kenya Demographic Health Survey
NACOSTI	National Commission for Science, Technology and Innovations
PEM	Protein-Energy Malnutrition
NRC	National Research Council
UNDP	United Nations Development Programme
USDA	United States Department Of Agriculture
WHO	World Health Organization

ABSTRACT

There is a steady increase in the world population and therefore more pressure on available natural resources such as land and water. Animal rearing contributes to pollution through greenhouse gas emission causing climate change, making it hard to continue depending on commonly available animal protein as a major source of protein. Therefore, there is a need for alternative animal protein source such as insects. This study aimed at determining the nutrient composition, the prebiotic potential of chitin from the farmed crickets, and their utilization to improve growth, haemoglobin and fatty acid levels of children in Kenya. The specific objectives for this study were; to determine the optimum harvesting age of crickets based on nutrient composition, to evaluate the potential of cricket chitin as a prebiotic, for selected probiotic bacteria, develop a cricket-based porridge and determine its consumer acceptability, and to evaluate the effect of cricket-based porridge on the growth, haemoglobin and essential fatty acids levels of school going children.

Crickets (*Acheta domesticus*) were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT) cricket farm and nutrients profiles for 13 weeks were determined using standard methods. Cricket chitin was used as a prebiotic on selected probiotic bacteria. In the evaluation, bacterial media supplemented with 0%, 1%, 5%, 10% or 20% chitin were prepared following the standard procedure. Probiotic and pathogenic bacteria were cultured for 0, 6, 12, 24, and 48 hours and bacteria colony forming units counted. To assess consumer acceptability, and sensory evaluation of cricket-based porridge, different flour containing either: maize millet (MMP), maize millet with skimmed milk (MP10) or cricket powder (CP5) at 5%, CP10 at 10% or CP20 at 20% were developed. Sensory evaluation was done using a 7 hedonic scale. Randomized control trial, with three arms, was set up to evaluate the acceptability of the porridge by children and the effect of cricket porridge on children growth, haemoglobin, and essential fatty acid levels. Children (n=138) aged 3-4.5 years were recruited and fed with either maize millet (MMP), maize millet with skimmed milk (MP10) or cricket powder [CP5] for 6 months with monthly follow-up. Food acceptance was evaluated for

the first 4 weeks of the trial, anthropometric and morbidity data were collected monthly from baseline to end line. Blood samples were collected using the finger prick method at baseline and endline for determination of haemoglobin and fatty acid levels.

Crude protein means ranged from 36.0–60.0g/100g, chitin 2.20- 12.40g/100g, crude fat 12.00– 25.00g/100g, over the 13 weeks period, the change was significantly different ($P \leq 0.05$) over time. Mineral concentration was optimum at week nine with magnesium ranges of 1.30- 11.30mg/100g, calcium 1.40-19.70mg/100g and zinc 0.20 -16.60mg/100g. Crude fat content 25g/100g was optimum at weeks 9 and 10. All chitin concentrations significantly increased the population of probiotic bacteria ($P \leq 0.05$) while decreasing the population of pathogenic bacteria. During growth, there was a significant pH change ($P \leq 0.05$) in the broth media with the growth of all probiotic bacteria. Inhibition zones from probiotic bacteria growth supernatant against *Salmonella typhi* were most apparent at 16 mm and statistically significant in comparison with a 10% chitin concentration ($P \leq 0.05$). Pathogenic bacteria were suppressed most in the presence of chitin and probiotic bacteria. The results clearly demonstrate the prebiotic potential of chitin from farmed crickets.

In the developed flour, microbial quality showed that the flour was safe for human consumption since there was no contamination of flour by enteric bacteria, with total bacteria counts, yeast and moulds in the flour having counts below the maximum acceptable limits. Maize millet porridge, milk based porridge, and cricket-based porridge 5% had the highest acceptability amongst the participants. There was a significant difference in appearance ($P=0.02$), taste ($P<0.001$), texture ($P=0.01$) and the general acceptability ($P<0.001$) of all the developed porridge. Therefore cricket-based porridge (5%) compared well with milk-based porridge hence used for the intervention.

Consumer acceptability of cricket porridge was >75% in the first week and by the fourth week, all the children were able to accept cricket porridge to 100%. By four weeks of the intervention, all the children were able to develop a liking for the cricket porridge. All

children in the three arms improved in anthropometric indices, weight for age Z score improved from -1.0 to 0.39, 035 and 0.41 in MMP, MP10 and CP5 respectively. There was a significant difference in haemoglobin levels at baseline and end line in all trial arms ($P \leq 0.05$).

In conclusion, farmed cricket should be harvested between weeks 9, 10 and 11 when the protein, mineral, and fat content is optimum. Additional benefits can be obtained from cricket chitin which serves as a prebiotic, leveraging on the wide consumer acceptability of cricket as an additive in porridge. Cricket-based porridge was shown to improve the nutritional status of children, there is need therefore to develop cricket-based products for child feeding. Crickets would then be used as an animal source protein to supplement the commonly available animal protein sources. Further research to exploit the pharmaceutical potential of cricket chitin should be conducted and more parents educated on the use of crickets as an animal source protein to improve child nutrition.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Access to food is a basic human right, and healthy food is key to proper growth and proper development to enable humans to achieve full potential in life (Pink, 2016, Belluco et al., 2013, Bytnerowicz et al., 2007, Nakagaki et al., 1987). Several governmental and non-governmental organizations work in policy formulation and promotion of research since good health is dependent on good nutrition (WFP, 2016, Pereira and Drimie, 2016, McIntyre et al., 2016, Belluco et al., 2013, Battisti and Naylor, 2009,).

Although there is so much effort from the governments, amidst population growth and environmental pressure, which have caused pollution and conflict for scarce resources, there has been lack of set target achievements, and therefore continual lack of sufficient healthy food by the population (WFP, 2018). The land resource has become limited, since there are more people to feed than it was before, and the population is estimated to continue rising in the coming years (Kuiper et al., 2018). This has led to an increase in world food prices, and more burden to the poor in society (Ruel et al., 2010, Mkhawani et al., 2016).

Millions of children in the world are affected by acute malnutrition (Bliss et al., 2016, Bartz et al., 2014, Black et al., 2013), with half of the deaths in children below 5 years, caused by undernutrition or nutrition-related complications (Nduati et al., 2015, Black et al., 2013). High prevalence of malnutrition characterized by stunting early in life has been reported in the Kenyan population (M'Kaibi et al., 2017). Lack of animal proteins has been rated high among the children (Kimani-Murage et al., 2015), and this is thought to be the largest contributor to malnutrition in Kenya. The burden of hunger in developing countries is high with protein-energy malnutrition leading to a compromised immune system (Ronoh et al., 2017). Children are mostly affected because their food frequency consists

of majorly cereals and tubers (Whitney and Rolfes, 2018). This causes recurrent infection in children, majorly from enteric disease, and even to already vaccinated disease such as Rotavirus infection (Rho et al., 2017).

The world population has tripled in the past 35 years, with current estimates of 8.7 billion world population in the next 100 years (Abel et al., 2016), leaving many people more vulnerable to poverty (Mohajan, 2014, Kuiper et al., 2018). Currently, a large proportion of the population lacks an essential quality diet (FAO WFP, 2013). In addition, there are about three million children deaths in the world every year, and almost half of the deaths are caused by undernutrition and those who survive to develop impaired cognitive problems (Kyu et al., 2016, Hunter and Prüss-Ustün, 2016). Childhood anaemia leads to a 2.5% drop in adult wage which in turn affects a country's productivity (Black et al., 2013).

Approximately 30% of Kenyan households are food insecure with more households lacking access to diverse diets within the year (Kotut et al., 2014). Moderate acute malnutrition in children is still unacceptably high with approximately 26% of children under five years were stunted, 11% underweight, and 4% wasted (KDHS, 2014). Energy malnutrition, common in children under five years mainly occurs where children consume insufficient proteins and calories (Bringas-Vega et al., 2018), and is not an isolated problem to Kenya, as it is present in other parts of the world like India (Tiwari et al., 2017). In western Kenya region, protein-energy malnutrition contribution to stunting is high up to 30% (Kwena, 2016).

Plant protein is usually common, cheaper and easily accessible than animal protein, which is rarely consumed due to its associated high cost (Van Huis et al., 2013b, Cook and Monsen, 1976,). Van Huis et al. (2013) pointed out the increase of animal protein cost, due to the increasing demand of animal protein directly attributed to sharp population rise, and has caused many people to consume fewer animal proteins. This has prompted a search for cheaper animal sourced proteins among them being insects.

Consumption of edible insects by human as food, is currently practiced largely in Africa, Asia, and Latin America (Nadeau et al., 2015), In the past, edible insects as food was considered a hobby where few people in communities would go to the wild to collect insects when the season allowed for non-commercial home consumption, but in recent times, insects are farmed and are increasingly sold in the markets and on the streets especially in countries of Asia (Durst and Hanboonsong, 2015), with recent data of edible insects increasing steadily in Europe (Van Huis, 2017). Approximately 2 billion people live in dietary cultures where insects have been eaten since ancient times (Yen, 2015). Insects can help address food and feed security since they can thrive under diverse environmental conditions, have a high feed conversion rate because they are cold blooded and can potentially convert 2 kg of feed to 1 kg of insects biomass (Abbasi and Abbasi, 2016). Some insect species such as crickets produce fewer greenhouse gases, some up to 100 times less than what the pigs and cows produce (Payne, 2018), and in dry areas insects can be reared since they use less water than livestock and also are less land-dependent (Nadeau et al., 2015).

Currently, there is great interest in insect consumption all over the world, since they have been shown to contain high nutrient content (van Huis, 2015, Halloran et al., 2015). In Kenya, use of insect for food is an ancient tradition in many communities, and currently a more sort after delicacy in the western region of Kenya, (Kinyuru et al., 2013, Ayieko et al., 2012, Christensen et al., 2006). Insect consumption has been practiced among some of the Kenyan communities and provided a refuge when there was food insufficiency (Evans et al., 2015). People were used to collecting insects in the wild, largely carried out by women and children, and insect farming is thus a new idea among the Kenyan population (Rumpold and Schlüter, 2013a, Kinyuru et al., 2012, Bukkens, 1997).

Crickets and termites are among the commonly consumed insect. Crickets belong to the same family phylogenetic tree with grasshoppers but grasshoppers and termites have a complex lifecycle that is not easy to rear artificially (Varelas and Langton, 2017). Cricket rearing requires less technical support, capital expenditure, basic farming, and harvesting

techniques, therefore can easily be reared and processed, as compared to livestock which is currently the largest source of animal proteins (Pimentel et al., 1997). Cricket farming is likely to benefit the society who can rear crickets, and provide increase protein intake and business opportunities leading to development, and increased good nutrition (Ayieko et al., 2012).

Crickets have a varied biochemical composition of both macronutrients and micronutrients, depending on their feed and habitat (Rumpold and Schlüter, 2013a). Their protein quality has been reported to be comparable to meat and fish (Van Huis, 2013). Crickets are relatively high in fat, minerals such as iron, selenium, copper, magnesium, and zinc (Acosta and Fanzo, 2012, Rumpold and Schlüter, 2013a). These insects have also been reported to have minimal risk of zoonotic disease transmission (Belluco et al., 2015), and are therefore considered a safe source of proteins and minerals. Cricket chitin has unknown nutritional and health impacts and maybe prebiotic with the ability to improve gut health (Brownawell et al., 2012). If each household in Kenya can adopt cricket farming, it may have a positive impact on increasing animal protein consumption and provide extra income raised from the surplus. Since cricket consumption is being introduced in Kenya, development of product may provide an entry point to avoid disgust by consumers. In Kenya use of porridge in child feeding is common, the government of Kenya has encouraged fortification of cereals which has partially helped improve micronutrient intake. Consumption of animal protein is low and we seek to determine the effect of the addition of cricket to porridge as an animal protein source.

1.2 Problem statement

Despite crickets having high protein and mineral content, there has been inadequate utilization of crickets as an animal protein source in child food. Crickets have been harvested at various stages of growth depending on consumer preference. However, there is a need to establish optimal harvesting age of crickets for maximum nutritional benefit. Chitin from crustaceans have been found to have prebiotic activity, there is, therefore,

need to establish whether cricket chitin possesses the similar potential for possible exploitation on the food sector. Child feeding practices involve the use of cereal or tubers based porridge. The porridge lacks animal proteins which make them inferior in terms of nutritional quality. Possibility of formulating baby food using cricket as an animal protein source has been suggested even though its acceptability and efficacy is unknown, and therefore this study seeks to determine, the acceptability of cricket porridge and its impact on child growth.

1.3 Justification

Currently, the consumption of animal proteins in children is low and animal protein is usually expensive and unaffordable to the most rural household in Kenya (Kinyuru et al., 2013). Mostly in rural households, children are fed with porridge made of maize or millet and tubers. Meat, pork, milk, and eggs are expensive and therefore rarely bought to supplement child nutrition. Cricket has high protein content, good fatty acid composition, as well as high micronutrient content (Nowak et al., 2016) and since they are easily reared with the minimal ecological impact it can be used to curb food insecurity through protein supplementation (Leung et al., 2013). Cricket based food can be made locally, which can then be packed with ease and distributed as fortified blended food products (Kinyuru et al., 2009).

Cricket farming has been seen as a success in other countries including Kenya and a possible source of high-quality animal protein (Halloran et al., 2016). Extensive modelling of this success would be beneficial in Kenya. However, wide adoption of farmed cricket as a source of food is been introduced in Kenya and has not yet been widely adopted, therefore this study seeks knowledge that will promote wide adoption of cricket as an animal protein source. By providing important information on the nutritional and chitin content of Kenyan farmed cricket based on their age, which will ensure crickets are harvested at their optimum age. Prebiotic potential of chitin from farmed cricket will be important to determine if crickets have additional health benefits when chitin is consumed.

Development of cricket porridge and assessing its consumer acceptability will help provide knowledge for future adoption of cricket product in Kenya. This study also seeks to feed children with cricket porridge to ascertain the effect on nutritional status, of school going children in Kenya. Knowledge gained will help in future nutrition interventions aimed at helping children to increase animal protein intake.

1.4 Objective

1.4.1 General objective

To assess nutrient and prebiotic potential of cricket and their utilization in promotion of growth, haemoglobin and fatty acids profiles of school going children in Kenya.

1.4.2 Specific objectives

1. To determine the nutrient composition of the farmed cricket based on the age of harvesting.
2. To evaluate the potential of cricket chitin as a prebiotic for selected probiotic bacteria.
3. To develop a cricket-based porridge and determine its consumer acceptability.
4. To evaluate the effect of cricket based porridge on the growth, haemoglobin and fatty acids profiles of school going children.

1.5 Hypotheses

1. Cricket age has no influence on nutrient content.
2. Cricket chitin has no impact on the growth of selected probiotic bacteria.
3. Cricket based porridge is not acceptable by consumers.
4. Cricket based porridge has no impact on growth, haemoglobin and fatty acids profiles in children.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition

Malnutrition is the largest contributor to disease in the world (Correia et al., 2014, Onis, 2006). Despite the intervention by governments and non-governmental organizations to try and curb malnutrition, it's still unacceptably high in many countries (Seshadri and Ramakrishna, 2018, Carroll et al., 2017, Chinnaswamy et al., 2017). Among the millennium development goals, and sustainable development goals, alleviation of malnutrition is key a component in accomplishing these goals and improving quality of life (Griggs et al., 2013, FAO WFP, 2013). Undernutrition is a cycle of poor nutrition and infection and is currently the biggest burden in most countries (te Lintelo et al., 2014). Undernutrition can be acute moderate or acute severe commonly referred to as wasting, while chronic undernutrition is characterized by stunting caused by compromised linear growth (FAO WFP, 2013).

Malnutrition in children is the most common problem in the developing world (Burroway, 2017), with Africa and Asia contributing to 94% of all underweight (Leung et al., 2013, FAO WFP, 2013). Protein deficiency is high in children due to less nutrient dense nature of the food most children eat, mainly composed of carbohydrates (Millward, 2017). Micronutrient deficiencies are also common (Miller et al., 2006, Allen et al., 2006), and there is a window of opportunity to optimally prevent undernutrition during foetal and early life period (Pepperberg, 2009). Micronutrient deficiency is commonly caused by lack of animal products in child diet (Hanson et al., 2015), though it's usually rarely noticed, commonly called hidden hunger. Kenyan children have been shown to be Vitamin B12 deficient, a vitamin that is mainly obtained from animal source food (Mohajan, 2014). Animal source protein is rarely consumed due to its high cost and studies show low intake of animal protein in Kenyan children (Kotut et al., 2014, Kinyuru et al., 2012).

Malnutrition in Kenya, is characterized by approximately 35% of children under five years being stunted with 4% severely underweight and 6% are wasted (Matanda et al., 2014, Konyole et al., 2017). Approximately 30% of Kenyan households are food insecure with more households lacking access to diverse diets within the year (Mohajan, 2014). To attain food security there is a need for continuous access to quality food and food frequency to provide nutrients which are essential for proper growth (Belton and Thilsted, 2014, Garstang et al., 2017). Mineral deficiency in children has been demonstrated with 40 % of pre-school aged below five years being vitamin A deficient while 69% are iron deficient (Lopez-Cepero et al., 2016). There is a high cost of food and fuel prices in Kenya, the food and non-alcoholic drinks index went up by 26% in November 2010 and November 2011. The cost of living, rose further in 2017 this has forced people to eat less due to the high prices (Grace et al., 2014, Chakravorty et al., 2017).

Table 2. 1: Micronutrient requirement in children

Reference nutrient intake during childhood, ages 4 to 8 years

Micronutrient	Males and Females
Folate	400 μg/day
Niacin	15 mg/day
Vitamin A	900 μg/day (3,000 IU/day)[c]
Vitamin B_6	40 mg/day
Vitamin C	650 mg/day
Vitamin D	75 μg/day (3,000 IU/day)
Vitamin E	300 mg/day (450 IU/day)[d]
Calcium	2,500 mg/day
Copper	3,000 μg/day
Fluoride	2.2 mg/day
Iodine	300 μg/day
Iron	40 mg/day
Magnesium	110 mg/day
Manganese	3 mg/day
Molybdenum	600 μg/day
Phosphorus	3,000 mg/day
Selenium	150 μg/day
Zinc	12 mg/day

Recommended nutrient intake(RNI) in children aged 4 to 8 years for both boys and girls (Payne et al., 2016b)

Acceptable standards in the formulation of food supplement to help improve nutritional status requires that the food provides the nutrients required and are present in sufficient quantities from the staple food (WHO, 2015). Micronutrient bioavailability in food especially that of iron is higher in animal source food, most common in beef, with current studies in crickets showing high micronutrient content (Kinyuru et al., 2013, Nakagaki et

al., 1987).

Better nutrition can be achieved by the production and distribution of high-quality protein (Kelemu et al., 2015). This has prompted a search for alternative protein sources that include insects which have been shown to have a varied biochemical composition, of both macronutrients and micronutrients (Finke, 2016b). Crickets, in particular, is a high protein source, since they are cheap to produce (Caparros Megido et al., 2016). Crickets, therefore, may provide a sustainable solution to existing and looming issues of low animal protein intake, which is key in helping meet growing demands of quality nutrition as the world population grows (Stull, 2015, Caparros Megido et al., 2016).

2.2 Insects as food

To ease the world burden on livestock dependent protein source, there is a need to invest in a farming system that would withstand drought and can thrive in less land and less water while giving out high results. Insects can be used as a protein source: to lower greenhouse effect, use less land, utilize high conversion rate, and potentially use organic by-products (Oonincx et al., 2010). Promotion of insects to supplement animal proteins is likely to be a solution to high population growth, especially in communities where edible insects are part of traditional meals, and there is need to enhance production to breach the gap of sufficiency (van Huis et al., 2015a).

A long time ago in the prehistoric time, insects containing sugar and fat became part of the human diet in different parts of the world (Meyer-Rochow and Changkija, 1997). Insects have been rated as traditional nutritious food and the culture is no longer seen as primitive (DeFoliart, 1992, Durst et al., 2010). More than 250 insect species have been identified in sub-Saharan Africa (Van Huis, 2003). In the recent decade, there has been loss of diversity due to climate change and overuse of pesticides and insects diversity has not been spared (Stoll-Kleemann and Schmidt, 2017, Pecl et al., 2017, Thom et al., 2017).

Table 2. 2: Micro-nutrients in commonly consumed meats, meat products, and insects

	Micronutrient content per 100 g edible portion							
	Calcium (mg)	**Iron (mg)**	**Iodine (mg)**	**Vitamin C (mg)**	**Thiamin (mg)**	**Vitamin A (mg)**	**Riboflavin (mg)**	**Niacin (mg)**
Source/Daily value	**1000**	**18**	**0.095**	**60**	**1.5**	**1.5**	**1.7**	**20**
Beef	5	1.95	10	0	0.08	0	0.23	4.7
	5–8.25	1.54–2.31	9–11		0.07–0.07	0–2	0.17–0.25	4.05–5.25
Chicken	8	0.88	6	1.1	0.075	0	0.16	6.5
	6.75–12	0.7–1	5–7.5	0–2	0.0675–0.12	0–16.5	0.125–0.22	4.87–7.65
Pork	7	0.8	5	0	0.77	0	0.235	5.6
	6–10	0.7–0.8		0–0.25	0.635–0.928		0.18–0.28	4.85–6.86
Offal (beef)	15	7.3	16	1	0.175	249	0.355	4.6
	11.3–23.5	3.8–10.5		0–5.5	0.11–0.28	128	0.185–1.13	3.48–6.65
Offal (chicken)	10	2.45	16	6	0.09	39.5	0.375	3.85
	7.75–13.3	1.25–6.07		1–14	0.05–0.125		0.123–0.578	2.25–6.45
Offal (pork)	10.5	4.8	7	6	0.27	5.5	0.47	4.18
	7.75–11.8	2.55–6.35		0–10.5	0.12–0.32	0–27.5	0.368–1.44	2.53–8.65
Cricket	104	5.46	0.021	3	0.04	6.53	3.41	3.84
	49.8 - 287	2.47–8.01				6.44–24.4		
Honeybee	30	18.5		10.25		25.7	3.24	
	22.7–37.3	15.2–21.9				19.1–27.4		
Silkworm	42	1.8			0.12		1.05	0.9
Mopane caterpillar	700							
Palm weevil larvae	39.6	2.58		0.00425		11.3	2.21	
	0.028–48	0.528–8.4						
Mealworm	42.9	1.87	0.017	1.2	0.24	9.59	0.81	4.07
	30	1.6–2.45				5.7–20.5		

All are daily reference values (DRV) with the exception of vitamins and minerals, which are recommended daily intake (RDI) values.

In Kenya, termite and grasshoppers are mostly consumed in most parts of the country when they swam (Kinyuru et al., 2013, Fombong and Kinyuru, 2018), however land cultivation, and use of pesticides has led to reduced insect population in the wild (Mishra, 2017). Insect farming would, therefore, provide sufficient supply throughout the year, though not all insects can be farmed (Van Huis et al., 2013a). Cricket has been successfully farmed in small and large scale (Rumpold and Schlüter, 2013b) and can be used as a source of protein.

2.3 Edible Crickets

Crickets have the ability to contribute positively to food security because of their ability to thrive in areas where other dense food cannot (Yen, 2015), including drought-stricken areas where lack of water cannot sustain crop and livestock farming. Nutrients from crickets have been shown to be of good quality, with a high digestibility (Wang et al., 2004). Domestication of cricket can increase the availability of edible crickets as opposed to traditional methods which relied on wild harvesting subjected to seasonal availability (Durst and Hanboonsong, 2015, Hanboonsong et al., 2013b).

Cricket farming is more advantageous when compared to major livestock farming, due to the high feed conversion rate in cricket, of about 12 fold when compared to other livestock (Ayieko et al., 2012). In crickets, feeding is efficient because 1.7 kg of feed is required to produce 1 kg cricket biomass, as compared to 10 kg of feed to produce 1 kg of beef (Rumpold et al., 2016). Crickets are also environmentally friendly since they do not emit methane, which is a major pollutant from livestock and has majorly contributed to climate change (Carson, 2015, Rao, 2016). Crickets emit low levels of carbon dioxide and nitrogen oxide which greatly reduced greenhouse ammonia (Rao, 2016).

2.4 Nutritional content of crickets

Edible crickets have varied nutritional values depending on the species, metamorphic

stage, habitat, and diet-fed on the cricket (Kinyuru et al., 2013, Nakagaki et al., 1987, Orinda et al., 2017). They have adequate amounts of energy and proteins with amino acids in amounts required by humans as supplements to common food (Finke, 2002). Crickets also contain sufficient micronutrients and monounsaturated and polyunsaturated fatty acids. The unsaturated fatty acid in insect, contribute up to 60% of the unsaturated fatty acid content of cricket, with the indicated presence of Omega 6 and Omega 3 which is good for child cognitive development (Kipkoech et al., 2017). In addition to the nutrients available, minerals and vitamins like copper, iron, magnesium, manganese, phosphorous, selenium, zinc, riboflavin, pantothenic acid, biotin, and folic acid have been shown to be available in sufficient quantities (Finke, 2016a).

2.5 Chitin content in crickets

A significant amount of chitin is found in crickets and is an insoluble exoskeleton derivative (Brownawell et al., 2012). Initial studies have shown that the cricket chitin content makes 8.7% of the total cricket content (Collavo et al., 2005), and this has been equated to cellulose from plants which are usually indigestible. However, human gastric juices contain chitinase enzyme which has the ability to digest chitin, though its availability in humans who rarely feed on insects may be low or lacking (Janiak, 2016). The close association between chitin and immune defence against parasitic, allergic reactions and disease has been shown in previous studies (Komi et al., 2017, Wagener et al., 2017b, Vannella et al., 2016, Esteban et al., 2001). Esteban et al. (2001) demonstrated increased sea bream innate immune protective response enhanced by dietary intake of chitin.

2.6 Insects and gut health

The human gut is home to different species of microorganisms which are established from infancy (Collado et al., 2008). Some of the microorganisms present are beneficial to the gut commonly referred to as probiotic and help in vitamin synthesis (Gibson and

Roberfroid, 1995), while others are harmful commonly referred to as pathogenic and cause disease (Boulangé et al., 2016). Balance of gut microbiota is important in ensuring that there is no shift to a disease state in the gut (Lopes, 2018). Probiotic bacteria are able to suppress the growth of pathogenic bacteria in the gut by acid production and production of metabolites that kill the pathogenic bacteria (Penders et al., 2006). Constant exposure to pathogens from the environment can cause environmental enteric dysfunction (EED) (Harper et al., 2018). EED is a disorder of the small intestines commonly affecting children in the poor resource set up, exposed to poor hygiene (Keusch et al., 2013). EED is characterized by poor gut structure and function, hence less absorption of nutrients and has been associated with stunting, where environmental contaminants are consumed with food due to poor hygiene (Mbuya and Humphrey, 2016). The microorganisms consumed alter the gut structure, causing reduced surface for food absorption, which leads to nutrient mal-absorption and anaemia (Denno et al., 2016).

Environmental enteric dysfunction (EED) is a pathway from poor hygiene to malnutrition (Keusch et al., 2013). Over the years, stunting could not be explained by poor diet, diarrhoea or reversed by optimized diet (Millward, 2017). Stunting has been because of villous atrophy leading to the reduced absorptive service area, and leaky gut caused by pathogenic microbes (Mbuya and Humphrey, 2016). Children living in unhygienic conditions are at risk of EED, because of constant exposure to pathogenic microbes in the environment and interventions aimed at improving EED are likely to improve stunting (Mbuya and Humphrey, 2016).

Probiotic bacteria commonly reside in the colon and feed by fermenting indigested material passing through the gut (Martín et al., 2013). Some indigestible material referred to as prebiotics promote the growth of probiotic bacteria, therefore, are important in nourishing probiotics. Prebiotic and probiotics that target intestinal health are currently an important segment of the functional food market in the world (Saarela et al., 2002, Al-Sheraji et al., 2013). Probiotics are important in processing polysaccharides present in the diet, and also promote absorption of nutrients from the gut lumen (Zhang et al., 2015).

Studies in India, Bangladesh, and Malawi, demonstrated that gut microbiota and undernutrition are closely related, and hence gut microbiota has been used as a predictor of good health in children (Gill and Finlay, 2011).

Intestinal microbial flora reflects the gastrointestinal function in human health and wellbeing (Lertsutthiwong et al., 2002, Nishijima et al., 2016). Initial studies have indicated that chitosan, a chitin derivative, has antitumor, cholesterol lowering, antifungal, and antibacterial effect. Importance of chitin in gut health was also demonstrated to have the ability to control metabolic inflammation with the probability of increased intestinal permeability (Boulangé et al., 2016). Health decline in the elderly has also been associated with diet driven microbiota alterations, which shifts the microbial balance and predisposes the elderly to disease (Biagi et al., 2016).

Altering composition of human intestinal microbiota by use of chitin can alleviate chronic metabolic disease which includes diabetes and obesity (Collado et al., 2008, Lin et al., 2012). Impact on metabolic disease may be caused by the antimicrobial and antifungal activity of chitin, through activation of host immune system, leakage and destruction of the bacterial cell wall or through other mechanisms (Khoushab and Yamabhai, 2010, Chlebowska-Smigiel et al., 2017). A most recent study demonstrated the ability of cricket derived chitin in influencing growth of probiotic bacteria leading to improved gut health (Stull et al., 2018). Ability to maintain healthy microbiota may constitute a new therapeutic strategy to prevent chronic disease, improve gut health and reverse malnutrition (Lin et al., 2012).

2.7 Insects as food for children and child feeding interventions

When hunger strikes, children are normally the worst affected because of their need for nutrients to grow and develop (WHO and UNICEF., 2003, Lieberman, 2003). More often hunger-stricken children are prone to malnutrition and the results are usually death or permanent disability with reduced cognitive development, and immunity (Shankar et al.,

2017, McIntyre et al., 2017). Children under five years of age are more likely to die if malnourished (Ibrahim et al., 2017). Studies have shown that children are able to eat diverse diets only when the food is available (M'Kaibi et al., 2017).

Traditional food which includes crickets, termites, grasshoppers, and black ants have been associated with health benefits, especially when used as complementary food (Ayieko et al., 2012). Studies on edible insects have gained momentum in this last decade. Kinyuru et al. (2013), demonstrated that insects have nutritional values of public health importance. On the other hand, termites were reported to have the potential to provide a high-quality diet with sufficient amounts of iron and zinc (Kinyuru et al., 2013). Children lack essential nutrients due to the high cost of animal proteins attributed to the rising cost of living (Meade and Thome, 2017). However, intervention on children using insects in complementary food has been shown to greatly improve child health (Konyole, 2014).

Crickets have approximately 50% to 74% crude protein content (Payne et al., 2016c, Payne et al., 2016a, Kinyuru et al., 2009). They can provide a good source of fatty acid important for childhood brain development, hence the ability of children to attain full cognitive potential and have a better adult life (Yang et al., 2006). In areas where studies have been undertaken, cricket based product acceptability is high among the Kenyan population (Konyole, 2014, Homann et al., 2017, Alemu and Olsen, 2018). Since crickets were part of the indigenous diet for most communities, they can provide the much-needed animal protein, minerals and fatty acid content lacking in the population (Bukkens, 1997, Davis et al., 2016, Kipkoech et al., 2017).

In an effort to improve acute malnutrition, the World Food Program (WFP) advocates using Corn Soy Blend plus (CSB+), a blend consisting of cereal-legume (corn and soybean), with premix as an additive. Soybean has been shown to have high phytate and fibre which may impair nutrient absorption. The protein content is low and therefore the desire to replace soybean with animal source protein. This study aimed at using edible cricket as an animal protein source in child porridge.

2.8 Conceptual framework

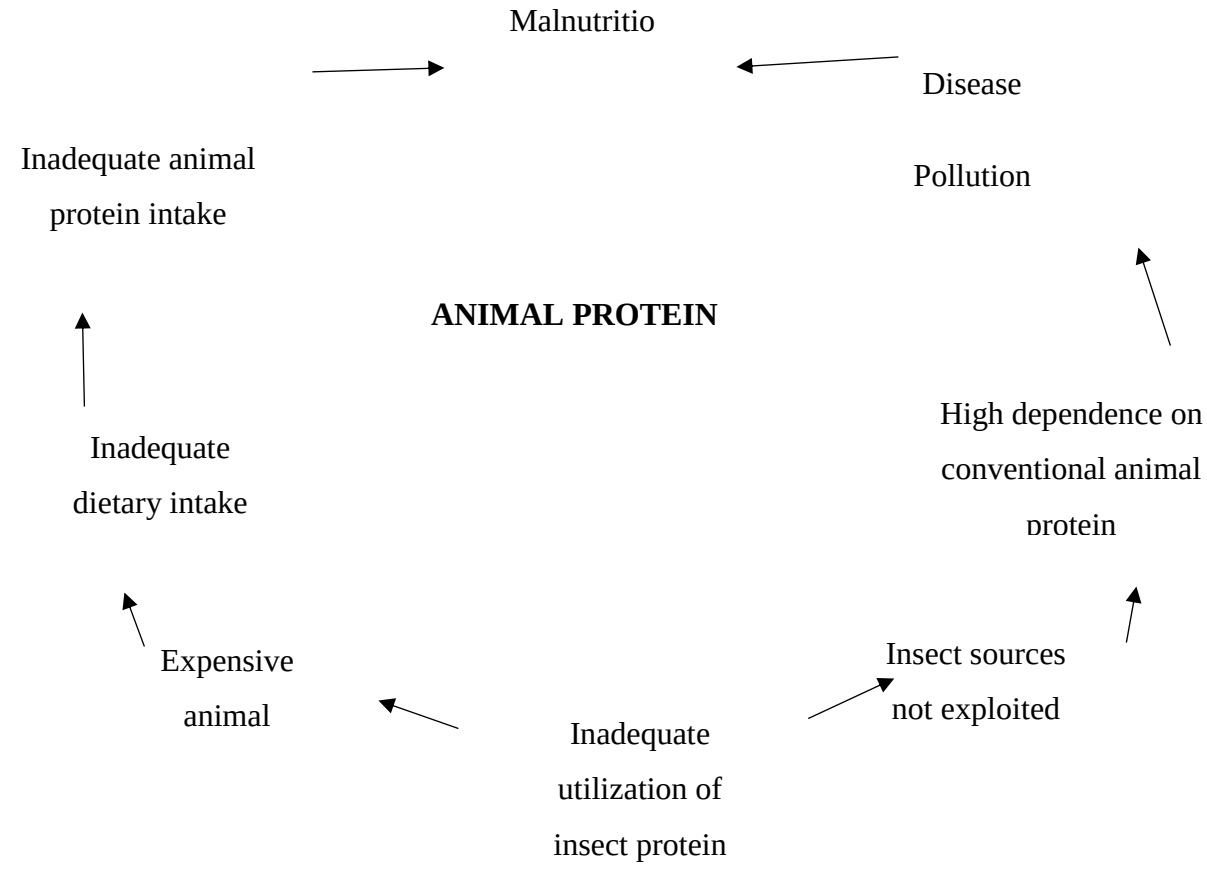

Figure 2. 1: Figure Conceptual framework showing a link between lack of animal protein and malnutrition

(Source; Kipkoech C, 2019).

CHAPTER THREE

NUTRIENTS AND CHITIN COMPOSITION OF FARMED CRICKETS

3.1 Introduction

Crickets are good for children especially those that are undernourished, due to high protein and fatty acid content (Acosta and Fanzo, 2012), and plenty of minerals such as iron, selenium, copper, magnesium, and zinc (Rumpold and Schlüter, 2013a). Currently, cricket farming is a feasible venture but is still under small-scale production in most parts of the world (Halloran et al., 2016). Importantly, more people are slowly adapting to cricket rearing largely for domestic consumption and cash at the local markets (Halloran et al., 2016) and it has demonstrated success in other countries such as in Thailand (Hanboonsong et al., 2013a).

Crickets are among the most important edible insects (DeFoliart, 1995). Their farming is cheap compared to livestock farming (Pali-Schöll et al., 2018), as it requires little space, less feeds as well as having a minimal ecological impact to the environment thus important in curbing food insecurity, pollution and climate change (Pali-Schöll et al., 2018).

Cricket rearing is economical, as it requires simple locally available raw materials, which includes egg trays, timber rails and simple structures with slippery lining (Orinda et al., 2017). In addition, it requires little water, with one litre of water sufficient for thousands of crickets in a day. Crickets can be fed with local herbs and kitchen waste (Orinda et al., 2017). Their rearing is also time efficient and less labour-intensive and is an environmentally safe way of alternative animal protein generation as they produce fewer greenhouse gases (Caparros Megido et al., 2016). Crickets have a high feed conversion rate, giving rise to high-quality nutrients, using less feed and this makes it cheaper, efficient and environmentally safe way of protein production (Payne et al., 2015).

To improve widespread utilization, the local community need to adopt cricket farming and

commercial production in an easily affordable way and incorporate cricket in the day-to-day diet. There is a need to farm crickets in large-scale and in a sustainable manner in order to supply a wider population (Van Huis et al., 2015b). Currently, cricket farming is still under small-scale production in most parts of the world (Halloran et al., 2016). More people are slowly adapting to cricket rearing largely for domestic consumption and cash at the local markets (Halloran et al., 2016)

In recent years, research on insect-based food has increased with Winfood trials in 2009-2013 showing improved child nutrition when termites and fish were used in complementary feeding (Konyole, 2014). There has been increased interest in insect food in Kenya. Cricket farmers' empowerment and training have been carried out at JKUAT to ensure insect reared are sufficient to provide adequate cheap protein.

The aim of this study was to assess the nutritional and chitin composition of farmed cricket as influenced by age in order to ascertain the optimal harvesting time for maximum nutrient composition in an effort to incorporate them in children diets. Crickets' rearing was preferred for this study since the crickets natural environment is easier to mimic and cheaper to produce in mass.

3.2 Materials and methods

3.2.1 Sample collection and preparation

Cricket samples were obtained from JKUAT Insect Farm (JIF) which had been reared as detailed by Kinyuru and Kipkoech, (2018). Sampling was done every week from week 4 to week 13. Three independent samples were collected weekly on day 1, day 3 and day 5 of every week. One kilogram of harvested cricket yielded from the three samples, was homogenized to represent the week's sample. After collection, the crickets were frozen at -20^{o}C overnight to kill, then washed in tap water and rinsed in distilled water. The crickets were then oven dried at 50^{o}C for 72 hours to dry and ground to very small fine particles

(250 mm), using a kitchen blender to get the cricket powder. The cricket powder was then stored at -20°C and during analysis only the required amount was taken from the freezer. All analysis was carried out in triplicates.

3.2.2 Proximate analysis

Standard methods for proximate analysis were adopted in this study. To determine crude proteins, total nitrogen obtained by digestion of protein using the micro-Kjeldahl method was multiplied by 6.25 factors to obtain crude protein content in crickets (AOAC, 2005 method 990.03). Crude fat was analysed by Soxhlet extraction method (AOAC, 2005 method 920.39), and AOAC 2005 Method 940.28 used to determine fatty acid profile.

Ash determination was done by 550°C muffle furnace incineration (AOAC 2005, method 920.48). The mineral analysis was determined using AOAC 2005, method 984.27 by dry ashing, and measured by flame atomic absorption spectrophotometry (Shimadzu 6300 AAS AA/AE Spectrophotometer) (AOAC, 2005). Quantification of minerals was done using commercial standards (Sigma – Aldrich Chemie, Steinheim, Germany). The in-house control sample, which was vacuum-packed and stored at -20°C consisting of known mineral concentrations were also used for quality control. Carbohydrate content was calculated from the proximate values by subtraction (AOAC, 2005), while energy levels from the proximate analysis, was obtained by multiplication of crude protein, available carbohydrates and crude fat content by a factor.

3.2.3 Determination of chitin content

Chitin was extracted and content per week calculated gravimetrically. To extract crickets chitin, 150 grams of ground cricket powder was placed in 1000 ml of 0.5M boiling sodium hydroxide and the mixture boiled for two hours for the powder to undergo deproteinization (Toan, 2009, Lertsutthiwong et al., 2002, Charoenvuttitham et al., 2006). The content was allowed to cool for 30 minutes, followed by centrifugation at 10000 rpm for ten minutes

(Galed et al., 2005). To dissolve minerals from the centrifuged residue, 600 ml of 1% hydrochloric acid was added and the residue placed in a rotor shaker (Sanyo electric company limited, Japan) rotating at 40 rpm for 24 hours at room temperature (Trung et al., 2006).

The content was then subjected to centrifugation at 10000 rpm for ten minutes, and the supernatant discarded while the remaining residue was treated with 500 ml of 0.5M boiling sodium hydroxide for one hour to decompose albumin (Puvvada et al., 2012). The content of albumin decomposition underwent centrifugation and the supernatant containing albumin discarded. The brown solid residue was washed three times with boiling distilled water to remove remaining polysaccharides and sodium hydroxide and dried at 70°C for 60 hours and the extracted chitin stored in zip lock bags at -20°C.

3.2.4 Amino acid analysis

The amino acid analysis was done using ion exchange chromatography. To analyze amino acids, two grams of the cricket flour sample was used. The sample was defatted using methanol before hydrolyzing it using 6 M HCl. The hydrolysed sample was broken down using sulfosalicylic acid and supernatants diluted in tridecafluoroheptanoic acid (Le et al., 2014). The amino acid sample was injected into the amino acid analyzer (Technicon Instrumentation Corporation, Dublin, Ireland) for separation and characterization of amino acids (Ertingshausen et al., 1969).

3.2.5 Data analysis

Data collected were recorded in Microsoft Excel. Analysis of variance within the means was used to determine the variation of means in energy, nutrient and chitin content in farmed crickets at a different age. Significant differences between ages were evaluated at $P \leq 0.05$.

3.3 Results and discussion

Figure 3.1, shows the nutrient and energy content of cricket based on the age of harvesting. Protein content was on a gradual rise from week 4 to week 11 and dropped at week 12 and week 13.

There is a significant increase of protein content from week four to week five, which further increase to week 11 before dropping slightly in week 12 and 13 though this difference is insignificant ($P = 0.68$). The protein content in cricket increased as the cricket aged, though the increase is not linear and maximum protein content was seen at between week 9 (58.3 g/100 g), week 10 (59.7 g/100 g) and week 11 (60.4 g/100 g). Based on the daily value in 2000 Kcal diet for children between 4 to 8 years, 100 g of cricket serving per day would provide the required 50 g protein (WHO and UNICEF., 2003). The crickets used in this study were fed on locally available diets, it is clear that local farmers who would like to adopt the simple diet can achieve the high protein content and this makes cricket rearing for protein supplementation a venture worth exploiting in Kenya and other parts with similar setups as Kenya.

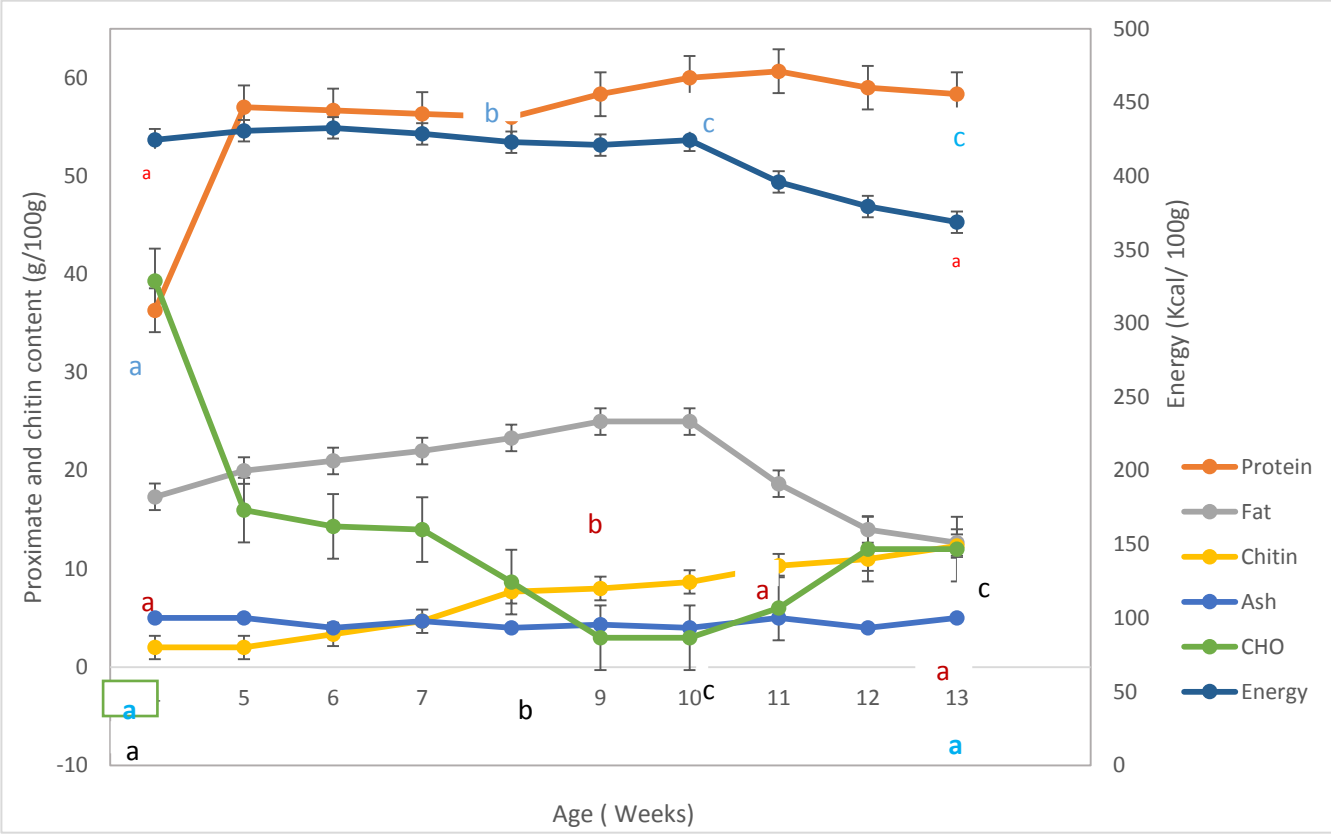

Figure 3. 1: Proximate, chitin and energy content of crickets harvested at different ages

Different letters within the figure in the same nutrient trend denote significant difference P≤0.05

n=3

The results concur with other studies that have shown high protein content in insects (Kinyuru et al., 2015, Collavo et al., 2005). According to a study by (DeFoliart et al., 1982), crickets were found to contain high protein content of 54 g/100 g but our study has demonstrated that cricket protein content can be higher than 54g/100 g. This may be attributed to cricket diet, species, or the ecological zone difference. Since protein content declined from week 12 and 13, it would be of importance to consider harvesting at week 9, 10 and 11 for optimal protein content.

Chitin content was on a steady rise as the crickets aged and at week 13 chitin content was highest at 12.3 g/100 g. The results of the study concur with other findings which reported chitin content in insects to range from 4.00 g/100 g to 21.00 g/100 g depending on age and other factors (Finke, 2015). Chitin content was between 2.00 g/100 g at 4 weeks and significantly increased to 12.33 g/100 g at 13 weeks (P = 0.04). Chitin content in cricket was at its lowest at a tender age and increased as cricket aged.

Ash content was almost constant with no significant rise across all ages (P = 0.12), with the approximate composition of between 4.00g/100g and 5.00 g/100 g. Past studies on insects have indicated that the ash content in the insect is less than 10.00 g/100 g (Finke, 2015). The ash content shows that there is minimal variation in the total mineral content in crickets as the cricket age, though individual mineral content may vary, with diet variation shown to have an influence on ash content, recent studies on fish and calcium-rich cricket diets have been shown to increase cricket ash content (Raubenheimer and Simpson, 2018).

The rise in crude fat is slow when compared to cricket protein content rise (Figure 3.1), the lowest crude fat content was reported at week 13 (12.67 g/100 g) which was significantly different from the highest at 25 g/100 g (P = 0.04) (Figure 3.1). Crude fat content reported in the cricket is within the range observed by Narzani and Sarmah (2015) where crude fat content in insect varied from 1.00 g/100 g to 40.00 g/100 g (Narzari and Sarmah, 2015). Children are given 100 g serving of crickets per day, the crude fat would provide about 38% of the total daily requirements of 65 g/100 (UNICEF., 2003). Other studies have shown low total fat content in crickets when compared to the fat content in termites (Payne et al., 2015, Fombong and Kinyuru, 2018). Low-fat content in cricket would be of importance for industrial exploitation of cricket, since high-fat content hinders processing and shelf life of food products, reducing its mass production and commercial potential (Kinyuru, 2014).

In the past, studies have demonstrated crickets having varied carbohydrate content ranging

from 1.00 g/100 g to 47.00 g/100 g (Belluco et al., 2013), (Narzari and Sarmah, 2015). In our study carbohydrate content was lower but within this range (Figure 3.1). The energy levels in cricket started at a high level and drop when fat content drops (Figure 3.1). Energy content was highest at week 6 (432.67 Kcal /100 g), though the energy content fluctuates, attributed majorly to the fluctuations in other proximate contents. As much as the energy contents drop, the drop is not significant since the overall energy content ranges from 368.67 Kcal /100 g and 432.67 Kcal/100 g ($P > 0.05$). Previous studies have shown insects to be energy dense with up to 577.99 Kcal/100 g (Finke, 2013). This study shows crickets are an energy dense food source, and can easily be an option to curb energy deficiency in children (Black et al., 2013), as long as the crickets are harvested at the right age to ensure a balance of nutrients, and energy.

Table 3.1 shows mineral content in farmed crickets. Mineral content started at a higher level and slightly dropped as the crickets aged and finally increased at week 11, 12 and 13.

Table 3. 1: Mineral composition in crickets as influenced by age

Age in weeks	*Magnesium*	*Calcium*	*Copper*	*Iron*	*Zinc*
4	11.33±0.3 [a]	5.67±0.1 [a]	8.67±0.1[a]	0.03±0.01 [a]	15.0±0.2 [a]
5	9.00±0.2 [a]	2.67±0.2 [b]	8.67±0.1[a]	0.01±0.01 [a]	12.3±0.2 [a]
6	10.33±0.1 [a]	1.67±0.1 [b]	9.33±0.1[a]	0.02±0.01 [a]	17.0±0.2 [a]
7	9.33±0.3 [a]	1.67±0.2 [b]	6.00 ±0.1[b]	0.01±0.01 [a]	15.0±0.3 [a]
8	9.00±0.3 [a]	1.33±0.3 [b]	4.67±0.2 [b]	0.03±0.01 [a]	14.6±0.2 [a]
9	9.67±0.4 [a]	1.00±0.3 [b]	5.67±0.2 [b]	0.03±0.01 [a]	15.3±0.2 [a]
10	9.33±0.5 [a]	1.67±0.3 [b]	4.33±0.1 [b]	0.01±0.01 [a]	14.3±0.1 [a]
11	9.33±0.2 [a]	2.00±0.3 [b]	5.67±0.1 [b]	0.02±0.01 [a]	16.3±0.3 [a]
12	9.00±0.3 [a]	1.00±0.3 [b]	5.67±0.2 [b]	0.03±0.01 [a]	14.6±0.1 [a]
13	9.67±0.3 [a]	1.33±0.3 [b]	5.33±0.2 [b]	0.04±0.01 [a]	15.6±0.1 [a]
RNI*	130	800	440	10	5

n=3. Values displayed as means ±standard deviation
Values within the same column under with different superscripts are significantly different $p < 0.05$
Minerals were measured in mg/100g.
*Age 4-8 years ((Payne et al., 2016b)

Magnesium high levels of 11.33 mg/100 g were seen at week 4, and at week 7 decreased insignificantly ($P > 0.05$). Copper highest level of 9.33 mg/100 g was seen at week 6, there was no significant difference of copper and iron level from week 4 to week 13 ($P > 0.05$). Cricket diet greatly affects the mineral content, with crickets fed on fish having higher mineral content (Raubenheimer and Simpson, 2018). Since the crickets reared were not fed on any special diet, there may need to include fish diet or other equivalent nutrient providers in insect rearing to increase mineral content in farmed insects.

Fluctuation in the mineral content may have been contributed by the increase in chitin content and probably most minerals are held up in the exoskeleton since mineral content in younger crickets was higher and most minerals especially calcium decreased significantly as the cricket aged (Table 3.1). Cricket mineral composition shows that crickets are not just energy-dense food, they are also good for curbing high mineral deficiency in children, since past studies have indicated a high mineral deficiency in children (Duong et al., 2015a). Data obtained from this study compared well with other studies that have analyzed the mineral content of crickets (Rumpold and Schlüter, 2015, Payne et al., 2016c, Taufek et al., 2016, Kinyuru, 2014). From the study results, crickets have higher mineral content before the age of 6 weeks, unless there is the improvement of diet to increase cricket mineral content.

Table 3.2 shows the fatty acid composition of cricket oil at different ages. Palmitic acid increased significantly after week 9, ($P = 0.04$) with EPA and DHA content in crickets oil showing that cricket oil is good for health since its presences are seen to protect children from disease (Dayrit, 2015). The oil found in cricket has Omega 3 and Omega 6 fatty acid, which clearly indicates that feeding children with cricket would boost their good fat content and n6:n3 ratio ranged from 0.33 to 2.40. Oil containing Omega 3 and Omega 6 fats has been rated positively in a child's diet (Brigham et al., 2018). Past studies have indicated that fatty acid is more desirable when the ration of Omega 6 to Omega 3 is almost equal to one (Yang et Al., 2006).

Table 3. 2: Fatty acid composition of cricket oil as influenced by age

Fatty acid									
Age (weeks)	***Palmitic acid***	***Oleic Acid***	***Linoleic acid***	***Linolenic acid***	***Arachidic acid***	***EPA***	***DHA***	***Omega 6/ Omega 3 ratio***	***Unidentified***
4	18.05±0.3[a]	23.9±0.4[a]	2.08±0.2[a]	1.0±0.2[a]	1.0±0.3[a]	1.0±0.1[a]	1.0±0.1[a]	1.04±0.2[a]	50.93
5	18.7±0.3[a]	23.0±0.4[a]	6.4±0.2[b]	1.0±0.3[a]	1.0±0.3[a]	1.0±0.1[a]	1.6±0.1[a]	2.4±0.1[a]	44.9
6	19.9±0.3[a]	29.5±0.4[b]	5.9±0.2[b]	1.0±0.2[a]	1.0±0.3[a]	1.3±0.2[a]	3.3±0.1[b]	1.2±0.2[a]	36.9
7	22.2±0.3[a]	35.3±0.4[b]	5.6±0.2[b]	1.0±0.2[a]	1.0±0.3[a]	2.0±0.1[b]	3.0±0.1[b]	0.3±0.1[a]	29.6
8	22.2±0.3[a]	31.2±0.4[b]	5.4±0.3[b]	2.0±0.2[b]	1.6±0.3[b]	2.0±0.2[b]	2.6±0.1[b]	1.1±0.1[a]	31.9
9	24.9±0.3[b]	37.4±0.4[b]	5.3±0.3[b]	2.0±0.1[b]	1.6±0.3[b]	1.6±0.1[b]	3.0±0.1[b]	1.7±0.2[a]	22.5
10	25.04±0.3[b]	37.0±0.4[b]	5.0±0.3[b]	3.0±0.1[b]	2.0±0.3[b]	1.0±0.2[a]	3.0±0.1[b]	1.2±0.2[a]	22.76
11	27.7±0.3[b]	51.1±0.4[c]	3.0±0.3[a]	3.0±0.2[b]	1.7±0.3[b]	1.0±0.1[a]	3.0±0.1[b]	0.7±0.2[a]	8.8
12	28.01±0.3	51.5±0.4	3.0±0.3[a]	3.0±0.1[b]	1.6±0.3[b]	1.0±0.2	2.6±0.1	0.8±0.1[a]	8.49

	b	c				a	b		
13	26.6±0.3[b]	48.8±0.4 c	3.3±0.3[a]	3.0±0.2[b]	2.0±0.3[b]	1.0±0.1 a	2.0±0.1 b	1.1±0.1[a]	12.2

n=3. Values displayed as means ±standard deviation

Values within the same column with different superscripts are significantly different $p < 0.05$

Fatty acid measured in %

A human being is believed to have evolved on Omega 6: Omega 3 balanced diet of 1:1, (Oddy et al., 2004) and increase the ratio of Omega 6: Omega 3, promotes obesity in children (Simopoulos, 2016). Growing children need sufficient amounts of Omega 3 and Omega 6, (Brenna et al., 2015). Therefore cricket meal is important if adopted to feed children to improve child nutrition.

Figure 3.2 shows saturated fats (SATU's), monounsaturated fats (MUFAs) and polyunsaturated fats (PUFAs) fatty acid fractions in cricket oil.

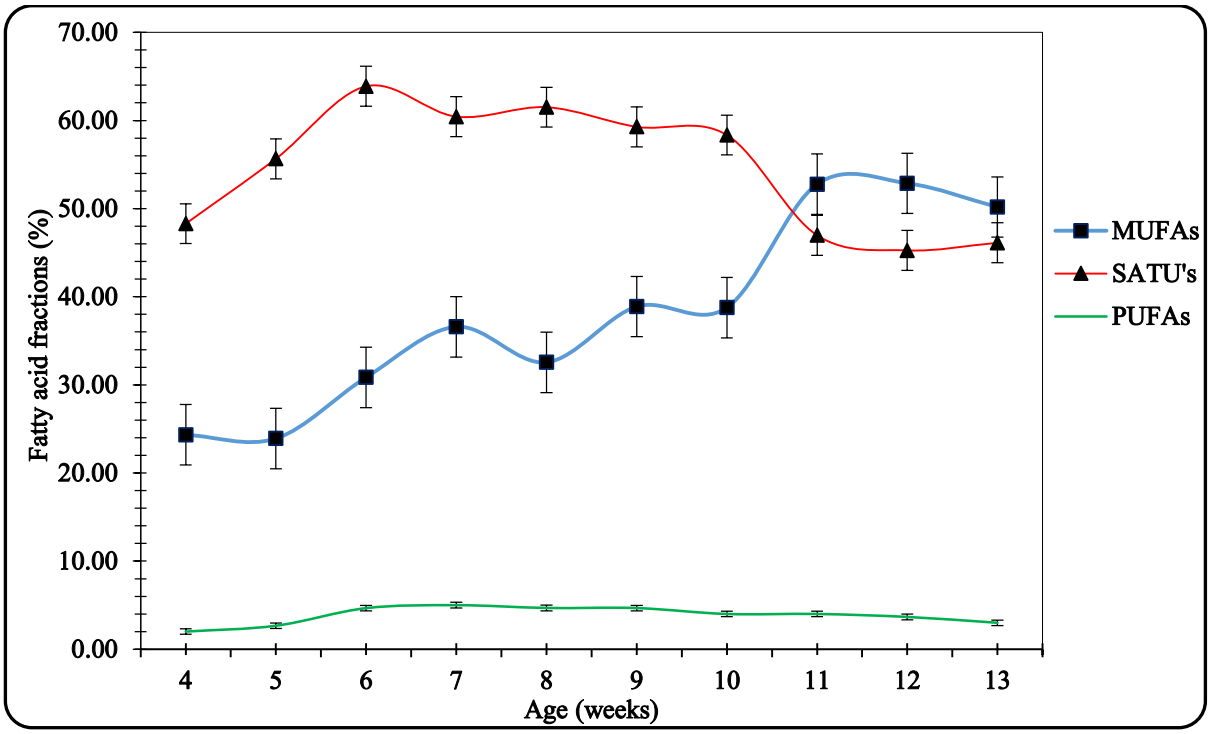

Figure 3. 2: Fatty acid fractions in cricket oil as influenced by age.

The fatty acid fraction of cricket oil in %

n=3

Polyunsaturated fatty acids (PUFAs) ranged from 2% to 5%, the PUFAs increased from week four to reach a maximum of 5 % at week 7 and drops again to 3% at week 13 (Figure 3.2). Saturated fatty acid (SATU's) increased as the cricket aged from 48% at week four

to 63.8% in week 6 then drops to 46% in week 13. Monounsaturated fatty acid (MUFAs) increased from week four to week 12 and slightly reduces at week 13.

Polyunsaturated fatty acid (PUFAs) content in crickets is an advantage especially in human nutrition since PUFAs in the diet has been linked to anti-obesity and increased activity in children (Średnicka-Tober et al., 2016). At week 4, cricket oil would provide a higher percentage of saturated fatty acid at 46 g/100g when compared to 33 g/100g in beef, and 34 g/100g in chicken (Ton et al., 2015), while PUFAs was at 5 g/100g in crickets which is the same as PUFAs percentage in beef (Ton et al., 2015, Średnicka-Tober et al., 2016). Supplementing children diet with cricket would give the children the needed lipids that would promote health and increased activity in children.

Table 3.3 shows the amino acid content of cricket protein in mg/g protein. Out of the 13 amino acids analysed, seven were detectable in the cricket protein.

Arginine was the most abundant amino acid present in cricket protein, followed by leucine and phenylalanine as shown in table 3.3. Available arginine in *Acheta domesticus* is higher when compared with arginine available in other insects (Ademola et al., 2017) (Arsenault and Brown, 2017). Leucine and phenylalanine have been found to be common in fish (Arsenault and Brown, 2017), while arginine is important in health and disease there is the need for higher quality protein content in children, to be able to meet the required amino acid patterns. Malnourished children require slightly higher protein intake, to enable them to get a higher amount of amino acids (Ricardo et al., 2015). Since cricket contains high protein content, its use in improving child nutrition is likely to boost the amino acid requirements in moderately malnourished children.

Table 3. 3: Amino acid content in cricket protein

Amino acid	Content	Recommended in (3-10) year old children
Histidine	ND	16
Arginine	67.3±3	
Lysine	ND	48
Glutamine	ND	
Serine	ND	
Glutamic acid	ND	
Proline	15.8±6	
Valine	12.4±6	40.00
Methionine	11.3±7	22.00
Tyrosine	11.3±8	22.00
Isoleucine	ND	31.00
Leucine	17.2±6	61.00
Phenylalanine	13.6±9	22.00

n=3 Values displayed as means ±standard deviation

Recommended reference range:(Ricardo et al., 2015, WHO and UNICEF., 2003)

ND= not detected.

3.4 Conclusions and recommendations

Age has an impact on cricket nutrient content. The optimum age for farmed cricket harvesting would be between weeks 9 and 11 when the protein, mineral and crude fat is optimum. Cricket Omega 6 to Omega 3 ratio at all ages was close to 1 and this makes cricket oil good for child nutrition. This study recommends harvesting of crickets at ages 9-11 for optimal nutrition to the consumer. Crickets harvested at this age may be utilized in child feeding as the nutrient present contributes significantly to RDA and RNI for children aged 4-8 years old.

CHAPTER FOUR

PREBIOTIC POTENTIAL OF CRICKET CHITIN ON SELECTED PROBIOTIC BACTERIA

4.1 Introduction

Prebiotics are a fermentable fiber that is able to benefit the growth of beneficial bacteria in the host's colon (Roberfroid, 2000, Gibson et al., 2017a). Positive alteration of the composition and metabolic activity of the host colon is of great interest owing to the important role of the intestinal microflora in human health (Banerjee and Dhar, 2018), in the synthesis of vitamins and boosting the immune system functions (Blaut, 2002). Prebiotics are important only if they can pass through the gut to the colon, be fermented by beneficial bacteria, and selectively promote the growth of useful intestinal bacteria (Ushakova et al., 2015).

Gut beneficial bacteria widely referred to as probiotic have been defined as live microorganisms that positively affect the host's organism by improving the intestine's microbial balance (Fuller, 1992, Gibson et al., 2017a). The intestinal microbiome consists of beneficial and harmful bacteria, constantly competing for space and nutrients (Zimmermann and Curtis, 2018). Probiotic bacteria are known to suppress the growth of pathogenic bacteria, by pH reduction to favour their growth, and production of growth metabolites able to suppress pathogenic bacteria (Slomka et al., 2017). When the growth of pathogenic bacteria is allowed to dominate, a disease state sets up (Vogt and Finlay, 2017). In recent years, consumption of prebiotics to increase the growth of probiotics has been on the rise with an increased search for new prebiotic candidates (Buruiana et al., 2017).

Consumption of prebiotics with dairy products stimulates the growth of *Bifidobacteria* and *Lactobacilli* in the colon (Scholz-Ahrens et al., 2016). Experimental studies in mice have shown an increase in microbial mass and improved gut health in fibre-fed mice

(Scholz-Ahrens et al., 2016). This and other findings which include recent findings on the use of chitin to improve gut health has led to increased interest in modulating gut microbiota through the use of prebiotics as a means to countering metabolic disease (Wong et al., 2016, Stull et al., 2018).

Although prebiotics support the growth of probiotic bacteria like *Bacillus subtilis*, *Bacillus licheniformis*, *Streptococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus helveticum* and *Bifidobacteria adolescentis* (Ushakova et al., 2015), they can, however, be only considered important if they stimulate growth and activity of specific beneficial bacteria such as *Bifidobacterium* species and *Lactobacilli* species. (Zaman and Sarbini, 2016). Currently, probiotic bacteria consumption has been encouraged through the consumption of fermented milk product to help increase the population of *Lactobacilli* species in the gut (Prodeus et al., 2016, Nikbakht et al., 2016).

Recent advancement in the important role of prebiotics includes a demonstration of prebiotics to stimulate the growth of beneficial bacteria and alleviate depression, by reversing the pathophysiology of depression (Kazemi et al., 2018). Consumption of cricket has been shown to promote the growth of probiotic bacteria when there is inflammation, through reduced plasma TNF-α (Stull et al., 2018). In a randomized control trial, on use of oligosaccharide as a prebiotic and *Bifidobacteria lactis* as a probiotic in infants, there were higher bifidobacterium and lactobacilli counts with lower clostridia counts in the group that consumed prebiotics (Radke et al., 2017). Use of prebiotics in oral health greatly increased the proportion of beneficial species while lowering the proportion of pathogenic species (Slomka et al., 2018).

Edible crickets contain a considerable amount of chitin, which has been exploited to a less extent (Kipkoech et al., 2017). Since chitin as initially been equated to cellulose, reduction of chitin content in cricket for its utilization as ruminant feed, allowed substitution of up to 50% of the animal feed with cricket (Jayanegara et al., 2017). Cricket chitin may reduce inflammation while improving health in the gut (Stull et al., 2018).

The most commonly used prebiotics have been fructooligosaccharides, inulin, galactooligosaccharides, lactulose, and dietary fibre with attention shifting to new candidates (Conlon and Bird, 2014), among them chitin and pectin (Ushakova et al., 2015). Chitin is one of the most abundant least utilized bio-products in the world (Bhattacharya et al., 2007). Chitin major function is in supporting the cuticles of insects, crustaceans and other arthropods (Merzendorfer and Zimoch, 2003). Crickets have been shown to contain approximately 9% chitin, rated to be of better quality than that of crabs (Wang et al., 2004).

Inulin as a prebiotic has been shown to selectively stimulate *Bifidobacteria adolescentis* and *Lactobacillus* species (Chlebowska-Smigiel et al., 2017, Nowak et al., 2017), with an immune-modulatory effect that inhibits the growth of pathogenic bacteria and restores the normal flora (Blaut, 2002). Besides inulin, chitin has also been shown to have activities similar to immune effector cells, which play an essential role in innate and adaptive immunity, at the same time increasing growth of probiotic bacteria (Singdevsachan et al., 2016, Stull et al., 2018).

Chitin contains antimicrobial and antifungal activity and activates the host's immune system (Khoushab and Yamabhai, 2010, Chlebowska-Smigiel et al., 2017). Introduction of chitin in diet can alleviate chronic metabolic diseases, including diabetes and obesity (Collado et al., 2008, Lin et al., 2012). Use of chitin and probiotic may constitute a new therapeutic strategy to prevent chronic disease and malnutrition, by altering the composition of the human intestinal microbiota and suppressing the growth of pathogenic bacteria.

Cricket farming is gaining popularity as a source of cheap high-quality protein, minerals, and fats able to curb malnutrition, with 12.40 g/100 g chitin, which has been exploited to a lesser extent (Kipkoech et al., 2017). Recent studies had suggested removal of chitin before feeding because chitin may hinder utilization of nutrients (Jayanegara et al., 2017), although, Paoletti et al. (2007) had pointed out the presence of chitinase enzyme in human

who has consumed insects over time enabling them to digest chitin (Paoletti et al., 2007). If chitin has prebiotic potential, then its consumption will be useful, and there will be no need to remove it before consumption. Therefore this study would like to explore the possible use of cricket chitin as prebiotic and possibly additional benefits of chitin in modulating gut health.

4.2 Materials and method

4.2.1 Prebiotic potential

Crickets were obtained and prepared as outlined in section 3.2.1. To determine the growth of pathogenic and probiotic bacteria on different concentrations of chitin, 0.1 ml of 16-hour old culture was incubated in nutrient broth with different concentrations of chitin 1%, 5%, 10% or 20%, at 37^0 C for 6 hours, 12 hours, 24 hours, and 48 hours. To determine the combined effect of chitin and probiotic bacteria on growth of *Salmonella typhi* as the selected pathogenic bacteria, bacterial species were incubated at probiotic-pathogenic ratios of 1:1, 1:2, and 2:1, and incubated in chitin supplemented nutrient broth at 37^0 C for 6 hours, 12 hours, 24 hours and 48 hours.

4.2.2 Preparation of chitin supplemented media

Crickets were obtained and prepared as outlined in section 3.2.1. Chitin was extracted as outlined in section 3.2.3. Media for probiotic bacteria, de Man, Rogosa and Sharpe Agar and broth (MRS) was used, (Invitro diagnostics-India), (De Man et al., 1960, Atlas, 2010). For *Salmonella typhi*, salmonella shigella agar was used, (Himedia laboratories PVT. Ltd), (Skariyachan et al., 2016, Rappaport et al., 1956, Atlas, 2010), for combined cultivation of probiotic and pathogenic bacteria, nutrient broth was used (Sigma –Aldrich Co. LLC-USA)(Ohta and Hattori, 1980).

Chitin supplemented media was prepared using different chitin concentrations, with 1%, 5%, 10% or 20% chitin added on media. The control medium consisted of media without

chitin, as either MRS, nutrient broth or salmonella shigella agar, which before use was sterilized at 121^0 C for 15 minutes (Atlas, 2010). Probiotic bacterial cultures: *Lactobacillus fermentum* ATCC 9338, *Lactobacillus acidophilus* ATCC 4356, and *Bifidobacteria adolescentis* ATCC 15703 were obtained from Chr. Hansen-Denmark through Promaco limited- Kenya. Selected pathogenic bacteria *Salmonella typhi* ATCC 6539 was obtained from Kenya Medical Research Institute- Kenya.

4.2.3 Bacterial growth and colony forming unit counts

Before growing bacteria in chitin supplemented media, the stock bacterial culture was defrosted and grown in nutrient broth, to reactivate growth of bacteria prior to inoculation. Enumeration of bacteria was done after incubation at 37^0 C for either 6 hours, 12 hours, 24 hours or 48 hours, where 0.1 ml of 10^{-6} of each replicate, was pour plate and cultivated on MRS agar and Salmonella-Shigella (SS) agar for cell colony counts and calculation of cell colony forming units, (Palframan et al., 2003). Before incubation, the pH was adjusted to neutral by the use of sodium hydroxide or hydrochloric acid, and after each time interval, pH was measured and monitored during the experiment period. The culture was serially diluted 10-fold, and 0.1 ml of 10^{-6} dispensed and pour plated on either SS agar or MRS agar and incubated at 37^0 C for 48 hours; the colonies were counted and colony forming units (CFU) calculated.

4.2.4 Effects of chitin and probiotic bacteria on growth of selected pathogenic bacteria

Cell-free supernatant from incubated probiotic bacteria, *Lactobacillus acidophilus, Lactobacillus fermentum,* and *Bifidobacteria adolescentis* as outlined in section 4.2.3, 1 ml of 16-hour old culture was dispensed into MRS, chitin-supplemented media and incubated for 24 hours at 37^0 C. The tubes were centrifuged at 4000 rpm for 30 minutes, to separate cells from the supernatant, and the supernatant used to determine inhibition zones on growth of *Salmonella typhi.* Pour plates of *Salmonella typhi* in salmonella

shigella agar were made using 1 ml of *Salmonella typhi* stock culture. Half centimetre radius holes were made in the *Salmonella shigella* pour plates, sealed at the bottom with 50 ul of agar and 1 ml of supernatant was dispensed in the holes and the plates incubated at 37^0 C for 48 hours. After 6 hours, 12 hours, 24 hours and 48 hours, inhibition zones were measured in millimetres (mm).

4.2.5 Data analysis

Data collected were recorded in Microsoft Excel. Statistical analysis of the data was performed using SPPS software. Data are presented as means ± standard deviation (SD) and were analysed by ANOVA and means separated by Tukey's multiple comparisons test across experimental groups. Difference between means was considered significant at $p \leq 0.05$. For each species as well as for the sum of pathogenic and probiotic species, a linear fixed model with experimental run as a random factor was applied. In all cases, comparisons with the control were set up and corrected for simultaneous hypothesis testing according to Dunnett 27.

4.3 Results and discussion

In chitin supplemented media, there was varied growth of cells at different chitin concentrations over time. When *Salmonella typhi* and probiotic bacteria were cultivated in the unmodified media with 0% chitin, all the bacterial strains had counts between 50 to 60 CFU/ml at 0 hours (Table 4.1).

There was a steady increase in bacterial growth between 0 and 24 hours before the growth slowly dropped as chitin concentrate on increased over time. The lowest increase in growth was seen at 20% chitin concentration especially in *Salmonella typhi*, this could be due to depletion of available nutrients in the growth media and the inability of *Salmonella typhi* to ferment chitin and utilize it for its growth. *Lactobacillus fermentum* and *Lactobacillus acidophilus* exhibited the highest growth although the growth of

Lactobacillus acidophilus picked up slowly initially. At 48 hours 20% chitin concentration, all bacterial cells were suppressed with *Salmonella typhi* being suppressed most. This could be due to early depletion of nutrients in the media and growth would be depended on the ability of the bacteria present to ferment chitin and utilize its products for growth.

Table 4. 1: Growth of bacterial cells in chitin supplemented media

Chitin Concentration %	Time (hours)	Bacterial species			
		S. typhi	***L. fermentum***	***B. adolescentis***	***L. acidophilus***
0%	0	54±1.5[a]	58±2.6[a]	60±2.0[a]	54±3.3[a]
	6	62±2.4[a]	61±3.4[a]	65±3.4[a]	60±3.3[a]
	12	96±4.3[a]	107±1.0[a]	60±3.5[a]	122 ±4.1[b]
	24	98±4.3[a]	102±3.8[a]	98±1.6[a]	132±3.3[b]
	48	102±5.1[a]	114±2.7[a]	112±3.3[a]	120±4.3[b]
1%	0	56±2.4[a]	56±3.4[a]	58±3.0[a]	60±6.3[a]
	6	64±3.4[a]	66±1.8[a]	64±2.4[a]	67±7.3[a]
	12	67±4.1[a]	110±3.4[b]	146±1.8[b]	137±3.3[b]
	24	68±2.3[a]	120±1.8[b]	156±3.3[b]	124±4.2[b]
	48	76±3.4[a]	101±2.4[b]	145±3.4[b]	138±4.3[b]
5%	0	60±3.5[a]	52±1.6[a]	54±1.0[a]	60±5.3[a]
	6	64±3.2[a]	58±3.0[a]	60±3.0[a]	71±2.4[a]
	12	72±1.4[a]	121±3.8[b]	146±2.3[b]	168±3.4[b]
	24	55±2.5[a]	127±2.4[b]	140±5.2[b]	167±3.7[b]
	48	57±3.3[a]	135±1.8[b]	132±3.3[b]	167±3.0[b]
10%	0	54±1.2[a]	56±3.4[a]	52±3.1[a]	60±2.4[a]
	6	62±3.2[a]	64 ±3.3[a]	66±3.2[a]	68±3.7[a]
	12	60±3.5[a]	117±1.7[b]	152±3.0[b]	169±3.0[b]
	24	54±2.6[a]	99±2.5[b]	144±3.6[b]	172±2.8[b]
	48	49±7.1[a]	101±3.2[b]	141±2.7[b]	154±3.6[b]
20%	0	52±3.3[a]	54±4.1[a]	52±3.1[a]	50±4.2[a]
	6	62±2.3[a]	64±4.3[a]	66±3.3[a]	68±3.2[a]
	12	52±4.2[a]	116±6.1[b]	130±4.2[b]	172±3.2[b]
	24	45±6.1[a]	113±4.3[b]	112±3.3[b]	136±2.3[b]
	48	37±4.3[a]	102±6.2[a]	98±4.1[a]	102±3.3[a]

n=3 Values displayed as means ±standard deviation

Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$

Abbreviation*: S. typhi: Salmonella typhi, L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus.*

Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored over time in 0, 6, 12, 24, and 48 hours. Bacterial growth was measured in colony forming unit per millilitre (CFU/ml)

All bacteria grew in chitin supplemented media, although *Salmonella typhi* growth was

limited by increased concentration of chitin, probably because *Salmonella typhi* was unable to break down chitin and therefore its growth would be limited by the availability of favourable growth media. The growth change of this bacteria was not significantly different at different chitin concentration at different times.

There was a significant change ($P < 0.05$) in probiotic bacterial growth at 12 hours, 24 hours and 48 hours at 1%, 5% and 10% chitin concentration. At 20% chitin concentration the growth was significantly different at 12 hours and 24 hours, but at 48 hours the growth dropped (Table 4.1). This could be due to the limitation of nutrients amid increased bacterial concentration. This shows that probiotic bacteria are able to degrade chitin only to a limited concentration and beyond this concentration higher chitin will limit the growth of probiotic bacteria.

This study demonstrates that chitin has the ability to increase the growth of probiotic bacteria and its use as a prebiotic would profoundly modify the gut microbial composition because beneficial bacteria are able to ferment chitin (Bird and Topping, 2008). When food ingested contains chitin, which is able to pass through the digestive system and gets to the colon almost unaltered, beneficial bacteria that reside in the colon can ferment chitin, and utilize the products for its growth (Buruiana et al., 2017).

Chitin in itself may limit the growth of *Salmonella typhi*, as seen in the limited growth in chitin supplemented media by deprivation of the required nutrients for growth. Past studies have shown prebiotics such as inulin having the ability to restrict the growth of pathogenic bacteria (Blaut, 2002). This demonstrates that chitin may prevent the colonization of the human gut by enteric pathogens as demonstrated by past studies (Roberfroid et al., 2010). Other studies have shown chitin derivatives having the antimicrobial ability (Shahidi et al., 1999) therefore chitin can be utilized to help improve gut health by directly suppressing the growth of *Salmonella typhi* through nutrient deprivation and possibly acting as an antimicrobial agent.

When chitin and probiotic bacteria effect on the growth of pathogenic bacteria was assessed, in chitin supplemented media at a ratio of 1:1 probiotic to the pathogen. All the bacterial cells started off at almost equal concentration. Increase in growth was seen in the first six hours before a drastic drop in *Salmonella typhi* was noted (Table 4.2). Even in media that did not contain chitin but contained the probiotic bacteria, *Salmonella typhi* growth was significantly suppressed at 24 hours ($P < 0.05$) (Table 4.2).

The drop in bacterial cell growth paralleled chitin concentration increase; only *Lactobacillus fermentum* seemed to thrive in high chitin concentrations and the presence of the pathogen in the media (Table 4.2). The number of colony forming units were significantly different in *Salmonella typhi* after 6 hours of growth. *Bifidobacteria adolescentis* and *Lactobacillus acidophilus* growth in different concentration over time did not change significantly. This was a different scenario with the initial growth of probiotic bacteria in chitin supplemented media where there was a significant increase in growth. This could be because of the presence of *Salmonella typhi* which was probably exerting a negative impact on probiotic bacteria growth probably as a survival strategy.

Probiotic bacteria grew well in chitin supplemented media, though the growth was slowed by the presence of *Salmonella typhi*, this could be due to lack of sufficient media to promote growth. Also, *Salmonella typhi* may probably produce toxins as a survival strategy since bacteria are known to produce toxins as a survival strategy. In the other hand *Salmonella typhi* was greatly reduced, this could have been due to the combined effect of the inability to ferment chitin and the by-products of probiotic bacteria have they grow in chitin which is probably probiotic bacteria survival strategy.

Table 4. 2: Effect of bacterial growth on chitin supplemented media in presences of probiotic-pathogenic in the ration of 1:1

Chitin Concentration (%)	Time (hours)	Bacterial species			
		S. typhi	***L. fermentum***	***B. adolescentis***	***L. acidophilus***
0%	0	64±2.3[a]	58±3.2[a]	60±2.4[a]	56±4.3[a]
	6	56±1.2[a]	80±1.3[a]	74±1.3[a]	76±6.4[a]
	12	5±0.4[b]	106±2.4[b]	80±4.3[a]	88±3.1[a]
	24	3±0.5[b]	104±2.3[b]	78±3.5[a]	72±3.3[a]
	48	1±0.4[b]	107±1.3[b]	76±3.4[a]	84±0.2[a]
1%	0	66±2.3[a]	58±3.4[a]	54±3.6[a]	56±2.3
	6	48±3.4[a]	80±3.5[a]	74±3.6 [a]	76±4.3[a]
	12	3±0.4[b]	122±3.2[b]	82±3.1[a]	82±3.0[a]
	24	1±0.3[b]	127±3.3[b]	90±4.4[a]	72±6.3[a]
	48	1±0.3[b]	125±3.4[b]	78±4.5 [a]	74±2.1[a]
5%	0	60±3.2[a]	58±3.6[a]	60±2.3[a]	56±5.1[a]
	6	54±3.4[a]	80±3.1[a]	73±4.4 [a]	76±4.2[a]
	12	2±0.5[b]	116±3.2[b]	66±5.6 [a]	72±1.3[a]
	24	1±0.3[b]	118±3.3[b]	67±2.5 [a]	68±2.3[a]
	48	1±0.2[b]	112±3.3[b]	69±3.4 [a]	72±5.3[a]
10%	0	48±3.6[a]	58±3.6[a]	46±3.3 [a]	52±7.3[a]
	6	30±3.2[a]	77±3.4[a]	68±3.2[a]	76±4.0[a]
	12	1±0.2[b]	102±3.2[b]	67±6.2 [a]	74±3.3[a]
	24	1±0.1[b]	100±3.2[b]	66±3.0 [a]	72±4.3[a]
	48	1±0.2[b]	107±3.3[b]	66±3.0[a]	64±1.3[a]
20%	0	66±31[a]	54±3.1[a]	58±4.0[a]	56±2.3[a]
	6	32±3.3[a]	65±3.2[a]	60±5.3[a]	70±5.4[a]
	12	1±0.3[b]	101±3.4[b]	57±2.0[a]	66±6.0[a]
	24	1±0.3[b]	98±3.2[b]	51±4.0[a]	65±2.3[a]
	48	1±0.3[b]	102±3.1[b]	52±3.2[a]	66±4.1[a]

n=3. Values displayed as means ±standard deviation

Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$

Abbreviation: *S. typhi: Salmonella typhi, L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus.*

Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored over time in 0,

6, 12, 24, and 48 hours. Bacterial growth was measured in colony forming unit per millilitre (CFU/ml)

Probiotic bacteria were able to grow since these bacteria are able to ferment chitin and therefore use it for growth. In the current study, as the probiotic bacteria grew, the pathogenic bacteria were suppressed. Recent studies have shown probiotic bacteria with the ability to suppress pathogenic bacteria (Slomka et al., 2017) and this would be one of the advantages of consuming chitin with probiotics to improve gut health. There can also be consumption of chitin alone targeting probiotics to suppress pathogenic bacteria and enhance the growth of probiotic bacteria already existing in the gut to help moderate gut health.

When the probiotic-pathogenic bacterial ratio was altered in favour of the probiotic bacteria, and the initial inoculum of probiotic bacteria increased, both the probiotic and pathogenic bacteria started off well at zero hours (Table 4.3), but at 12 hours there was a significant drop in *Salmonella typhi* growth, even in the media that did not contain chitin. At 20% chitin concentration, *Salmonella typhi* did not even have time to acclimatize and grow in media because at 6 hours the drop in growth was already significant $p< 0.05$ (Table 4.3).

Apart from nutrient depletion, there would be more metabolites produced by the growing probiotic bacteria which means that *Salmonella typhi* is eliminated faster, this is why at 12 hours the suppression of *Salmonella typhi* was significant at ($p < 0.05$). There was a significant increase in probiotic bacteria at 0%, 1% and 5% chitin concentration. Presence of *Salmonella typhi* and high chitin concentration beyond 5% did not favour the growth of *Lactobacillus acidophilus* since the difference in growth at 10% and 20% chitin concentration was not significant ($p > 0.05$). Growth of *Lactobacillus fermentum* and *Bifidobacterium adolescents* was still significantly at 10% chitin concentration but at 20% chitin concentration and presence of *Salmonella typhi*, there was no significant growth in all probiotic bacteria over time.

Table 4. 3: Effect of bacterial growth on chitin supplemented media in presences of probiotic-pathogen in the ration of 2:1

Chitin Concentration %	Time (hours)	Bacterial species			
		S. typhi	***L. fermentum***	***B. adolescentis***	***L. acidophilus***
0%	0	48±4.2[a]	42±3.2[a]	56±3.3[a]	43±3.2[a]
	6	16±3.0[a]	66±5.1[a]	58±3.2[a]	64±4.2[a]
	12	3±0.3[b]	102±4.2[b]	82±3.0[b]	71±2.0[b]
	24	2±0.7[b]	92±4.0[b]	88±3.1[b]	121±4.1[b]
	48	2±0.6[b]	127±1.4[b]	108±2.3[b]	148±7.1[b]
1%	0	48±7.0[a]	47±3.2[a]	56±4.0[a]	43±3.0[a]
	6	16±3.0[a]	66±3.0[a]	58±1.4[a]	64±3.2[a]
	12	1±0.1[b]	94±3.1[b]	82±4.0[b]	71±2.0[b]
	24	2±0.2[b]	92±2.0[b]	88±6.3[b]	71±4.0[b]
	48	1±0.8[b]	90±1.4[b]	80±1.3[b]	72±2.1[b]
5%	0	40±3.0[a]	45±2.3[a]	49±4.0[a]	40±3.1[a]
	6	10±0.9[a]	62±3.0[a]	47±2.3[a]	50±2.0[a]
	12	3±0.7[a]	92±2.1[b]	86±2.3[b]	77±1.7[b]
	24	1±0.6[a]	87±6.0[b]	80±1.3[b]	76±2.0[b]
	48	2±3.1[a]	82±7.0[b]	84±4.0[b]	80±2.1[b]
10%	0	38±0.7[a]	40±2.3[a]	38±2.6[a]	40±2.0[a]
	6	12±2.3[a]	62±5.3[a]	47±4.1[a]	50±1.3[a]
	12	1±0.3[b]	97±2.3[b]	88±3.0[b]	61±5.0[a]
	24	1±0.3[b]	56±2.0[b]	46±1.3[a]	38±4.2[a]
	48	2±0.3[b]	77±4.0[b]	50±4.5[a]	45±1.3[a]
20%	0	42±3.2[a]	40±2.1[a]	48±3.0[a]	37±2.3[a]
	6	8±3.3[b]	62±7.0[a]	47±6.0[a]	50±4.3[a]
	12	2±0.6[b]	80±1.3[a]	47±1.7[a]	44±4.1[a]
	24	2±0.3[b]	76±4.0[a]	44±3.4[a]	54±2.1[a]
	48	1±0.3[b]	66±4.0[a]	46±2.0[a]	48±3.0[a]

n=3. Values displayed as means ±standard deviation

Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$

Abbreviation: *S. typhi: Salmonella typhi, L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus.*

Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored over time in 0, 6, 12, 24, and 48 hours. Bacterial growth was measured in colony forming unit per millilitre (CFU/ml)

This would mean that nutrient present in the media was depleted faster due to the increased probiotic bacteria concentration since the initial inoculum of probiotic bacteria was high their multiplication would probably be higher and therefore faster chitin fermentation.

Despite the initial chitin concentration, the pathogenic bacteria were at the end outgrown. This is probably due to the combined effort of the probiotic bacteria, low pH and chitin (Marques et al., 2006). Studies in fish fed with chitin showed increased survival, although there was no evidence of increased cellular immunity, and the researchers pointed out that increased survival was as a result of the suppression of pathogenic bacteria by chitin (Powell and Rowley, 2007). Growth of probiotic bacteria may also have aided the suppression of pathogenic bacteria through the production of bacteriocin, exopolysaccharides and biogenic amines (Prodeus et al., 2016, Boskey et al., 1999). This points to the importance of using chitin as a prebiotic, and these findings encourage the promotion of chitin consumption to suppress pathogenic bacteria in the gut.

When the initial bacterial concentration was altered in favour of pathogenic bacteria, the growth of probiotic bacteria slowed, but still, the elimination of pathogenic bacteria occurred although delayed (Table 4.4). At 24 hours pathogenic bacteria were eliminated at 0% and 1% chitin concentration. With the increase in chitin concentration and the presence of probiotic bacteria, pathogenic bacteria were eliminated faster at 5% and 10% chitin concentration. There was no significant increase in the growth of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* in different chitin concentration over time with an initial high concentration of *Salmonella typhi.* At 6 hours in 20% chitin concentration there was a significant decrease in bacterial growth in *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* while at 48 hours in 20% chitin concentration, growth of *Lactobacillus fermentum* was significantly reduced.

Pathogenic bacteria were able to survive longer due to high initial concentration and therefore higher cell multiplication. The slowest increase in probiotic cell growth could have been caused by a high concentration of pathogenic bacteria hence fast depletion of

nutrients.

Table 4. 4: Effect of bacterial growth on chitin supplemented media in presences of probiotic-pathogen in the ration of 1:2

Chitin Concentration %	Time (hours)	Bacterial species			
		S typhi	***L. fermentum***	***B. adolescentis***	***L. acidophilus***
0%	0	67±2.4[a]	42±4.2 [a]	40±2.4[a]	43±1.7[a]
	6	42±3.0[a]	66±5.2[a]	58±1.5[a]	64±2.8[a]
	12	34±1.2[a]	67±4.3[a]	64±2.4[a]	70±2.3[a]
	24	3±3.0[b]	72±2.3[a]	66±3.0[a]	74±1.7[a]
	48	1±2.0[b]	76±1.7 [a]	70±3.0[a]	74±2.4[a]
1%	0	64±4.3[a]	38±1.4[a]	44±3.0[a]	42±1.8[a]
	6	38±5.0[a]	68±2.0[a]	66±1.3[a]	66±1.8[a]
	12	12±2.0[a]	67±3.2 [a]	66±4.1[a]	76±1.2[a]
	24	2±0.3[b]	78±1.4[a]	45±4.0[a]	70±2.0[a]
	48	2±0.3[b]	78±2.3[a]	30±3.2[a]	54±3.0[a]
5%	0	66±4.0[a]	44±2.0[a]	46±3.0[a]	38±4.0[a]
	6	32±2.4[a]	68±2.0[a]	58±2.1[a]	58±1.2[a]
	12	1±0.3[b]	66±3.1[a]	60±4.0[a]	66±4.1[a]
	24	2±0.3[b]	65±4.0[a]	64±3.3[a]	67±1.8[a]
	48	1±0.3[b]	70±4.4[a]	66±2.0[a]	68±4.0[a]
10%	0	64±3.4[a]	38±2.0[a]	40±2.0[a]	32±2.3[a]
	6	22±5.4[a]	40±4.0[a]	66±2.0[a]	56±4.0[a]
	12	2±0.3[b]	42±3.3[a]	50±4.0[a]	46±3.0[a]
	24	2±0.3[b]	60±1.3[a]	52±4.4[a]	58±2.3[a]
	48	3±0.3[b]	54±2.4[a]	42±4.3[a]	52±2.1[a]
20%	0	66±1.3[a]	34±3.0[a]	36±1.7[a]	36±2.0 [a]
	6	12±2.3[b]	54±3.0[a]	66±1.5[a]	44±1.3[a]
	12	1±0.3[b]	46±3.0[a]	37±2.3[b]	26±4.3[b]
	24	2±0.3[b]	42±1.4[a]	32±1.8[b]	28±4.0[b]
	48	2±0.3[b]	32±2.3[b]	21±2.0[b]	27±2.0[b]

n=3. Values displayed as means ±standard deviation

Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$

Abbreviation: *S. typhi: Salmonella typhi, L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus*.

Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored over time in 0, 6, 12, 24, ad 48 hours. Bacterial growth was measured in colony forming unit per millilitre (CFU/ml)

Metabolites produced as the pathogenic bacteria grew might have also contributed to the slowed growth of probiotic bacteria. At 20% chitin concentration, suppression of all bacterial cells was likely caused by depletion of nutrients in growth media and pollution of media by dying bacterial cells. Good prebiotic candidates would selectively support the growth of specific beneficial bacteria (Macfarlane and Cummings, 1999) leading to positive modulation of gut microbiota (Gibson et al., 2017a). In the gut, pathogenic bacteria are the main cause of diarrhoea and the use of probiotic bacteria and chitin can suppress pathogenic bacteria, by nourishing the probiotic bacteria which in turn produce metabolites to kill the pathogenic bacteria (De Vrese and Offick, 2010). Chitin can also act directly as an antimicrobial in the human gut, suppressing the growth of pathogenic bacteria (Wagener et al., 2017a).

As the probiotic and pathogenic bacterial cells grew the pH changed, being initially neutral. However, differences were apparent (Table 4.5). The pH in *Salmonella typhi* growth in chitin barely changed and was almost neutral at all chitin concentrations over time ($P > 0.05$). While in *L fermentum*, *B adolescentis*, and *L acidophilus* the pH decreased as the bacteria grew (Table 4.5). In *L acidophilus*, a species that seemed to thrive well at low pH, the highest drop in pH was noticed with bacteria growing in chitin. This species was able to grow at a pH in chitin concentration as low as pH 3.6, occurring in 20% chitin supplemented media at 24 hours. All other probiotic bacteria growth media pH drop was seen after 12 hours of growth in all growth media, even in the unmodified media. At 20% chitin concentration, the pH drop was delayed up to 24 hours (Table 4.5). This may be due to the slow growth of probiotic bacteria in high chitin concentration. Lower pH during growth is likely to be a survival strategy for probiotic bacteria. This may be lacking in *Salmonella typhi* and could be the reason why probiotic bacteria was able to suppress pathogenic bacteria growth in the presence of probiotic bacteria and chitin.

Table 4. 5: Response of pH in chitin supplemented media during bacterial cell growth

Chitin Concentration (%)	Time (hours)	Bacterial species			
		S. typhi	***L. fermentum***	***B. adolescentis***	***L. acidophilus***
0%	0	7.2±0.3[a]	7.1±0.3[a]	7.2±0.3[a]	7.2±0.3[a]
	6	7.3±0.3[a]	6.5±0.3[a]	6.7±0.3[a]	6.2±0.3[a]
	12	7.4±0.3[a]	5.9±0.3[a]	5.6±0.3[a]	5.2±0.3[a]
	24	7.0±0.3[a]	4.4±0.3[b]	4.6±0.3[b]	4.8±0.3[b]
	48	6.9±0.3[a]	4.1±0.3[c]	4.2±0.3[b]	4.1±0.3[b]
1%	0	6.9±0.2[a]	7.0±0.3[a]	7.2±0.1[a]	6.8±0.1[a]
	6	7.0±0.2[a]	6.6±0.3[a]	6.6±0.1[a]	5.4±0.1[a]
	12	7.1±0.1[a]	5.4±0.3[a]	5.5±0.1[a]	5.2±0.1[a]
	24	7.0±0.2[a]	4.6±0.3[b]	4.8±0.1[b]	4.2±0.1[b]
	48	7.1±0.2[a]	4.2±0.3[c]	4.3±0.1[b]	3.8±0.2[b]
5%	0	7.1±0.1[a]	6.9±0.1[a]	6.8±0.1[a]	6.7±0.1[a]
	6	7.3±0.2[a]	6.1±0.2[a]	6.2 ±0.1[a]	5.5±0.5[a]
	12	7.3±0.3[a]	5.4±0.2[a]	5.0±0.4[b]	4.9±0.2[b]
	24	7.3±0.3[a]	4.6±0.3[b]	4.4±0.2[b]	4.3±0.3[b]
	48	7.3±0.3[a]	4.2±0.3[b]	4.0±0.3[b]	3.8±0.3[b]
10%	0	7.2±0.2[a]	7.2±0.4[a]	7.1±0.3[a]	7.0±0.3[a]
	6	7.3±0.1[a]	6.1±0.3[a]	6.0±0.2[a]	5.8±2.3[a]
	12	7.4±0.1[a]	5.8±0.2[a]	5.2±0.1[a]	4.4±0.3[b]
	24	7.0±0.2[a]	4.8±0.3[b]	4.6±0.2[b]	3.9±0.5[b]
	48	6.9±0.3[a]	4.2±0.3[b]	4.4±0.3[b]	3.7±0.3[b]
20%	0	7.0±0.1[a]	6.8±0.1[a]	6.8±0.3[a]	7.1±0.4[a]
	6	6.9±0.3[a]	7.1±0.3[a]	6.7±0.2[a]	6.2±0.2[a]
	12	6.9±0.2[a]	5.9±0.1[a]	5.6±0.4[b]	5.2±0.3[a]
	24	7.0±0.2[a]	4.4±0.1[b]	4.6±0.2[b]	3.8±0.3[b]
	48	7.1±0.3[a]	4.1±0.3[b]	4.2±0.3[b]	3.6±0.3[b]

n=3. Values displayed as means ±standard deviation

Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$

Abbreviation: *S. typhi: Salmonella typhi, L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus*.

Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored over time in 0, 6, 12, 24, ad 48 hours. Bacterial growth was measured in colony forming unit per millilitre (CFU/ml)

Fermented milk products have been consumed with perceived health benefits due to the presence of live bacteria responsible for milk fermentation, and recent years have seen

increased interest due to their health benefits (Hill et al., 2017). Combining fermented milk products with a prebiotic is likely to increase the benefits of its consumption. Nutrient limitations and a low pH have been indicated as the main inhibitors of the growth of pathogenic bacteria in the gut (Charalampopoulos et al., 2002, Di Gioia and Biavati, 2018).

This was likely the main reason for *Salmonella typhi* restricted growth. The low pH during chitin breakdown may have stimulated the growth of probiotic bacteria which are well adapted to a low pH, and lowering the pH in their environment is a survival strategy in many bacterial species, helping to suppress pathogenic species (Blaut, 2002).

Consumption of prebiotics would increase the colonization by probiotic bacteria, which in turn would suppress pathogenic bacteria (Wagner and Johnson, 2017). The gut microbiota would be highly regulated and therefore alleviate infections caused by a microbial imbalance in the gut (Christensen et al., 2017). Intestinal microbiota depends on non-digestible fibre, (Shang et al., 2017) and cricket chitin is a possible prebiotic candidate. Fermentation of chitin leads to lower colonic pH due to the production of acetate, propionate, and butyrate by probiotic bacteria (Scott et al., 2013) and these weak acids then influence the microbial composition, by suppressing the growth of pathogens, favouring the growth of probiotic bacteria.

When probiotic bacteria were cultivated on chitin supplemented media and the supernatant of the growth media, used in the experiment to inhibit the growth of pathogenic bacteria, inhibition zones were seen, even in the media that did not contain chitin although this inhibition was not significantly different ($p > 0.05$) (10 mm).

Table 4. 6: Inhibition of pathogenic bacteria growth by probiotic bacteria supernatant

Chitin concentration	Bacterial species		
	L. fermentum	***B. adolescentis***	***L. acidophilus***
0%	10 ± 0.3^{a}	10 ± 0.2^{a}	10 ± 0.3^{a}
1%	11 ± 0.3^{a}	10 ± 0.3^{a}	11 ± 0.2^{a}
5%	11 ± 0.3^{a}	10 ± 0.1^{a}	12 ± 0.2^{b}
10%	16 ± 0.3^{b}	12 ± 0.2^{b}	12 ± 0.3^{b}
20%	13 ± 0.3^{c}	14 ± 0.3^{c}	10 ± 0.1^{a}

n=3. Values displayed as means ±standard deviation
Values within the same column under the same chitin concentration with different superscripts are significantly different $p < 0.05$
Abbreviation: *L. Fermentum: Lactobacillus fermentum, B. adolescentis: Bifidobacteria adolescentis, L. acidophilus: Lactobacillus acidophilus.*
Bacterial growth in 0%, 1%, 5%, 10% and 20% chitin supplemented media was monitored at 24 hours. Bacterial inhibition zones were measured in millimetres (mm)

The highest inhibition was seen with *L fermentum*, which was significantly different at 10% chitin concentration at 16 mm (Table 4.6). An increase in chitin concentration to 20% did not increase the diameter of the inhibition zone.

The difference in the inhibition distance was significantly different at $p < 0.05$ for *L fermentum* at 10% and 20% chitin concentration, for *B adolescentis* at 10% and 20% chitin concentrations and for *L acidophilus* at 5%, 10%, and 20% chitin concentration. In the media that did not contain chitin, the difference was not significant $p > 0.05$. Presence of chitin leads the bacteria to ferment in order to utilize the products of fermentation for its growth. During fermentation, there is the production of weak acids and other unknown metabolites that are responsible for restricting the growth of *Salmonella typhi.* There is a need to profile the metabolites in the media to ascertain the exact cause of pathogenic bacterial growth inhibition in the presence of chitin and probiotic bacteria.

Chitin fermentation metabolites have been documented by other studies to inhibit bacterial growth (Ross et al., 2017). Chitosan a derivative of chitin has been found to contain antimicrobial activities that are useful in inhibiting gram-positive bacteria (Cremar et al., 2018). In a recent study, chitosan a derivative of chitin was shown to inhibit all of the bacterial strains tested (Abdelmalek et al., 2017). Chitin hydrogels and other products, have also been developed to help in wound dressing and antimicrobial properties, and enhance healing (Shao et al., 2017).

There is a need to explore additional benefits of combined probiotic and chitin as they impact human health. Insects as components of traditional medicines have been used since time immemorial (Meyer-Rochow and Changkija, 1997), and chitin can help solve gut health in children either directly by acting as an antimicrobial or as a prebiotic to nourish probiotic bacteria. Trials of child feeding with food containing chitin should provide more insight into the use of chitin in modulating gut health in a positive way and in connection with treatments of environmental enteric dysfunction. Environmentally enteric dysfunction (EED) is a pathway from poor hygiene to malnutrition (Keusch et al., 2013).

Over the years, stunting could not be explained by poor diet or diarrhoea but could be reversed by optimizing the diet partially to counteract villous atrophy known to lead to the reduced absorptive service area and a leaky gut caused by pathogenic microbes (Mbuya and Humphrey, 2016). Children living in unhygienic conditions are at risk of EED, because of constant exposure to pathogenic microbes in the environment while interventions aimed at improving EED are likely to improve stunting (Mbuya and Humphrey, 2016). There is a need to utilize the probiotic, pathogenic and chitin interaction in restoring microbial balance in the gut and restore health.

4.4. Conclusions and recommendations

This study has demonstrated the ability of probiotic bacteria to breakdown chitin, lower pH of growth media and inhibit pathogenic bacterial growth. Chitin may be a functional

prebiotic due to its ability to stimulate the growth of specific beneficial bacteria, in this case, Lactobacillus and Bifidobacteria. There is a need to explore the effect of chitin to help improve gut health in children. There is also a need to exploit active sites in chitin and its pharmaceutical potential.

CHAPTER FIVE

DEVELOPMENT AND CONSUMER ACCEPTABILITY OF CRICKET-BASED PORRIDGE FLOUR

5.1 Introduction

According to van Huis (2015), there is food security if the four pillars of access, utilization, availability, and affordability apply to all people. Kenya is not yet food secure since there are many children lacking essential nutrients in the available diets, with rain-fed agriculture increasingly becoming unpredictable due to climate change (Zhang et al., 2017, Shisanya et al., 2017).

Animal protein in child diet has been shown to be important in the provision of healthy nutrients required for proper child growth (Lind et al., 2017, Mohamed et al., 2018). The major problem faced by parents is not lack of protein since plant protein is abundant and mostly consumed but it's the lack of animal protein because its expensive (van Huis, 2015). World Health Organization (WHO) provides guidelines on child feeding (WHO, 2006) and requires that any developed child food should contain balanced nutrients for proper child growth and should include animal sourced protein. Most parents often fail to provide the required balanced food for their children due to either lack of knowledge or access to the required food, causing poor growth (UNICEF., 2003).

Annual production of animal products in Kenya falls below the national consumption levels (Ogello and Munguti, 2016), with part of the population not able to afford the conventional animal proteins and therefore protein-energy malnutrition remains high. Protein undernourishment in the world has remained high, due to overdependence on animal proteins which are on the constant decrease due to land pressure, and related cost of buying animal proteins (Briedenhann et al., 2017). In the world, 75% of cereals produced is used as animal feed, and animals occupy slightly over 60% of the available land (Eisler et al., 2014). Approximately 50% of households in Kenya suffer from poverty

and insufficient food production and therefore are food insecure, with the added burden of morbidities' attributed to malnutrition (Bor et al., 2016), the poor are more food insecure with the central bureau of statistics showing that the availability of income and social service favours the rich (Shisanya et al., 2017).

Insect based foods developed in the past have been shown to be safe in presence of microbes and other chemical seen as food contaminants (Poma et al., 2017), though it's important to adhere to food safety standards during production and processing of edible insects products (Schlüter et al., 2017). Edible insect food products developed are highly acceptable by the consumers (Van Huis, 2017, Menozzi et al., 2017), as long as the food is developed to balance the best attributes and inserted in commonly consumed products (Alemu et al., 2017).

Most commonly available child food and snacks are composed of carbohydrates with little or no proteins (Michaelsen et al., 2009), children worldwide are deficient of minerals and vitamins, most of which are exclusively found in animal-sourced diets (Cusick et al., 2018). In Kenya nutrient deficiency leading to poor growth in children is common (Kujinga et al., 2018), and there is a need for a better alternative in child foods to improve child nutrition in Kenya.

Insects have high protein source and can be used in place of conventional animal protein commonly eggs, meat, pork and fish (Van Huis, 2013). In Kenya crickets has been introduced in farming systems (Ayieko et al., 2016, Kipkoech et al., 2017), and in bid to increase cricket consumption while reducing disgust, food products with cricket have been developed with previous cricket products having used 5% and 10% cricket flour (Alemu et al., 2017), and this study tested if 20% can make better porridge. Skimmed milk powder was used for comparison because milk is acceptable by the World Health Organization as a standard for moderately malnourished children interventions (WHO, 2015).

For any new product, sensory evaluation is important because it will help in the collection

of best-desired attributes to enable adjustment of a product to fit consumer liking. New products are best introduced has blends of existing products. In this study, various percentages of cricket-based porridge are blended with maize and millet. The product is then subjected to an untrained panel for sensory evaluation, to pick out the cricket-based porridge with best attributes for child feeding trials.

5.2 Materials and methods

5.2.1 Study area

The study was accomplished at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

5.2.2 Material acquisition and preparation

Crickets were harvested from JKUAT insect farm (JIF), frozen, blanched at 98^0C for one minute, oven dried at 50^0C for 72 hours, milled to cricket flour, and sieved through 250 um mesh sieve size (Kipkoech et al., 2017). Maize flour, skimmed milk powder, millet flour, sugar and soybean oil were sourced from the local shops in Nairobi, while vitamin and mineral premix was sourced from DSM nutritional products (South Africa).

5.2.3 Porridge flour formulation

Cricket-based porridge flour was developed with varied composition as shown in Table 5.1. The porridge flours were formulated with varied cricket powder content as follows, cricket 5% (CP5), cricket 10% (CP10) or cricket 20% (CP20). Porridge with the skimmed milk powder was used as the positive control and denoted as MP10. The mixture of maize and millet (MMP) without any animal-source protein added was used as the negative control and denoted MMP.

Table 5. 1: Porridge flour formulation

Flour type	Millet (%)	Maize (%)	Milk (%)	Cricket (%)	Soybean oil (%)	Sugar (%)	Vitamin and mineral Premix
MMP	25	59	0	0	10	4.2	1.8
MP10	25	49	10	0	10	4.2	1.8
CP5	25	54	0	5	10	4.2	1.8
CP10	25	49	0	10	10	4.2	1.8
CP20	25	39	0	20	10	4.2	1.8

MMP; millet maize-based porridge, MP10; milked based porridge, CP5; cricket 5% based porridge, CP10; cricket 10% based porridge and CP20; cricket 20% based porridge.

To ensure the near similarity in the developed porridge, millet, soybean oil, sugar, and premix was equal in all the groups. Interventions contained added animal protein source in the form of cricket powder, which lacked in the negative control flour, while the positive control contained milk powder. Maize was varied to fill in the gap created by the addition or subtraction of an animal protein.

MMP flour production is outlined in the flow diagram below, (Figure 5.1,). Maize and millet were cleaned and extruded, the product of extrusion was milled and mixed with sugar and soybean oil to produce the MMP flour (Figure 5.1).

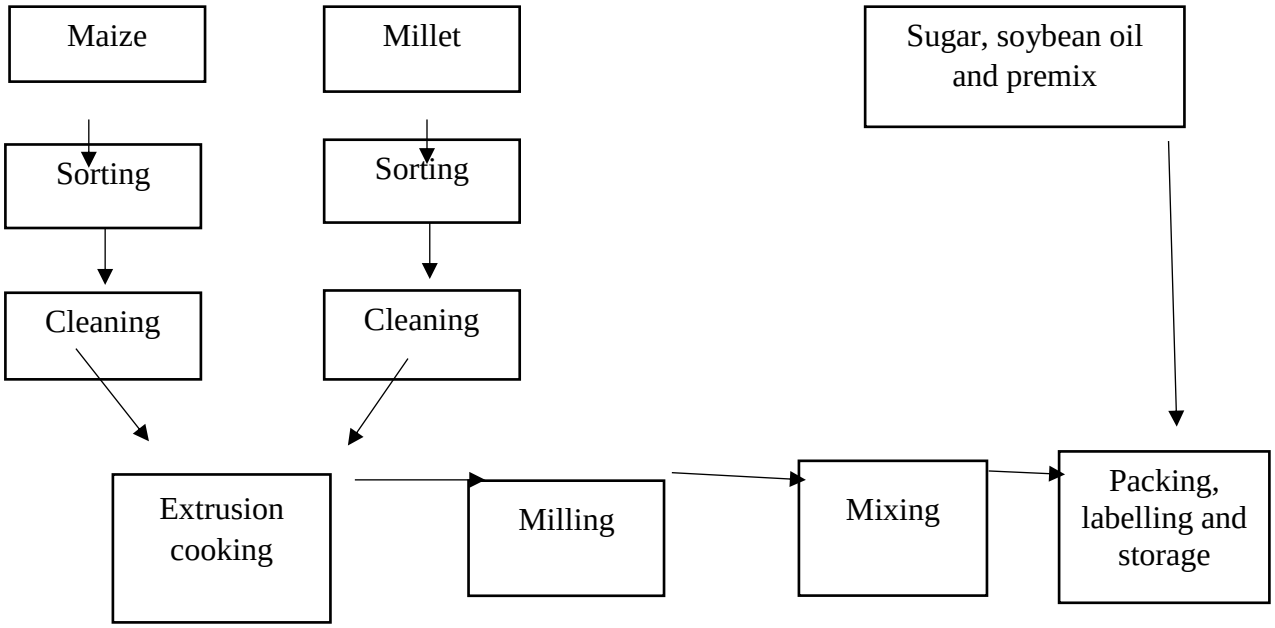

Figure 5. 1: MMP flour production

To produce MP10, maize and millet were cleaned and extruded, the product of extrusion was milled and mixed with 10% skimmed milk, sugar, and soybean oil (Figure 5.2).

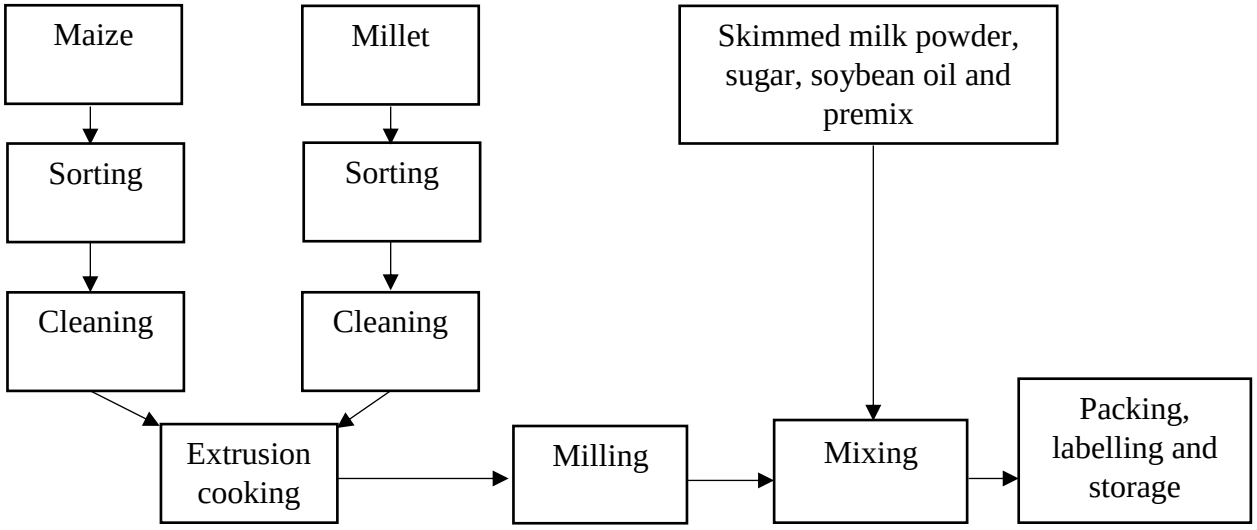

Figure 5. 2: MP10 flour production

During the production of cricket based porridge flour (Figure 5.3), the cricket powder was varied by the addition of 5%, 10% or 20% cricket powder. The same process was then repeated to produce CP5, CP10 or CP20. Soybean oil, sugar, and premix were added after each extrusion cooking to avoid loss of nutrients in premix by high heat extrusion (Boyaci et al., 2012).

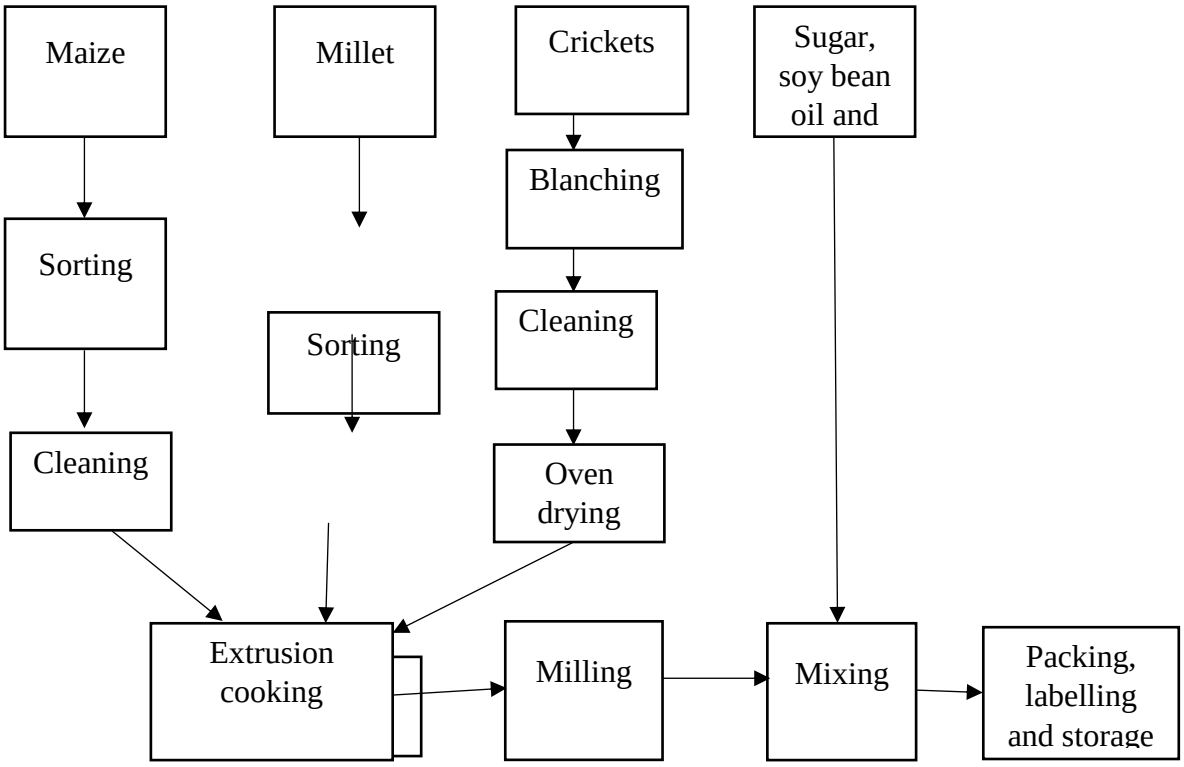

Figure 5. 3: CP5, CP10, and CP20 flour production

5.2.4 Determination of the microbial safety in developed porridge flour

To determine the overall microbial load in the developed flour, 4 g of each flour type was mixed separately in a stomacher bag and homogenized (Bag mixer Interscience-France) at a speed of 200 revolutions per minute for 30 seconds. Serial dilution of the homogenate to tenfold was done and 50 µl spread on plate count agar. Cultivation of *Escherichia coli (E coli),* was accomplished using MacConkey agar (March and Ratnam, 1986) test method

TES/MIC/TM/17. *Salmonella typhi (S typhi),* was cultivated on Salmonella-Shigella agar (Madigan et al., 2017) test method TES/MIC/TM/08. Nutrient agar was used for total viable bacteria count, test method EAS782. Finally, peptone glucose agar was used for yeast and moulds cultivation, test method TES/MIC/TM/11.

5.2.5 Nutrient content in the developed porridge

The nutrient content in the developed porridge was analysed as per the methods described in section 3.2.2, 3.2.3 and 3.2.4.

5.2.6 Porridge preparation

Porridge was prepared in the morning by addition of one part of flour to four parts of water to get 250 ml of porridge for a daily serving. The porridge was then cooled to approximately 60^0C and maintained at this temperature until the end of sensory evaluation. Porridge was self-served by the participant and immediate filling of the questionnaire (Appendix 2) provided with the seven hedonic scales (Amegovu et al., 2014). The freshness of porridge was evaluated by characteristic taste, texture, colour, appearance and general acceptability. Presentation of the porridge was single-blinded, each sample coded with letters, with an interval of mouth rinsing with water between samples (San José et al., 2018).

5.2.7 Sensory evaluation by a semi-trained panel

There was a call for willing adults to sign up for sensory evaluation test. A total of 73 participants agreed to participate in the study and were all allowed to participate. The sample size was based on recommendations on sample size in consumer test and descriptive analysis, which proposes the use of 40 to 100 participants (Maximo et al., 2006). Sensory evaluation participants were adults, who are part of the JKUAT community. Porridge was served between 10 am to 12 pm. The importance of the sensory evaluation was to select a cricket-based porridge to be taken for the child feeding trial.

This was to be the porridge that would compare well with milk-based porridge (MP10) in terms of sensory properties. Milk-based porridge (MP10) was to be used as positive control while maize-millet porridge (MMP) was to be used a negative control.

Porridge was prepared from the five sets of developed flour, MMP, MP10, CP5, CP10, and CP20. Sensory test of the different attributes was rated based on appearance, texture, taste, aroma, mouth-feel and general acceptability (Kinyuru et al., 2009). The questionnaire (Appendix 2) laid out the sensory attributes using seven hedonic scales with the template used by Kinyuru et al. (2009). All the participants were requested to rate this as dislike extremely =1, dislike very much=2, dislike moderately=3, neither like nor dislike=4, like moderately=5, like very much=6, and like extremely=7.

5.2.8 Viscosity test

Porridge from the developed flour was prepared as outlined in section 5.5.6. Each participant was allowed a serving of at least 50ml of the porridge and provided with a questionnaire (Appendix 2) to rate the porridge viscosity. The expected porridge viscosity known by the participant would be rated as 100% while the provided porridge was rated in a percentage depending on its deviation from the expected.

5.2.9 Data analysis

Descriptive statistics were used to present the data, and analysis of variance was used to test differences among means at 5% level of significance for sensory evaluation results.

5.3. Results and discussion

5.3.1 Nutrient composition of the developed porridge flour

Proximate nutrients in the different types of porridges are shown in table 5.2. Porridge served to the children provided 27% proteins from the MP10 type and 26% proteins in the

CP5 type. Porridge being a snack, there was the assumption that children would get more protein at home having more than a quarter of their protein requirement already met. Both MMP, CP5 and MP10 could provide an equal 25% of the daily fat requirement for the children. Since the porridge was for child feeding, energy density (E %) from proteins, carbohydrates, and fats in different porridge types were also calculated. MP10 E% from protein = 11%, Lipids 25% and 27% in CP5. This was within the recommended 10-35% (WHO and UNICEF., 2003).

Table 5. 2: Nutrient values on 65 g serve/day of porridge flour

	Porridge type					
Proximate / Flour type	MMP	MP10	CP5	CP10	CP20	RDI/RNI
Water (g)	6.32	8.45	6.12	5.91	5.50	1100
Energy (Kcal)	251.07	250.63	251.41	251.75	252.42	2000
Protein (g)	4.28	6.74	6.47	7.69	11.11	25
Total lipid (g)	8.75	8.97	9.01	9.67	10.59	35
Available carbohydrate (g)	43.18	40.22	40.75	38.32	33.47	140
Fibre (g)	3.24	2.58	3.34	3.45	3.65	25
Calcium (mg)	40.04	151.83	39.14	38.24	36.44	800
Phosphorus (mg)	119.71	183.19	115.34	110.97	102.23	500
Magnesium (mg)	49.68	41.29	46.99	44.31	38.95	130
Potassium (mg)	167.19	141.08	172.65	178.11	189.03	3800
Sodium (mg)	16.61	13.43	19.49	22.37	28.13	1200
Iron (mg)	5.94	5.19	5.64	5.34	4.74	10
E% Protein (%)	6.81	10.76	12.22	12.22	17.60	10-35
E% Lipids (%)	24.40	25.06	26.89	34.57	37.75	25-40
E% CHO (%)	68.79	64.18	60.89	60.89	53.04	45-65

MMP; millet maize-based porridge, MP10; milked based porridge, CP5; cricket 5% based porridge, CP10; cricket 10% based porridge and CP20; cricket 20% based porridge.
RDI; recommended daily intake (WHO and UNICEF., 2003)
RNI; recommended nutrient intake (4-8 years) (WFP 2016)

5.3.2 Microbial safety in the developed porridge flour

There were no *Escherichia coli*, or *Salmonella typhi* detected in all the flour sampled. Total viable counts, yeast, and moulds varied as shown in table 5.3. The results were based on the Kenya Bureau of Standards (KEBs) acceptable limits. The results showed that the flour was fit for human consumption and a certificate was issued for the flour to be used to for human consumption. Much has been done on yeast and mould spoilage in flour, (Lund et al., 2000), but there has been little on microbial spoilage, of porridge flour with farmed cricket powder The microbial load in the prepared flour was at an acceptable level without coliforms contamination.

Table 5. 3: Bacterial cells content in CFU/g of developed porridge flour

Flour type	MMP	MP10	CP5	CP10	CP20	Maximum Limits*
E coli	ND*	ND*	ND*	ND*	ND*	Nil
Salmonella spp	ND*	ND*	ND*	ND*	ND*	Nil
Yeast/Moulds	$0.5x10^2$	$1.5X10^3$	$1.7x10^2$	$1.2x10^3$	$1.2x10^3$	1.0×10^5
Total viable count	$0.8x10^4$	$1.3x10^3$	$1.4x10^2$	$1.3x10^3$	$0.4x10^3$	1.0×10^5

n=3 ND bacterial cells non-detectable

**Kenya Bureau of standards requirements*

MMP; millet maize-based porridge, MP10; milked based porridge, CP5; cricket 5% based porridge, CP10; cricket 10% based porridge and CP20; cricket 20% based porridge.

Presence of spoilage bacteria especially coliform bacteria in the food renders the food unfit for human consumption (Gibson et al., 2017b). The flour was to be used for sensory analysis and child acceptability tests, and therefore this was an important step to certify microbial safety. This also indicates that the rearing of crickets and handling of purchased ingredients was done with high standards of hygiene since most coliforms and high

bacterial counts in food are caused by poor hygiene (Lee et al., 2017). Past studies had rated cricket products safe for human consumption (Schlüter et al., 2017) and the results of this study concur well with this.

5.3.3 Porridge viscosity

Porridge thickness varied with the cricket flour percentage as observed during porridge preparation (Table 5.4), this was useful to inform the preparation and serving for child acceptability. The MMP porridge was considered of normal viscosity, upon a rating of porridge by participants (Table 5.4), MP10 was slightly lighter, while CP5, CP10, and CP20, porridge thickness increased with increase cricket concentration (Table 5.4). CP20, upon cooling the porridge was too thick to flow, and difficult to drink, this might be the reason why most participants could not finish 100 ml of CP20 porridge.

World Health Organisation recommends spoon feeding in children to avoid dilution of nutrients. Child porridge is usually prepared to a consistency that allows spoon feeding. In this study, the viscosity measure was subject to the participant rating (Kevin, 1995, O'Mahony, 2017).

Table 5. 4: Viscosity of porridge types as rated by participants

Porridge type	Light	Normal	Thick
MMP	8%	82%	10%
MP10	76%	20%	4%
CP5	6%	75%	19%
CP10	2%	60%	38%
CP20	1%	29%	70%

MMP; millet maize-based porridge, MP10; milked based porridge, CP5; cricket 5% based porridge, CP10; cricket 10% based porridge and CP20; cricket 20% based porridge.

Recent studies on porridge show that flour composition plays a major role in viscosity (Syahariza and Yong, 2017). Participants were encouraged to pour the porridge and rate immediately before cooling and thickening, which would discourage them from drinking the porridge since the results showed that the highest cricket percentage in porridge caused the porridge to thicken more. If cricket porridge is used by rural mothers, they should be encouraged to feed the children with a spoon instead of adding too much water to obtain a lighter viscosity that can be drunk from a cup, since this will cause a reduction in nutrients by dilution (Onwurafor et al., 2017).

5.3.4 Sensory attributes

Table 5.5 presents the results of the major sensory attributes of different porridges and the rating based on seven hedonic scales. There were significant differences in appearance P = (0.02), taste P= (0.001), texture P= (0.01) and the general acceptability P = (0.004) among the different types of porridge. The general acceptability of most porridge was moderately liked above a score of 4 while the acceptability of CP20 was disliked (score 2.33), by most participants. The general acceptability of MMP and MP10 porridge was moderate (5.51). CP5 (4.85), and CP10 (4.81). The general acceptability of CP20 was rated worst and was significantly different from other types of porridge (P = (0.004). The rating of sensory, increased with the increase of cricket flour up to 10%, but increase in cricket flour to 20% did not increase the sensory attributes, it made the porridge more disliked.

Table 5. 5: Sensory attributes of different types of porridge

Porridge type	Appearance	Texture	Taste	Aroma	Mouth-feel	General acceptability
MMP	4.79±1.32[a]	2.97±0.92[a]	5.11±1.27[a]	5.59±1.02[a]	5.26±1.21[a]	5.51±1.18[a]
MP10	6.38±0.71[b]	3.63±0.70[a]	4.99±1.41[a]	5.49±1.18[a]	5.33±1.19[a]	5.51±1.30[a]
CP5	5.93±0.88[b]	4.10±0.78[b]	5.38±1.29[a]	5.42±1.14[a]	5.77±1.13[a]	4.85±1.16[a]
CP10	4.71±1.17[a]	4.30±1.03[b]	5.32±1.17[a]	5.44±1.23[a]	5.81±1.07[a]	4.41±1.26[a]
CP20	2.37±1.21[c]	3.10±1.22[a]	3.81±1.43[b]	5.11±1.56[a]	5.18±1.41[a]	2.33±1.43[b]
p-value	0.02	0.001	0.01	0.34	0.44	0.004

Values within each column followed by different superscripts differ significantly (P≤ 0.05)

Values are presented as Mean ± SD, n=73

MMP; millet maize-based porridge, MP10; milked based porridge, CP5; cricket 5% based porridge, CP10; cricket 10% based porridge and CP20; cricket 20% based porridge.

The MP10 porridge was rated equally with CP5 in general acceptability. CP5 was liked very much for the mouthfeel while the taste of CP20 was disliked moderately. Addition of cricket gave the porridge a better mouthfeel since MMP and MP10 porridge is associated with children food and most adults usually like a different porridge, slightly rough textured porridge, which was obtained by addition of cricket powder. Therefore CP10 was liked very much in terms of texture.

The high concentration of cricket flour reduces the viscosity of porridge, changed the texture and mouthfeel hence making the porridge least acceptable. Addition of more water to reduce viscosity reduces the energy and nutrient density of the porridge. Therefore, 5% or 10% cricket powder in porridge is considered enough to attain acceptable porridge viscosity, taste, texture, and mouthfeel.

Past studies have rated cricket based products as highly acceptable (Homann et al., 2017, Van Thielen et al., 2018), and there is need to exploit and develop more insect-based food products to increase animal protein availability and consumption.

5.4 Conclusion and recommendations

Addition of cricket to millet and maize increased the nutritional content of porridge with 5% of cricket providing comparable nutrient content to 10% skimmed milk powder. Addition of 5% and 10% cricket powder to porridge flour had high acceptability and therefore can be used at household levels to improve nutrition.

CHAPTER SIX

ACCEPTABILITY AND EFFECT OF CRICKET BASED PORRIDGE ON GROWTH, HAEMOGLOBIN AND ESSENTIAL FATTY ACID LEVELS OF SCHOOL GOING, CHILDREN

6.1 Introduction

Poor nutrition with a concentration of mainly plant-based diet has been, demonstrated to cause poor growth, increased morbidities and stunting in children living in poor hygiene (Headey et al., 2017, Neumann et al., 2002, Crane et al., 2015, Millward, 2017). Mineral and vitamin deficiency is a public health problem worldwide (WHO, 2018). Iron deficiency can affect cognitive development (Larson et al., 2017), and supplementing anemic and non-anaemic children with iron has been shown to improve their immunity and cognitive development (Ip et al., 2017).

Children with low animal product consumption are likely to have low nutrient diversity which may lead to altered growth and immune function since iron deficiency is associated with reduced cell-mediated immunity (Lind et al., 2017). Children with Vitamin B12 deficiency a vitamin largely derived from animal source food, have a risk of low education outcomes (Duong et al., 2015b).

Malnutrition is not the only challenge in children living in poor resource setup, poor hygiene causing environmental enteric dysfunction (EED) is a major setback too (Millward, 2017). EED is a syndrome that causes reduced absorption in the small intestine and is often the reason why nutrition interventions are not successful (Crane et al., 2015).

Dietary interventions with improved quality diets directly improve nutritional status (Menon et al., 2017). Dietary intervention with iron-fortified biscuits, significantly increased serum retinol, ferritin, and haemoglobin levels in children, and led to the increased cognitive function of children in a rural community (Van Stuijvenberg et al.,

1999). Feeding interventions with milk has been shown to influence linear growth in children (Millward, 2017). Analysis of intervention over time revealed consistent positive effects of child nutritional intervention (Jomaa et al., 2011).

Hunger prevents children from attaining physical, educational, and health benefits, since they gain reduced growth and became restless (Powell et al., 1998). When the Human Immunodeficiency Virus (HIV) affected children were fed with nutrient dense biscuit, they showed improved nonverbal cognitive performance (Khee Loo et al., 2017).

In cultures where insects are not part of the traditional culture, the inclusion of invisible insects in food with known flavours, by grinding the insects into flour, would provide an easy way to consume these insects (Van Huis et al., 2013b, van Huis, 2015). This study was aimed at evaluating safety and consumer acceptability of crickets based porridge which is not common in Kenyan households, the first step was to ensure that cricket flour is safe for human consumption, and thereafter collect the best attributes that would allow use of cricket flour for child feeding, and find the highest percentage of cricket powder in porridge flour that would help boost children protein intake but maintain the taste of acceptable porridge.

This study was a single-blind randomized control trial using maize millet, cricket and skimmed milk powder in porridge to assess their impact on child nutritional status and gut health. We aimed at evaluating the impact of intervention porridge on anthropometric and biochemical measures and the effect of feeding on stool microbial diversity. The information gained is likely to influence future interventions on cricket-based products.

6.2 Materials and methods

6.2.1 Porridge production

The porridge flour was formulated, developed, processed and packed at JKUAT as per the method outlined in section 5.2.3.

During the field intervention, children were served 250ml of porridge. The nutrient content of porridge in child-serving are outlined below (Table 6.1)

Table 6. 1: Nutrient content of the intervention porridge per 100ml

Proximate / Flour type	MMP	MP10	CP5	RDI/RNI
Water (g)	2.528	3.38	2.448	1100
Energy (Kcal)	100.428	100.252	100.564	2000
Protein (g)	1.712	2.696	2.588	25
Total lipid (g)	3.5	3.588	3.604	35
Available carbohydrate (g)	17.272	16.088	16.3	140
Fibre (g)	1.296	1.032	1.336	25
Calcium (mg)	16.016	60.732	15.656	800
Phosphorus (mg)	47.884	73.276	46.136	500
Magnesium (mg)	19.872	16.516	18.796	130
Potassium (mg)	66.876	56.432	69.06	3800
Sodium (mg)	6.644	5.372	7.796	1200
Iron (mg)	2.376	2.076	2.256	10
E% Protein (%)	2.724	4.304	4.888	10-35
E% Lipids (%)	9.76	10.024	10.756	25-40
E% CHO (%)	27.516	25.672	24.356	45-65

RDI; recommended daily intake (WHO and UNICEF., 2003)

RNI; recommended nutrient intake (4-8 years) (WFP 2016)

6.2.2 Study area

The study was conducted at Cheptigit primary school in Uasin Gishu County. Uasin Gishu County borders Elgeyo Marakwet County, Nandi County, Bungoma County, and Tran's Nzoia Country. It lies between longitude 34^0 50'' East and 35^0 37'' West and Latitudes 0^0 03'' South and 0^0 55'' North. It covers an area of 3,345.2KM^2 with a population of 10,446,750 according to the projection of 2009 housing and population census (KNBS, 2010). Among the under-fives, 115,000 (7%) are underweight while 314,000 (19%) have stunting growth; this data clearly demonstrates chronic malnutrition.

Uasin Gishu County was chosen for this study because it is a cosmopolitan County. Its proximity to Western Kenya where insect-eating is common in their diet may have led to exposure to insect-eating which may help reduce disgust. Among the schools in Uasin Gishu County, the most desired school had to be accessible, in a rural setup, with a high student population and therefore since Cheptigit fitted our selection criteria, it was purposively chosen for this study. The student population of the school was high with 200 students in the pre-primary section and therefore it was easy to get all the children under intervention in one school. The population in the surrounding was also diverse and it would be possible to get parent ethnic diversity since some ethnic groups in Kenya are known to have insect-eating habits that have been maintained over time. The diversity would help reduce insect-eating phobia since parents who regularly eat insects would encourage the other parents during recruitment.

6.2.3 Intervention study design

This study was a single-blind randomized controlled dietary intervention in children aged between 3-4.5 years old at baseline. For a child to be included in the study, the parent had to accept by signing the consent form and the child had to be willing to take porridge, follow the study procedure, and therefore assent to the study. Children allergic to any ingredients in the porridge or those whose parents did not consent were excluded from the study. Children not consuming porridge due to allergy or any other reasons were allowed to withdraw from the study.

6.2.4 Participant recruitment

All children in the pre-primary section of Cheptigit primary section were included in this intervention. The sample size was calculated using the formula for a cross-sectional survey to estimate a population parameter. (Charan & Biswas, 2013)

N = $Z1 - \frac{\alpha}{2} p(1-p)$

d^2

Where: n=sample size,

$Z_{1-\alpha/2}$= standard normal variate at 5% type 1 error ($p<0.05$) is 1.96

P= expected proportion in the population in Uasin Gishu underweight = 1%

D= absolute error (0.05)

$$n = \frac{1.96^2 \times 0.03(1-0.03)}{0.05^2}$$

=45

Therefore a minimum of 45 children in each trial arm was expected, and since the trial was 3 arm we expected a minimum total of 135 children.

Inclusion criteria

6.2.4.1 Inclusion criteria

The inclusion criteria shall include

i. Children attending Cheptigit nursery school
ii. Children at the age of 3 – 4.5 years
iii. Weight for Height (WHZ) – ≥-3 Z scores
iv. Haemoglobin (Hb) ≥9 g/dl
v. Mothers who will accept that their children participate in the study and sign the consent form.
vi. Children who are willing to take porridge and follow the study procedure.
vii. Mothers who consent on their children participation will fill in the

questionnaires

6.2.4.2 Exclusion criteriaChildren n belowthe age of 3 years or above the age of 4.5 years.

i. Weight for Height (WHZ) ≤ 3 Z scores
ii. Haemoglobin (Hb) ≤ 9 g/dl
iii. Children allergic to any ingredients in the porridge
iv. Children whose parents do not consent to the study
v. Children with obvious signs of disease

A qualified clinician from Cheptigit dispensary helped monitor the children for any allergic reactions. The guardians and the teachers responsible for taking care of the children were also notified to check out for any allergic reactions and report immediately to the research team who ensured that the child was treated at the dispensary. In the current Kenyan government policy, the children are treated freely at the government facilities, therefore there was no cost associated with treatment at Cheptigit dispensary. At the beginning of the study period, children were dewormed by the local dispensary staff.

6.2.5 Randomization

There were two stages of randomization in this study: Individual randomization of the children to the intervention or the control, and block randomization stratified for sex. To ensure an equal number of boys and girls, two envelopes each containing three differently coloured random numbers were presented to the children one envelope for boys and the other for girls.

To ensure blinding and ease of identification by the children, three different colours and codes were used for each intervention group and all the children would belong to either group one MMP (blue) containing maize and millet without any added protein, which was the negative control, group two (MP10) green containing maize millet and skimmed milk

powder, which was the positive control, or group three (CP5) yellow containing maize, millet and 5% cricket powder, which was the intervention.

Randomization and study procedure allocation was carried out as shown below (Figure 6.1). On recruitment, 159 children fitted the inclusion criteria, though 21 children were excluded before the trial due to reasons highlighted in the randomization chart. Participation in the trial was by volunteering and therefore not all parameters had an equal number of participants.

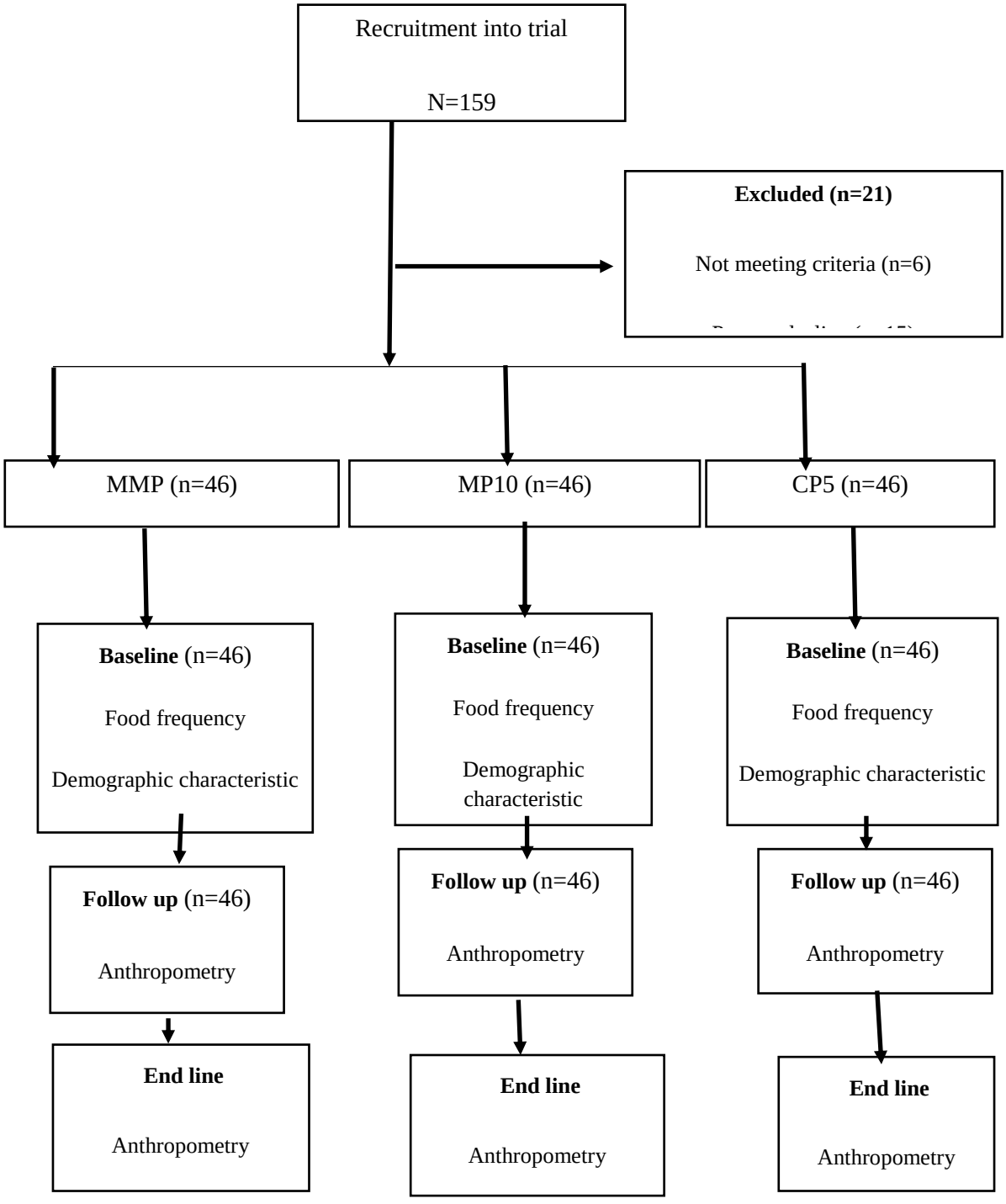

Figure 6. 1: Flowchart of child enrolment, randomization and treatment allocation

6.2.6 Training and quality assurance

The research assistants were trained on porridge preparation, the importance of children adherence to their allocated group, data collection, and entry. A pre-test was done to ensure the research assistants understood the standard operating procedures. The principal investigator and university supervisors, with the help of experts in various study procedures, were involved in research assistant training.

6.2.7 Ethical approval

The study protocol obtained ethical clearance from Mt Kenya University Ethical Review Board (ERC), Ref. No. MKU/ERC/0274: ISRCTN10920322. Study participation was voluntary with verbal consent and agreement by signing up for the study. Participants were informed about the study procedure and the content of different porridge. All participants were informed of cricket and milk in the porridge which may be a source of allergy.

6.2.8 Dietary intervention

All baseline measures were taken before the children were fed. All children were served 250 ml of porridge for 6 months from February 2017 to July 2017. This was done in a school setup and children fed at midmorning break, every 5 days of the week, from Monday to Friday inclusive of school and public holidays.

In addition to the porridge, the children were served with available fruits once a week, to improve their diet diversity. The porridge was prepared and served in the school kitchen by researchers who had been trained on the study procedure and adherence to randomization. Children were given coloured numbered cups, to identify themselves within accordance with their intervention groups.

The researchers prepared the porridge every morning and the porridge was cooled to

approximately 40^0C. The ratio of four parts of water to one part of flour was adhered to, and spoons were provided to the children to facilitate consumption. Any sick child was referred to the local dispensary, which was 100 meters away from the school. Children who missed school for other reasons but came for their porridge ratio were allowed to take their share.

6.2.9 Consumer acceptability by children

Children were served porridge as outlined in section 6.2.7. Acceptability was rated by the amount of porridge consumed over the study period. The research assistant responsible for the group measured all the unfinished porridge and recorded in a questionnaire (Appendix 2). The porridge consumed was ranked >75% highly acceptable, 50-75% moderately acceptable, < 50% not acceptable (Konyole, 2014).

6.2.10 Anthropometric measurements

Child anthropometry data was collected on a monthly basis, from baseline to end line, using WHO standard procedure (WHO 2006). The weight was measured on Seca scale with an accuracy of 100 grams, the length was measured using a marked platform with a sliding footboard (Seca), head circumference was measured using a flexible fabric tape measure, while mid-upper arm circumference (MUAC), was measured using a MUAC tape. Anthropometric indices weight for age (WAZ), height for age (HAZ), and BMI for age Z score (BAZ), head circumference Z score (HCZ) and MUAC Z scores (MUACZ), were obtained using WHO Anthro software (WHO, 2010a).

6.2.11 Haemoglobin and essential fatty acids analysis

Haemoglobin and fatty acids were analysed from blood collected from a finger prick at baseline and end line. The tip of the third finger was cleaned with 70% ethanol and wiped with dry cotton wool before pricking. Finger prick needles used were child-friendly. Blood for haemoglobin test was collected following the World Health Organization standard

operating procedures (WHO, 2010b). Rapid analysis of haemoglobin was done using HemoCue machine (Von Schenck et al., 1986). A blood drop was spotted on a dry spot paper and dried for fatty acid analysis. Blood was spotted on dry paper containing butylated hydroxytoluene. Fatty acids were then extracted with acetyl chloride in methanol to form methyl esters of fatty acid which were measured using gas chromatography (Kandar R, et al., 2016, Misaiko K, & Seiji Y 2002).

6.2.12 Food intake

Food intake was assessed using Food frequency questionnaires (Appendix 2) among the study participants. The parent of the participant was asked the number of times they ate a certain food in the family within a period of one week. All food and beverage consumed were recorded, and grouped into different categories, as either; plant protein, animal protein, cereals, or fruits for ease of analysis.

6.2.13 Morbidity

Morbidity data were collected by asking parents about any sickness and visits to the health facility within the last five days (Appendix 2). Parents were encouraged to visit the dispensary whenever their children were sick to seek treatment. Morbidity was scored as healthy, self-limiting illness, moderate or severe illness.

6.2.14 Data analysis

Both descriptive and inferential statistics were used to analyse data. All data collected was entered into excel sheets. Data were cleaned and analysed using One Ways and two-way Analysis of Variance and post hoc Tukey HSD β- the value of 0.01 and alpha value of 5% was used and statistically, significant difference accepted $P < 0.05$ for anthropometric and biochemical measures.

6.3 Results and discussion

6.3.1 Demographic characteristics at baseline

A total of 159 children were recruited to the study. On initial screening, 21 children were excluded; 6 were above 4.5 years at baseline, while 15 children were excluded because parents withdrew their consent after recruitment and consenting. The remaining 138 children were randomized into the three different groups with each intervention arm having 46 children. Demographic characteristics of the children are presented below (Table 6.2). Most of the children in the study were taken care of by their biological parent, or by their older siblings. More than 90% of the children in CP5, 87% in MMP and 80% in MP10, were delivered in a health care facility and therefore had a clinic card or a birth certificate and only 9% in CP5, 24% in MMP and 20% in MP10 were delivered with the help of a traditional birth attendant and their date of birth could only be relied upon from caretakers recall.

In Kenya more than 30% of mothers lack skilled birth attendant during delivery (Naanyu et al., 2018, Cheptum et al., 2017), home deliveries may be contributed by lack of knowledge, poor infrastructure or lack of transport fee since delivery in government facilities if free in Kenya (Dennis et al., 2018).

Majority of the parents had primary school education 70% in MMP, 54% in MP10 and 65% in CP5. Maternal education has been shown to have an impact on child nutrition, mothers who have post-primary education and beyond are likely to feed their children better with higher nutrient diversity (Alderman and Headey, 2017). None of the mothers in the study population had attained university education in this community, a few of the mothers had gone to post-primary and college but the majority were primary school certificate holders. Maternal education is a contributor to poor feeding and less diversity in child diet which contributes to malnutrition (Debela et al., 2017).

Table 6. 2: Demographic characteristic of intervention participants at baseline

		Intervention group		
Characteristic		**MMP**	**MP10**	**CP5**
Sex	Male %	61	65	61
	Female %	39	35	39
Relationship with caretaker	Parent %	78	59	77
	Step parent %	7	15	7
	Grandparent %	4	7	4
	Sibling %	11	20	12
Source of birth information	Clinical card %	70	63	80
	Birth certificate %	7	17	11
	Care takers recall %	24	20	9
Caretakers level of education	Didn't attend formal school	9	20	7
	Primary level %	70	54	65
	Secondary level %	22	22	20
	College %	0	4	9
Source of drinking water	Piped %	4	2	4
	Borehole %	61	57	76
	River %	35	37	20
	Dam %	0	4	0
Source of income	Formal employment %	13	9	9
	Non formal employment %	37	59	54
	Farming %	50	33	37

n=46.

A portion of children caretakers in this community had never attended formal schooling. This is a major hindrance to knowledge sharing, despite the information provided by the government health institutions for mothers during antenatal care when the mother is expectant or sick. There is a need for continuous nutrition education to these mothers at the community because most of them, with little education, are not able to read

supplementary materials provided in health care facility charts and books. Less educated mothers have also been shown to be poor in decision making in regards to their household and often depends on the household heads normally the husband to make decisions including food choices (Khan et al., 2017, Ickes et al., 2018).

Most households used boreholes water in their homes for cooking and drinking, while others got their cooking and drinking water from the river, < 4% had access to clean piped water. Access to clean water is still a problem in Kenyan rural population and has been closely associated with diarrhoea in children (Cheptum et al., 2017). Use of clean water is important in child hygiene to avoid loss of nutrient through diarrhoea (Dearden et al., 2017, van der Meij et al., 2017). Poor hygiene is also known to predispose children to EED, which is a pathway from poor hygiene to malnutrition (Keusch et al., 2013). Over the years, stunting could not be explained by poor diet, diarrhoea or reversed by optimized diet. Stunting has been partly because of villous atrophy leading to the reduced absorptive service area, and leaky gut caused by pathogenic microbes (Mbuya and Humphrey, 2016). Children living in unhygienic conditions are at risk of EED, because of constant exposure to pathogenic microbes in the environment and interventions aimed at improving EED are likely to improve stunting (Mbuya and Humphrey, 2016).

The community where the study school was situated was from low economic setup, the main source of income was farming and non-formal employment, characterized by casual irregular poor defined jobs, which has been shown as an indicator of poor nutrition because caretakers are unable to constantly buy sufficient food (Kamau et al., 2018). From observation, most parents engaged in illicit alcohol which has been shown to contribute to child neglect, poor feeding, and malnutrition in children (Ssewanyana et al., 2018).

6.3.2 Study participants

There were 28 males (61%) and 18 females (39%) in MMP group, 30 males (65%) and 16 females (35%) in MP 10 group and 28 males (61%) and females 18 (39%) in CP5

group (Figure 6.2).

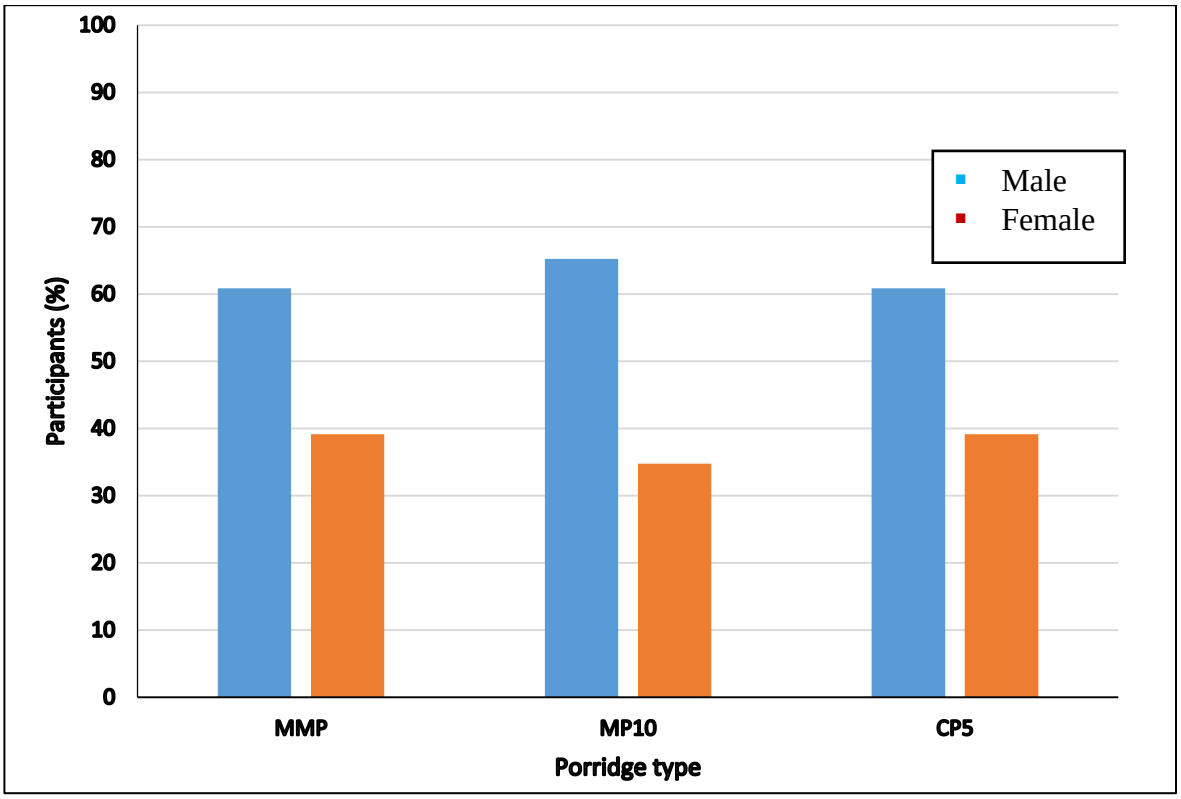

Figure 6. 2: Distribution of children in the study

6.3.3 Consumer acceptability by children

During the intervention, porridge acceptability by children was also accessed. Best attributes and comparison of nutrients in MP10 porridge was a basis for determining the cricket-based porridge to be chosen between CP5, CP10, and CP20. CP5 was found to compare well with MP10 in nutrient composition and sensory evaluation.

Acceptability of porridge was rated differently depending on the type. At the start of acceptability trial, MMP porridge which was similar to what the children were used to in school and at home had the highest acceptability (Figure 6.3).

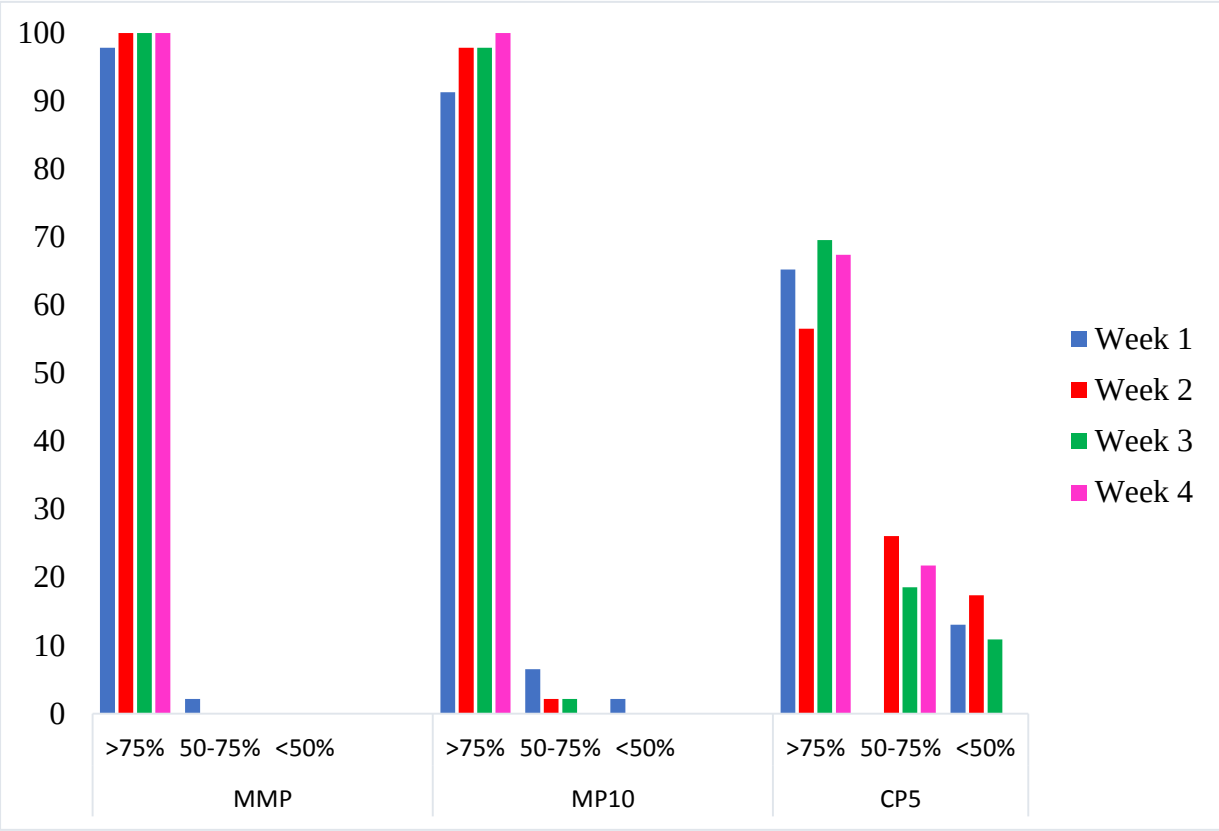

Figure 6. 3: Porridge acceptability among children

The intake of the different types of porridge increased significantly during the first four weeks of acceptability test with MMP and MP10 recording the highest rate of acceptance > 75%. Most children were comfortable with MMP porridge since this is what they would normally drink at home, and none of the children showed < 50% acceptability of this porridge. MP10 porridge had higher intake as compared to CP5 porridge during the first four weeks of the study period. The children liked MMP and MP10 porridge by week three, while it took longer for all the children to like the CP5 porridge. After four weeks all the children had completely liked all the three porridge and the rating was >50%.

Addition of cricket powder to porridge can provide a cheap protein source with the porridge comparing well with milk porridge. The current market price for crickets in

Kenya is KES 1000 per Kg of ground cricket flour, while 1 kg of skimmed milk powder costs KES 1500 (Price sourced from local shops and JIF). Milk powder contains 26.3 g/100g proteins and using milk powder in child complementary food doubles the price of the product (Lukmanji et al., 2008), while protein content in cricket is 60 g/100g and crickets are easily reared, with short maturity period (Kipkoech et al., 2017, Halloran et al., 2017), there is need for a study in cost analysis of cricket production.

6.3.4 Baseline anthropometric indices

At baseline Z scores measured nutritional status in children; most children >50% were normal with Z score between ≥-2 SD + 2≤ (WHO, 2006). Stunting which is an indicator of chronic malnutrition was not evident with children having the height for age Z score (HAZ) of between -0.27 to -0.1 HAZ a range within the WHO recommendation for normal children. Although stunting and wasting were rated high in Uasin Gishu County with under-five being 7% underweight while 19% showing stunted growth (KNBS, 2010), this was not evident in the study population.

Table 6. 3: Anthropometric Indices at baseline

	Intervention group		
Indices	**MMP**	**MP10**	**CP5**
Age(M)	38.34 [a]	39.09 [a]	37.69 [a]
Weight(Kg)	14.67 [a]	15.35 [a]	14.97 [a]
Height(cm)	102.73 [a]	105. 85 [a]	105.67 [a]
HAZ	-0.27 [a]	-0. 1 [a]	-0.13 [a]
WAZ	-1 [a]	-1 [a]	-1.13 [a]
BAZ	-1.58[a]	-1.4 [a]	-1.26 [a]
HC(cm)	48.4 [a] 5	48.76 [a]	48.73 [a]
MUAC (Cm)	13.97 [a]	13.32 [a]	13.85 [a]
HCZ	-0.76 [a]	-0.66 [a]	-0.36 [a]
MUACZ	-1.66 [a]	-1.87 [a]	-1.31 [a]

n=46. Values within the same row with different superscripts are significantly different $p < 0.05$

There was no significant difference in nutritional baseline characteristics in all arms of the study ($P>0.05$). Children were within the normal nutritional ranges as per WHO recommendations of either normal or moderately malnourished. Poor nutrition is seen as a common phenomenon in poor set up where there is no sufficient food and little knowledge on the importance of exclusive breastfeeding to the mothers (Vanderlinden and Van de Putte, 2017), in this setup, the children were likely well fed.

6.3.5 Anthropometric measurements during follow-up

There was a change in height for age Z scores (HAZ) across the three interventions arms, over the six months that was significant ($P = 0.05$) (Table 6.4).

The change in weight for age Z score (WAZ) was significant from baseline to end line ($P = 0.02$). Though the difference within the interventions was not significantly different ($P = 0.68$). BMI for age Z score (BAZ) increased but was not significantly different across all the three intervention over time $P = 0.08$, from baseline to end line.

There was an increase in Z score from baseline to end line in all intervention arms, though the increase was not equal in all the anthropometry measures. There was a significant increase in HAZ, WAZ, and MUACZ ($P < 0.05$) but BAZ and HCZ increase was not significantly different across the months ($P > 0.05$). Past studies have shown that different anthropometry measure responds differently to different nutrition interventions and some indicators such as height need longer periods of interventions for noticeable change to occur (Scharf et al., 2018, Nguyen et al., 2017).

Increase in child weight would have been a contribution to a healthy diet with sufficient minerals and fruits weekly from the intervention. The children were given energy dense porridge, containing proteins, vitamins, mineral with fresh fruit once a week. This was because even the children who could not get any other meal at home were able to get at

least one dense meal in school in the form of porridge. Diets containing essential nutrients help improve physical activity (Borde et al., 2017). Parents reported that their children had developed a smooth skin, were cleaner, healthier and without common irritating mucus, this would have been an attribute of sufficient minerals and proteins in the diet (Krutmann et al. 2017).

Minerals and proteins are important in skin repair, skin being one of the most commonly replaced body parts, it's also exposed to harsh environmental conditions and constantly requires repair (Krutmann et al., 2017, Liakou et al., 2017, Pearson, 2018). Improved health may have been due to the healthy diet which is essential in building better nutrition for the children (Freeman et al., 2017). Inclusion of minerals, fatty acid and vitamins have been indicated for improved nutrition in children (Pai, Chandrasekhar, Carvalho, & Kumar, 2017) (Baye, 2017). Past studies have shown nutrition interventions to have the ability to boost child health and reduce morbidities (Calder et al., 2006; Chandra, 1992; Friedman et al., 2005; Van Ginneken & Muller, 1984).

Table 6. 4: Baseline, follow up and end line anthropometric indices

Variable	Intervention	Follow up months					
		Baseline	**1**	**2**	**3**	**4**	**End line**
HAZ	MMP	-1.3±1.4 [a]	-0.94±1.3 [a]	-0.42±1.5 [a]	-0.19±1.4 [b]	0.67±1.4 [c]	1.03 ± 1.3 [d]
	MP10	-1.4±1.2 [a]	-1.15±1.2 [a]	-0.58±1.5 [a]	-0.25±1.4 [b]	0.03±1.2 [c]	0.65±1.4 [d]
	CP5	-1.7±1.2 [a]	-1.31±1.0 [a]	-0.87±1.0 [a]	-0.47±1.1 [b]	0.25±1.1[c]	1.07±161 [d]
WAZ	MMP	-1.0±1.0 [a]	-0.80±1.0 [a]	-0.48±1.1[b]	-0.30±1.0[c]	0.07±0.975 [c]	0.39±0.82 [d]
	MP10	-1.0±1.1 [a]	-0.91±1.1 [a]	-0.57±1.0[b]	-0.35±1.0[c]	-0.13±1.1 [c]	0.35±1.07 [d]
	CP5	-1.0±1.1 [a]	-1.00±1.11 [a]	-0.72±1.29[b]	-0.50±1.34[c]	-0.04±1.15 [c]	0.41±1.22 [d]
BAZ	MMP	-0.7±0.8 [a]	-0.44±.80 [a]	-0.33±.86 [a]	-0.36±0.89 [a]	-0.33±0.926 [c]	-0.28±0.88 [a]
	MP10	-0.8±1.0 [a]	-0.59±1.04 [a]	-0.45±0.9 [a]	-0.44±0.98 [a]	-0.39±0.95 [c]	-0.29±1.0 [a]
	CP5	-0.6±1.1 [a]	-0.34±1.03 [a]	-0.23±1.11 [a]	-0.20±1.12 [a]	-0.14±1.00 [c]	0.00±1.10 [a]
HCZ	MMP	-0.7±0.8 [a]	-0.44±.80 [a]	-0.33±.86 [a]	-0.36±0.89 [a]	-0.33±0.926 [a]	-0.28±0.88 [a]
	MP10	-0.8±1.0 [a]	-0.59±1.04 [a]	-0.45±0.9 [a]	-0.44±0.98 [a]	-0.39±0.95 [a]	-0.29±1.00 [a]
	CP5	-0.6±1.1 [a]	-0.34±1.03 [a]	-0.23±1.11 [a]	-0.20±1.12 [a]	-0.14±1.00 [a]	0.00±1.10 [a]
MUACZ	MMP	-2.0±1.1 [a]	-1.69±1.14 [a]	-1.33±1.14 [b]	-0.86±0.89 [c]	-0.81±0.889 [c]	-0.81±0.98 [c]
	MP10	-2.2±.89 [a]	-1.94±1.01 [a]	-1.39±0.93 [b]	-1.19±0.85 [c]	-0.97±0.75 [c]	-0.97±0.75 [c]
	CP5	-1.7±1.2 [a]	-1.38±1.07 [a]	-0.87±0.71 [b]	-0.57±.6.79c [c]	-0.39±0.73 [c]	-0.30±0.72 [c]

n=46. Values within the same row with different superscripts are significantly different $p < 0.05$

Abbreviation: MMP; Maize millet porridge, MP10; milk-based porridge, CP5; cricket based porridge

HAZ; Height for age Z score, WAZ; Weight for age Z score, BAZ; BMI for age Z score, HCZ; Head circumference for age Z score, and MUACZ; MUAC for age Z score.

6.3.6 Haemoglobin and essential fatty acids

Haemoglobin levels increased from 11.93 g/dl to 13.37 g/dl (P=0.01) in MMP, 10.63 g/dl to 13.18 g/dl (P=0.01) in MP10, and 10.05 g/dl to 13.59 g/dl (P=0.02) in CP5. The difference was not significant within the three interventions arms at baseline P = 0.68 and at end line P = 0.99.

Table 6. 5: Haemoglobin and fatty acid levels in different interventions at baseline and endline

	Interventions		
Variables	**MMP**	**MP10**	**CP5**
Baseline Hb g/dl	11.93 [a]	10.63 [a]	11.05 [a]
End line Hb g/dl	13.37 [b]	13.18 [b]	13.59 [b]
Baseline PUFAs	25.37 [a]	24.86 [a]	26.73 [a]
End line PUFAs	24.75 [a]	27.06 [b]	27.36 [b]
Baseline HUFAs	10.00 [a]	9.86 [a]	10.16 [a]
End line HUFAs	8.68 [a]	10.24 [a]	10.11 [a]
Baseline EPA+DHA	1.11 [a]	1.00 [a]	1.16 [a]
End line EPA+DHA	0.88 [a]	1.34 [a]	1.11 [b]
Baseline n-6/n-3	12.94 [a]	12.64 [a]	12.97 [a]
End line n -6/n-3	11.90 [a]	15.07 [a]	14.28 [a]

n=33. Values within the same row with different superscripts are significantly different $p < 0.05$

Abbreviation: MMP; Maize millet porridge, MP10; milk-based porridge, CP5; cricket based porridge

Past studies have shown positive improvement of haemoglobin in children fed on a nutrient dense diet (Konyole, 2014). Children in developing world have had low Hb levels majorly contributed by cereal-based diets with fewer minerals and fewer animal proteins which are a good source of iron (Harika et al., 2017), due to poor diet diversity and

insufficient nutrients in diets. Iron supplementation has been indicated to improves Hb levels in children, improve growth and reduce mortality (Clemens et al., 2011, Mosites et al., 2017).

Presence of mineral in the porridge caused improvement in haemoglobin levels from baseline to end line in all the interventions groups. Standard premix that was present in all the porridge contained iron that has been shown to increase haemoglobin levels in interventions (Le Port et al., 2017; Mei et al., 2005). When baseline and end line fatty acid analysis in blood was done, there was a significant increase in polyunsaturated fatty acids (PUFAs) in MP10 and CP5 from baseline to end line though there was a decrease in MMP that was not significantly different ($p < 0.05$). When the n6/n3 ratio was determined, there was a significant increase in MP10 and CP5 groups ($P < 0.05$). Past studies on cricket nutritional profiles have shown that cricket fat contains low n6/n3 ratio (Kipkoech et al., 2017). There was no significant increase in the n6/n3 ration in the MMP group ($P > 0.05$), this ratio has been shown to be important in determining good fat in child nutrition (Barbara, 2013).

In the study area, a lower percentage of the population had access to fish and meat products in the diet, the larger population were dependent on cereals and plant proteins, which has been shown to be low in docosahexaenoic acid and other important fatty acid fragments (Michaelsen et al., 2011). These are the fatty acid fractions that have been shown to be important in child growth and cognitive development, (MacKinnon et al., 2017, Parks et al., 2017). Cricket has been shown to contain EPA and DHA (Kipkoech et al., 2017) and therefore intake of cricket would be important in the provision of these fatty acid fragments if used as a complementary food additive.

6.3.7 Food intake

Cereals were the most common foods in this community since all the children ate cereals during the week. Half of the children reported having taken any type of fruit in the last

week in the food frequency questionnaire. More than 50% of the children reported to have eaten plant protein while less than half of the children reported having eaten animal protein, milk was the popular source of animal protein in the community, while fish was most rare (Table 6.3).

Table 6. 6: Food intake in the study community

		Intervention group		
Food	**Intake**	**MMP**	**MP10**	**CP5**
Fruits within the last one week	Yes	43	50	52
	No	57	50	48
Starch within the last one week	Yes	100	100	100
	No	0	0	0
Animal Protein within the last one week	Yes	35	48	39
	No	65	52	61
Plant Protein within the last one week	Yes	65	52	76
	No	35	48	24

n=46.

Abbreviation: MMP; Maize millet porridge, MP10; milk-based porridge, CP5; cricket based porridge

This population clearly showed that they depend mostly on a plant-based diet. Other studies have shown that a plant-based diet is most commonly consumed (Tontisirin et al., 2002, Murphy and Allen, 2003, Konyole, 2014) in Kenya and other parts of the world. Plant-based diet has been shown to be low in fatty acids, minerals and lack important amino acids which are available in animal-sourced diet, and children from communities who depend on plant-based diet are likely to be malnourished due to lack of the essential nutrients found in animal source diets (Neumann et al., 2002, Millward, 1999, Gregory et al., 2017). Studies have also shown that children brought up by parents rearing dairy cows to have a better nutrient diversity score and better nutritional outcomes (Konyole, 2014), which was common in this community since most children consumed milk.

Children living in communities who dependent majorly on plant-based diets, need nutrient supplementations prompting the World Health Organisation and individual governments to ensure fortification of food to provide the most important minerals and vitamins lacking in cereal-based diets, (Allen et al., 2006, Garrett and Bailey, 2018, Manjeru et al., 2017).

A good example is the cereal fortification with vitamin A in Kenya. Vitamins have been shown to be important in carbohydrate utilization and lack of minerals with vitamins has been shown to cause poor utilization of carbohydrates in diets and largely contributes to hidden hunger (Nabwera et al., 2017) (Phillips Jr, 1969, Huskisson et al., 2007, Low et al., 2017, Luoma et al., 2010).

6.8 Morbidity

During the study period, children were all reported to be well with no major disease, expect to cough, wheezing and running nose, the average body temperature, and respiratory rates were within the normal range as shown in (Table 6.6).

Table 6. 7: Common indications of illness in children during the study period

		Intervention period					
Illness indication	**Intervention group**	**Baseline Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Coughing (%)	MMP	10	11	9	4	3	5
	MP10	12	7	6	4	4	4
	CP5	11	8	8	3	5	2
Wheezing (%)	MMP	14	15	9	10	5	6
	MP10	12	12	10	6	4	3
	CP5	12	11	6	5	4	3
Running nose (%)	MMP	34	27	10	8	8	5
	MP10	38	24	13	7	8	4
	CP5	30	29	12	6	6	5
Watery diarrhea%	MMP	10	4	3	2	1	1
	MP10	7	3	0	2	0	0
	CP5	6	2	1	0	0	0

Means were reported as absolute means in % since they were not subjected to any statistical analysis.

Episodes of loose watery diarrhoea in the 4^{th} to 6^{th} month of the study were greatly reduced across the study groups. Giving children nutrient-rich food has been shown to help improve health in children and would be the reason the children appeared healthy with reduced episodes of diarrhoea during the study period.

6.4 Conclusion and recommendations

Cricket porridge was highly acceptable by children. Use of cricket in child feeding helps improve child health and increase linear growth. The importance of cricket in the diet was equal to that of milk and therefore crickets can supplement milk as a protein, mineral and fatty acid source in child porridge. Feeding children with cricket as a protein source can help improve child nutrition in Kenya, therefore there is a need to explore ways to include farmed crickets in child meals or snacks.

CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In Kenya, most farming practices, especially by small scale farmers, is done for consumption with occasional sale of surplus. Cricket farming in Kenya is still under-utilized with very few farmers taking up the technology due to lack of cricket rearing knowledge, technical know-how and few cricket eggs available in the institutions doing cricket farming not able to supply all the farmers willing to try cricket farming. Though JKUAT has done cricket farmers training, there is a need to expand this training to reach wider areas in Kenya.

There are communities in Kenya who still detest cricket consumption and it's a hindrance to cricket protein consumption rollout in Kenya. Nutritional profiling of Kenyan farmed crickets have shown that locally reared crickets are rich in proteins, fatty acids, and minerals that compare well with meat and have even better values of nutrients such as EPA and DHA. There is need therefore for sensitization and processing of crickets into powder to be used as a food additive to derive the nutrients at the same time reduce insect-eating phobia.

Cricket chitin has been shown to be a prebiotic candidate and can be used to improve gut health. There is a need to exploit these desirable impact for improved gut health and cricket chitin can be used as a stand-alone prebiotic or in combination with a probiotic. There is a need to carry out extensive research on the structure and active sites of cricket chitin and exploit its potential as a pharmaceutical product.

Cricket porridge was highly acceptable by children and adults and there is a need to develop other products with cricket powder to improve animal protein uptake by all Kenyans. There is a need to replace the high cereal-based diet which is currently exposing

the population to malnutrition and obesity, with an affordable locally produced cricket protein source. This can be achieved by the addition of cricket powder to commonly consumed cereal products such as porridge, cakes, and bread.

Nutrition intervention using cricket powder has shown that cricket compared well to the traditionally used milk powder. Protein content in cricket is higher than the protein content in milk, there is need therefore to invest is cricket as a workable solution to improve child health.

7.2 Recommendations

From the study findings, the following recommendations were made from the study results:

1. Support insect food policy. In food industries, insects are considered as contaminants, and there is a need to review policy to include insects as food. There is a need for farmer training and empowerment to rare crickets.
2. Encourage insect consumption. There is a need for mothers to be empowered through education on the use of crickets to improve child nutrition and health.
3. There is a need for more research on the role of myeloperoxidase and neopterin enzyme in child immunity and its relationship to nutrition.
4. There is a need to developed chitin as a prebiotic for commercial use and to test chitin as stand-alone prebiotic and in combination with a probiotic to determine its effect on gut health.
5. Food and nutrition stakeholders should team up to commercialize cricket rearing, develop and commercialize cricket products to reach a wider population.
6. There is a need to make available cricket powder to be used as an additive in homemade meals

7.3 Areas requiring further research

1. Explore cricket chitin as a pharmaceutical product
2. Explore the metabolites responsible for pathogenic bacteria inhibition
3. Determine specific species responsible for the gut microbial shift
4. Evaluate optimum prebiotic/probiotic ration in cricket products

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APPENDICES

Appendix 1: Informed Consent forms

Parent informed consent

Your child is invited to participate in a research study on **the** use of farmed edible crickets (*Acheta domesticus*) to improve child nutrition in Kenya in Cheptigit primary school located in Uasin Gishu County, Kenya. We ask that you read this form and ask any questions you may have before agreeing that your child is in the study. The study is being conducted by Carolyne Kipkoech for academic purpose to address malnutrition in Kenya.

Study Purpose: The purpose of this study is to determine the impact of porridge on nutritional status and gut health of school going children. Knowledge gained from the study will help in future policy formulation in regards to food formulation, edible insect use and farming in Uasin Gishu and the entire country.

Study Procedures: If you agree that your child is in the study, your child will be one of 135 children in Cheptigit primary who will be participating in this research. Your child will be given porridge, every weekday for six months, we will ask you and your child questions and you and your child will give us feedback to the best of your knowledge, we will also require blood and stool from your child.

Questionnaires

At the beginning, and at the end of the study will ask questions, you will be asked questions about the family situation, your child's health and development, breastfeeding pattern and your child dietary intake. You do not have to answer any questions that you do not want to answer.

Clinical examination

In the beginning, and the end of the study, the child will be examined by a health professional, where temperature and blood pressure will be measured. The health professional will look for signs of symptoms of diseases or malnutrition. If your child is severely anaemic or malnourished, you and your child will be referred for treatment.

Body size

At the beginning and every month until the end of the study, weight, height, mid-upper arm circumference, head circumference and thickness of fat at several places on the body will be measured.

Blood samples

In the beginning, and at the end of the study, we will take about a drop of blood from your child's finger. We will test your child's blood for anaemia, and some blood will be put in a dry paper for fatty acid analysis. The blood will not be used for anything else.

Stool collection

We will request your child to give us a stool at the beginning and at the end of the study.

Your participation is entirely your choice. Whether you choose to participate or not, it will not affect the other services you and your family receive from, school, health centers or other government authorities. Although we hope you will continue with the study for 6 months, you can stop participating in the study at any time during the study.

Confidentiality: You will be asked to keep all information confidential; Efforts will be made to keep your personal information confidential.

Costs: The study will not cost you any finances, all we will need is you and your child's time.

Benefits: If your child is part of this study, he/she will benefit from the nutrients from the porridge for six months, which will improve the nutritional status, cognitive function and gut health of your child.

Risks: There are no adverse risks in the study, the children will feel a little pain during blood draws, the children might also develop an allergy to any ingredient in the porridge, and if this happens the child will be stopped from taking the porridge and taken for treatment at Cheptigit dispensary. Also, children will be given tissue and stool collection bag and taught on proper stool collection methods.

Voluntary nature of the study: Taking part in this study is voluntary. You may choose not to take part or to leave the study at any time. Leaving the study will not result in any penalty and will not affect your relations with the health care facilities, school or any other person in the community.

Contact: For questions about the study, contact Carolyne Kipkoech on phone number 0721-481324 or E-mail address: Kipkoechcarolyne@gmail.com. If you feel your right are infringed by this study kindly contact Dr. John Kinyuru on phone number 0723 66732 Email address: jkinyuru@agr.jkuat.ac.ke. In case of any concerning this study kindly forward to The Chairman, Mount Kenya Ethical Review Committee P.O Box 342-01000. Thika: Tel. 0672820000: Email: research@mku.ac.ke

Subject's Consent:

In consideration of all of the above, I give my child's consent to participate in this research study. I will be given a copy of this informed consent document to keep for my records. I agree that my child takes part in this study.

Subject's Code: _____

Subject's Signature: _____ Date: _____

(Must be dated by the subject)

Name of Person Obtaining Consent: _____

Signature of Person Obtaining Consent: _____Date:_____

Child Assent

I have understood the study procedure and I am willing to participate

Child code: _____Date_____

Informed consent: Swahili Version

RIDHAA TAARIFA KATIKA UTATHMINI

Umekaribishwa kujumuika nasi katika utafiti ya matumizi ya Kricket inayofukwa (*Acheta domesticus*) kuimarishe lishe bora nchini Kenya. Tunakuagiza usome fomu hii vizuri, ukiwa na maswali uulize kabla ya kukubali kujumuika katika utafiti huu. Utafiti huu unaendeshwa na Carolyne Kipkoech, mwanafunzi wa cheti cha shahada kwa chakula na lishe bora katika chuo kikuu cha kilimo na technologia cha Jomo Kenyatta.

Kusudi la utafiti: Kusudi kuu la utafiti huu ni kutumia uji kuimarisha lishe kwa watoto wa shule ya msingi ya Cheptigit. Majibu ya uthafithi huu utatumika kuimarisha lishe kwa watoto wa Uasin Gishu na chi ya Kenya.

Taratibu ya utafiti: Kama unakubali kwamba mtoto wako awe katika utafiti, mtoto wako atakuwa mmoja wa watoto 135 katika shule ya msingi ya Cheptigit ambao watashiriki katika utafiti huu. Mtoto wako atapewa uji, kila siku ya wiki kwa miezi sita, tutakuuliza wewe na mtoto wako maswali, mtatupa majibu kwa kadri ya ufahamu wenu, sisi pia tutahitaji damu na choo kutoka mtoto wako.

Maswali: Mwanzoni, na mwishoni wa utafiti tutakuuliza maswali, kuhusu hali ya familia, afya ya mtoto wako na maendeleo. Sio lazima kujibu maswali yeyote ambayo wewe hutaki kujibu. Mwanzoni, na mwisho wa uthafiti huu, mtoto atachunguzwa na wataalam wa afya, ambapo joto na shinikizo la damu itapimwa. Mtaalamu wa afya itaangalia dalili za magonjwa au utapiamlo. Kama mtoto wako ako na damu kiwango cha chini au utapiamlo, wewe na mtoto wako mtaarifiwa kwa ajili ya matibabu.

Vipimo vya mwili: Mwanzoni, na mwishoni wa utafiti, uzito, urefu, duara ya mkono wa juu, duara ya kichwa na unene wa mafuta katika maeneo kadhaa juu ya mwili itapimwa.

Sampuli za damu na choo: Mwanzoni, na mwishoni wa utafiti, tutachukua, choo kidogo

kutoka kwa mtoto wako, na tone la damu kutoka kidole ya mtoto wako. Tutapima upungufu wa damu, na baadhi ya damu itawekwa katika karatasi kavu kwa ajili ya uchambuzi wa fatty acid. Damu haiwezi kutumika kwa kitu kingine chochote.

Umuhimu ya utafiti: Matokeo ya utafiti huu utatumuka kubadilisha sera ya lishe Uasin Gishu na chi ya Kenya.

Usiri: Utaulizwa uweke taarifa yote uliyonayo kwa usiri, Jina lako halitatumika katika nakala na pia jina lako halitatumika katika ripoti zozote za utafiti. Taasisi ya Utafiti na Maadili itaweza kukagua na kukiri kuwa hakuna jina lako au la mwenzako litakalotumika katika ripoti yoyote.

Gharama: Utafiti huu hautakugharimu kitu chochote. Mtoto wako atasikia uchungu kidogo damu itakapotolewa. Anawesa pia kupata shida kama ya kujikuna utokana na uji, kama jambo ili litafanyika, mtoto atapelekwa kwa sahanati ya Cheptigit, pia hatarudia kunywa uji tena.

Hiari asili ya utafiti: Kuchukua hatua ya kujumuika katika utafiti huu ni kwa kujitolea. Unaweza kuamua kuhusika katika utafiti huu ama kuacha wakati wowote. Kukosa kuhusika katika utafiti huu wakati wowote hautahusisha fidia yoyote na pia haitaharibu uhusiano wowote baina yako na shule.

Ukiwa na maswali yetote: Kwa maswali kuhusu utafiti huu, wasiliana na Carolyne Kipkoech, 0721481324, au Daktari John Kinyuru wa chuo kikuu cha kilimo na technologia cha Jomo Kenyatta jkinyuru@agr.jkuat.ac.ke. Unawaeza pia kuwasiliana na Taasisi ya chuo kikuu cha mlima Kenya, kitengo cha Utafiti na Maadili orofa ya pili pale Chuo Kikuu kama una maswali kuhusu haki yako kama mhusika katika huu utafiti. Unawesa pia kutuma malaamishi yako kwa Mwenye kiti Taasisi ya uthafiti sandulu la posta 342-01000. Thika /; Tel: 0672820000: Barua pepe; research@mku.ac.ke

Maamuzi yangu: Kutokana na kuzingatia yale yote yamesemwa hapo awali, nimmeamua

na kukubali kuhusika katika utafiti huu. Nitapatiwa nakala hii ya kukubali kuhusika katika utafiti huu ili iwe rekodi yangu. Nimekubali kuhusika katika utafiti huu.

Kodi la Mhusika: _____

Sahihi ya mhusika: _____ Tarehe _____ (tarehe ijazwe na mhusika)

Jina la mtafiti: _____

Sahihi ya mtafiti: _____ Tarehe _____

Kibali kutoka kwa mtoto

Nimeelewa uthafiti huu na nitasingatia yote yaliyosemwa na yanayotakikana kwa uthafiti huu.

Kodi ya mtoto_____Tarehe_____

Informed Consent form (Parent)

You are invited to participate in a research study on the use of farmed edible crickets (*Acheta domesticus*) to improve child nutrition in Kenya in Cheptigit primary school located in Uasin Gishu County, Kenya. We ask that you read this form and ask any questions you may have before agreeing that you are in the study. The study is being conducted by Carolyne Kipkoech for academic purpose to address malnutrition in Kenya.

Study Purpose: The purpose of this study is to determine the impact of porridge on nutritional status and gut health of school going children. Knowledge gained from the study will help in future policy formulation in regards to food formulation, edible insect use and farming in Uasin Gishu and the entire country.

Study Procedures: If you agree that you are in the study, you will be among the parents participating and your child will be given porridge, every weekday for six months, we will ask you questions and you will give us feedback to the best of your knowledge.

Questionnaires

At the beginning, and at the end of the study will ask questions, you will be asked questions about the family situation, your child's health and development, breastfeeding pattern and your child dietary intake. You do not have to answer any questions that you do not want to answer.

Confidentiality: You will be asked to keep all information confidential; Efforts will be made to keep your personal information confidential.

Costs: The study will not cost you any finances, all we will need is your time.

Benefits: If you become part of this study, your child will benefit from the nutrients from the porridge for six months, which will improve the nutritional status, cognitive function

and gut health of your child.

Risks: There are no risks associated with the study.

Voluntary nature of the study: Taking part in this study is voluntary. You may choose not to take part or to leave the study at any time. Leaving the study will not result in any penalty and will not affect your relations with the health care facilities, school or any other person in the community.

Contact: For questions about the study, contact Carolyne Kipkoech on phone number 0721-481324 or E-mail address: Kipkoechcarolyne@gmail.com. If you feel your right are infringed by this study kindly contact Dr. John Kinyuru on phone number 0723 66732 Email address: jkinyuru@agr.jkuat.ac.ke. In case of any complaints concerning this study kindly forward to The Chairman, Mount Kenya Ethical Review Committee P.O Box 342-01000. Thika: Tel. 0672820000: Email: research@mku.ac.ke

Subject's Consent:

In consideration of all of the above, I give my consent to participate in this research study. I will be given a copy of this informed consent document to keep for my records.

Subject's Code: _____

Subject's Signature: _____ Date: _____

(Must be dated by the subject)

Name of Person Obtaining Consent: _____

Signature of Person Obtaining Consent: _____Date:_____

Informed consent: Swahili Version (Mzazi)

RIDHAA TAARIFA KATIKA UTATHMINI

Umekaribishwa kujumuika nasi katika utafiti ya matumizi ya Kricket inayofukwa (*Acheta domesticus*) kuimarishe lishe bora nchini Kenya. Tunakuagiza usome fomu hii vizuri, ukiwa na maswali uulize kabla ya kukubali kujumuika katika utafiti huu. Utafiti huu unaendeshwa na Carolyne Kipkoech, mwanafunzi wa cheti cha shahada kwa chakula na lishe bora katika chuo kikuu cha kilimo na technologia cha Jomo Kenyatta.

Kusudi la utafiti: Kusudi kuu la utafiti huu ni kutumia uji kuimarisha lishe kwa watoto wa shule ya msingi ya Cheptigit. Majibu ya uthafithi huu utatumika kuimarisha lishe kwa watoto wa Uasin Gishu na chi ya Kenya.

Taratibu ya utafiti: Kama unakubali uwe katika utafiti, utakuwa mmoja wa wazazi wa watoto 135 katika shule ya msingi ya Cheptigit ambao watashiriki katika utafiti huu. Tutakuuliza wewe maswali, utatupa majibu kwa kadri ya ufahamu wako.

Maswali: Mwanzoni, na mwishoni wa utafiti tutakuuliza maswali, kuhusu hali ya familia, afya ya mtoto wako na maendeleo. Sio lazima kujibu maswali yeyote ambayo wewe hutaki kujibu.

Umuhimu ya utafiti: Matokeo ya utafiti huu utatumuka kubadilisha sera ya lishe Uasin Gishu na inchi ya Kenya.

Usiri: Utaulizwa uweke taarifa yote uliyonayo kwa usiri, Jina lako halitatumika katika nakala na pia jina lako halitatumika katika ripoti zozote za utafiti. Taasisi ya Utafiti na Maadili itaweza kukagua na kukiri kuwa hakuna jina lako au la mwenzako litakalotumika katika ripoti yoyote.

Gharama: Utafiti huu hautakugharimu kitu chochote.

Hiari asili ya utafiti: Kuchukua hatua ya kujumuika katika utafiti huu ni kwa kujitolea. Unaweza kuamua kuhusika katika utafiti huu ama kuacha wakati wowote. Kukosa kuhusika katika utafiti huu wakati wowote hautahusisha fidia yoyote na pia haitaharibu uhusiano wowote baina yako na shule.

Ukiwa na maswali yetote: Kwa maswali kuhusu utafiti huu, wasiliana na Carolyne Kipkoech, 0721481324, au Daktari John Kinyuru wa chuo kikuu cha kilimo na technologia cha Jomo Kenyatta jkinyuru@agr.jkuat.ac.ke. Unawaeza pia kuwasiliana na Taasisi ya chuo kikuu cha mlima Kenya, kitengo cha Utafiti na Maadili orofa ya pili pale Chuo Kikuu kama una maswali kuhusu haki yako kama mhusika katika huu utafiti. Unawesa pia kutuma malaamishi yako kwa Mwenye kiti Taasisi ya uthafiti sandulu la posta 342-01000. Thika /; Tel: 0672820000: Barua pepe; research@mku.ac.ke

Maamuzi yangu: Kutokana na kuzingatia yale yote yamesemwa hapo awali, nimmeamua na kukubali kuhusika katika utafiti huu. Nitapatiwa nakala hii ya kukubali kuhusika katika utafiti huu ili iwe rekodi yangu. Nimekubali kuhusika katika utafiti huu.

Kodi la Mhusika: _____

Sahihi ya mhusika: _____ Tarehe _____ (tarehe ijazwe na mhusika)

Jina la mtafiti: _____

Sahihi ya mtafiti: _____ Tarehe _____

Appendix 2: Data collection forms

GENERAL DATA COLLECTION FORM

(Interviewer ID):_____ (Child´s ID): _____

(School): _____ *(Date of data collection)* _____ / _____ / _____

Consent obtained from primary caretaker Yes No

(First, I would like to ask some general questions about the child)

1. *(CHILD´S NAME)* : _____ *(FATHER'S NAME)*: _____ *(MOTHER'S NAME)*: _____	
2. *(CARE TAKER´S NAME)* : _____ *(HEAD OF HOUSEHOLD NAME)* :_____	
3. *(WHAT IS YOUR RELATIONSHIP TO (name)?* *(Circle ONLY ONE answer)*	*(Biological mother)* 1 *(Grandmother)* 2 *(Sister)*3 *(Stepmother)* 4 *(Aunt)* 5 *(Another female relative)* 6 *(Brother)* 10 *(Father)* 11

	(Other (specify) 7 *(Answer refused)* 8 *(Don't know)* 9
4. *(HAVE YOU HAD PRIMARY RESPONSIBILITY FOR TAKING CARE OF (name) FOR AT LEAST THE LAST TWO WEEKS?)* *(Circle ONLY ONE answer)*	*(Yes)* 1 *(No)* 2 *(Answer refused)* 8 *(Don't know)* 9
5. *(IS (name) A BOY OR GIRL?)* *(Circle ONLY ONE answer)*	*(Boy)* 1 *(Girl)* 2
6. *(WHAT IS (name) ´S DATE OF BIRTH?)* *(Even if the mother knows the exact date of birth, ask if she has an immunization card or other document and checks that the date is correct.)* *(Mark 01.01.2099 if not known)*	*(Day)(Month)(Year)*
7. *(SOURCE OF DATE OF BIRTH INFORMATION)* *(Circle ONLY ONE answer)* *(If a date of birth is available, jump to question 10.)*	*(immunization or vaccination card)* 1 *(Birth certificate)* 2 *(Caretaker's recall)* 3 *(Other (specify)* 7 _____
8. *(HOW OLD IS (name)?*	*(Age in months)*

(Demographic and socioeconomic variables)

I will now ask you about the people who live here and other things about your household.

9. *(HOW MANY CHILDREN UNDER 5 YEARS OF AGE USUALLY LIVE IN THIS HOUSEHOLD?)* *Including the study child*	*(Number children under 5 years)*
10. What is the parents highest level of education	*1 primary* *2 secondary* *3 college* *3 university* *5 didn't go to school*
11. Where do you get water to drink	*1 tank* *2 piped* *3 borehole* *4 river* *5 dam*
12. How many acres of land do you own	*1 less than 1* *2 less than five* *3 more than 5* *4 we don't have land*

13. What is your primary source of income	*1 formal employment* *2 farming* *3 dairy* *4 non-formal employment*
14. *(WHERE DO MEMBERS OF YOUR HOUSEHOLD USUALLY GO TO RELIEVE THEMSELVES?)* *(Circle ONLY ONE answer)* *From the DHS survey*	*(Flush or pour flush toilet)* *(Flush to piped sewer system)*1 *(Flush to septic tank)* 2 *(Flush to pit latrine)* 3 *(Flush don't know where)* 4 *(Pit latrine)* *(Ventilated improved)* 5 VIP 6 *(Pit latrine with slab)* 10 *(Pit latrine without slab/open pit)* 11 *(Composting toilet)* 12 *(Bucket toilet)* 13 *(Toilet over water)* 14

	(No Toilet/field/forest) 15 *(Other (specify)* 7 _____ *(Answer refused)* 8 *(Don't know)* 9
15. *(DO YOU SHARE THIS TOILET FACILITY WITH OTHER HOUSEHOLDS?)* *From the DHS survey*	*(Yes)* 1 *(No)* 2 *(Answer refused)* 8 *(Don't know)* 9
16. *(HOW MANY HOUSEHOLDS USE THIS TOILET FACILITY?)* *From the DHS survey*	*(No of household)* *(Answer refused)* 88 *(Don't know)* 99
17. *(WHAT IS THE PRIMARY SOURCE OF INCOME FOR THIS HOUSEHOLD?)* *Circle ONLY ONE answer)*	*0 = None* *1= Farming* *2=Self Employed* *3=Salaried* *4=Not applicable(dependent)*

	5= Remittance
18. *(DOES THIS HOUSEHOLD OWN ANY LIVESTOCK, HERDS OR FARM ANIMALS?)* *From the DHS survey*	*(Yes)* 1 *(No)* 2 *(Answer refused)* 8 *(Don't know)* 9
20. *(I WILL NOW MENTION SOME ANIMALS, AND I WOULD LIKE YOU TO TELL ME HOW MANY ANIMALS OF EACH TYPE YOU HAVE.)* *(Fill in NUMBER of each type of animal)* *From the DHS survey*	*(Cows/bulls)* : *(goats)* : *(pigs)* : *(Chicken)* : *(ducks)* :
21. *(DOES ANY MEMBER OF THIS HOUSEHOLD OWN ANY LAND THAT CAN BE USED FOR AGRICULTURE?)* *From the DHS survey*	*(Yes)* 1 *(No)* 2 *(Answer refused)* 8 *(Don't know)* 9

Thank you very much for taking your time for this interview.

(Signature of team Interviewer):

SENSORY EVALUATION QUESTIONNAIRE FOR PORRIDGE

Participation in the Taste Panel Trial is Voluntary!!

RECORDING SHEET

Date:

Taster Name: **Gender:**

Age group (Please tick) : <20: 20-30: 30-40: 40-50: 50-60 >60

You are provided with coded porridge. Please score the samples from 1-5 according to the scale provided below by filling in the table against each sample attributes.

1. Dislike extremely (worst)
2. Dislike very much
3. Dislike moderately
4. Neither like nor dislike
5. Like moderately
6. Like very much
7. Like extremely

For general acceptability

I cook and eat this food in every opportunity I have.....

I frequently eat this.....

I would eat this if available but would not go out of my way.....

I would hardly ever eat this.....

I would eat this only if I were forced to

SAMPLE CODE	APPEARANCE	TEXTURE	TASTE	AROMA	MOUTHFEEL	GENERAL ACCEPTABILITY	VISCOSITY
MMP							
MP10							
CP5							
CP10							

CP20							

In the last three month, about how often have you drink porridge? (Tick where applicable)

□ Not a single time

□ Once a month

□ More than twice a month

□ Once a week

□ More than once a week

General comments

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Thank you for your participation in our taste panel trial. Your contribution is very much appreciated

MORBIDITY – ROUTINE CLINICAL EXAMINATION

Health professional ID:

Child´s ID:

(Morbidity – Routine Clinical Examination)

(School): _____ *(Date of data collection)* _____ / _____ / _____

(No of data collection: 1 / 2

2.1 *(Temperature)*		
I__I__I●I__I Cº		
2.2 *(Blood pressure)*		
Diastolic: I__I__I mmHg	Systolic: I__I__I mmHg	
2.3 *(Signs of under nutrition-related diseases)*		
(Oedema: yes no)	*(Vitamin A deficiencies)*: yes no	
2.4 *(Haemoglobin)*		
I__I__I●I__I g/L		

1.	*(GENERAL CONDITION)*	*(Well/alert)* 1 *(Restless/irritable)* 2 *(Abnormally sleepy)* 3 *(Sleeping, could not be assessed)* 4
2.	*(RESPIRATORY RATE/MINUTE)*	1: 2:

	(count for 60 sec and repeat)	
3.	*(COUGHING)*	*(Yes)* 1 *(No)* 2
4.	*(RUNNING NOSE)*	*(Yes)* 1 *(No)* 2
5.	*NASAL FLARING*	*(Yes)* 1 *(No)* 2
6.	*AUDIBLE WHEEZING OR GRUNTING*	*(Yes)* 1 *(No)* 2
7.	*SEVERE CHEST IN DRAWING*	*(Yes)* 1 *(No)* 2
8.	*BULGING FONTANEL*	*(Yes)* 1 *(No)* 2
9.	*(EYE PROBLEM) (redness, discharge)*	*(Yes)* 1 *(No)* 2
10.	*(EAR PROBLEM) (e.g. discharge)*	*(Yes)* 1 *(No)* 2

11.	*(SKIN RASH OR PUSTULES)*	*(Yes)* 1 *(No)* 2
12.	*(JAUNDICE)*	*(Yes)* 1 *(No)* 2
13.	*(TREATMENT REQUIRED)*	*(None)* 1 *(Home care without medicine)* 2 *(Home care with medicine)* 3 *(Hospitalization)* 4

FOOD RECALL QUESTIONNAIRE

Interviewer ID: Child´s ID:

School _____No of data collection: Baseline / 1 / 2

Date of data collection _____ / _____ / _____, Day of week: _____,

Time of Recall: _____ / _____

Time	Place	Food & Description	Serving Size	Raw ingredients		Weight of total cooked ingredients (g)	Amount Eaten (g)
				Foods items	Weight (g)		

Breastfeeding record

1 HAS *(name)* EVER BEEN BREASTFED? *Circle ONLY ONE answer*	Yes 1 No 2 Answer refused 8 Don´t know 9
2. SINCE THIS TIME YESTERDAY, HAS *(name)* BEEN BREASTFED? *Circle ONLY ONE answer*	Yes 1 No 2 Answer refused 8 Don´t know 9
3. AT WHAT AGE DID *(name)* START EATING COMPLEMENTARY FOOD? COMPLIMENTARY FOOD IS ANY FOOD WHICH IS NOT BREAST MILK EXCEPT MEDICATIONS.	Number of months
4. DOES HE USUALLY FINISH HIS/HER SHARE	Yes 1

	No 2 Answer refused 8 Don't know 9

Porridge consumer acceptability form (to be filled weekly)

Sno	Child code	Finished Porridge		Amount Remained
		Yes	No	
1	B1			
2	B2			
3	B3			
4	B4			
5	B5			
6	B6			
7	B7			
8	B8			
9	B9			
10	B10			
11	B11			
12	B12			
13	B13			
14	B14			
15	B15			
16	B15			
17	B16			
18	B17			
19	B18			
20	B19			

21	**B20**			
22	**B21**			
23	**B22**			
24	**B23**			
25	**B24**			
26	**B25**			
27	**B26**			
28	**B27**			
29	**B28**			
30	**B29**			
31	**B30**			
32	**B31**			
33	**B32**			
34	**B33**			
35	**B34**			
36	**B35**			
37	**B36**			
38	**B37**			
39	**B38**			
40	**B39**			
41	**B40**			
42	**B41**			
43	**B42**			
44	**B43**			
45	**B44**			
46	**B45**			
47	**B46**			
48	**G1**			
49	**G2**			
50	**G3**			
51	**G4**			
52	**G5**			
53	**G6**			
54	**G7**			
55	**G8**			

56	**G9**			
57	**G10**			
58	**G11**			
59	**G12**			
60	**G13**			
61	**G14**			
62	**G16**			
63	**G17**			
64	**G18**			
65	**G19**			
66	**G20**			
67	**G21**			
68	**G22**			
69	**G23**			
70	**G24**			
71	**G25**			
72	**G26**			
73	**G27**			
74	**G28**			
75	**G29**			
76	**G30**			
77	**G31**			
78	**G32**			
79	**G33**			
80	**G34**			
81	**G35**			
82	**G36**			
83	**G37**			
84	**G38**			
85	**G39**			
86	**G40**			
87	**G41**			
88	**G42**			
89	**G43**			
90	**G44**			

91	**G45**			
92	**G46**			
93	**Y1**			
94	**Y2**			
95	**Y3**			
96	**Y4**			
97	**Y5**			
98	**Y6**			
99	**Y7**			
100	**Y8**			
101	**Y9**			
102	**Y10**			
103	**Y11**			
104	**Y12**			
105	**Y13**			
106	**Y14**			
107	**Y15**			
108	**Y16**			
109	**Y17**			
110	**Y18**			
111	**Y19**			
112	**Y20**			
113	**Y21**			
114	**Y22**			
115	**Y23**			
116	**Y24**			
117	**Y25**			
118	**Y26**			
119	**Y27**			
120	**Y28**			
121	**Y29**			
122	**Y30**			
123	**Y31**			
124	**Y32**			
125	**Y33**			

126	**Y34**			
127	**Y35**			
128	**Y36**			
129	**Y37**			
130	**Y38**			
131	**Y39**			
132	**Y40**			
133	**Y41**			
134	**Y42**			
135	**Y43**			
136	**Y44**			
137	**Y45**			
138	**Y46**			

Appendix 3: Standard operating procedure for anthropometry

ANTHROPOMETRY SOP

Measuring weight

- Put the scale on the floor on a flat surface.
- Turn on the scale
- Allow the scale to Zero until it shows 0.00 then ask the child to stand on the scale, with feet on the right place.
- Repeat this three times and record all the readings.

Measuring head circumference

- The person measuring should stand at the left side of the child.
- Place the tape just above the supraorbital ridges covering the most prominent part of the frontal bulge and over the part of the occiput that gives the maximum circumference.
- Measure the circumference to the nearest millimetre.
- Repeat three times and record all the measurements.

Measure mid-upper arm circumference

- Gently bend the left arm through 90 degrees at the elbow and then place the forearm with the palm down across the body.
- Locate and mark the tip of the shoulder.
- Locate the tip of the elbow.

- Measure the distance between the two points using fiberglass insertion tape and mark the midpoint with a soft pen directly in line with the point of the elbow and shoulder
- Relax the arm so that the elbow is extended and hanging just away from the side of the trunk with the palm facing the thigh. Then wrap the tape gently but firmly around the arm at the midpoint, care taken to ensure the arm is not squeezed. Measurements are taken to the nearest mm.
- Repeat three times and record all the measurements

Measure skinfold

- Obtain these measurements with the child seated.
- Alternatively, children may be measured lying down.
- It is helpful to demonstrate the caliper on the hand of the measurer and on the hand of the child, measuring total palm thickness, before beginning to measure skinfold thickness.

Measure the trice skinfold

- Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
- Locate and mark the tip of the acromion process of the shoulder blade at the outermost edge of the shoulder.
- Locate the tip of the olecranon process of the ulna.
- Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.
- Extend the child's arm so that it is hanging loosely by the side.

- Grasp a vertical fold of skin plus the underlying fat, 1cm above the marked midpoint, in line with the tip of the olecranon process, using the thumb and forefinger.
- Gently pull away from the skinfold from the underlying muscle tissue, and apply the caliper jaws at right angles, exactly at the marked midpoint.
- Hold the skinfold between the fingers while measuring.
- Repeat three times and record all measurements.

Measure subscapular skinfold

- The site is just inferior to the inferior angle of the shoulder and can be identified more readily by placing the child's arm behind the back.
- To locate the site, the health professional will run a finger along the shoulder blade until the inferior angle is identified.
- Relax the shoulder and the arm, and pick up a skinfold on a 450 angle from horizontal, in the same direction as the inner border of the shoulder (i.e., medially upward and laterally downward).
- Skinfolds should be recorded to 0.2 mm on the Harpenden skinfold callipers three times. Skinfold measurements made with precision callipers should normally agree to within 1mm.
- Record all 3 measurements on the form.

Appendix 4: Data collection form for anthropometry

Form for data recording

Child´s ID:

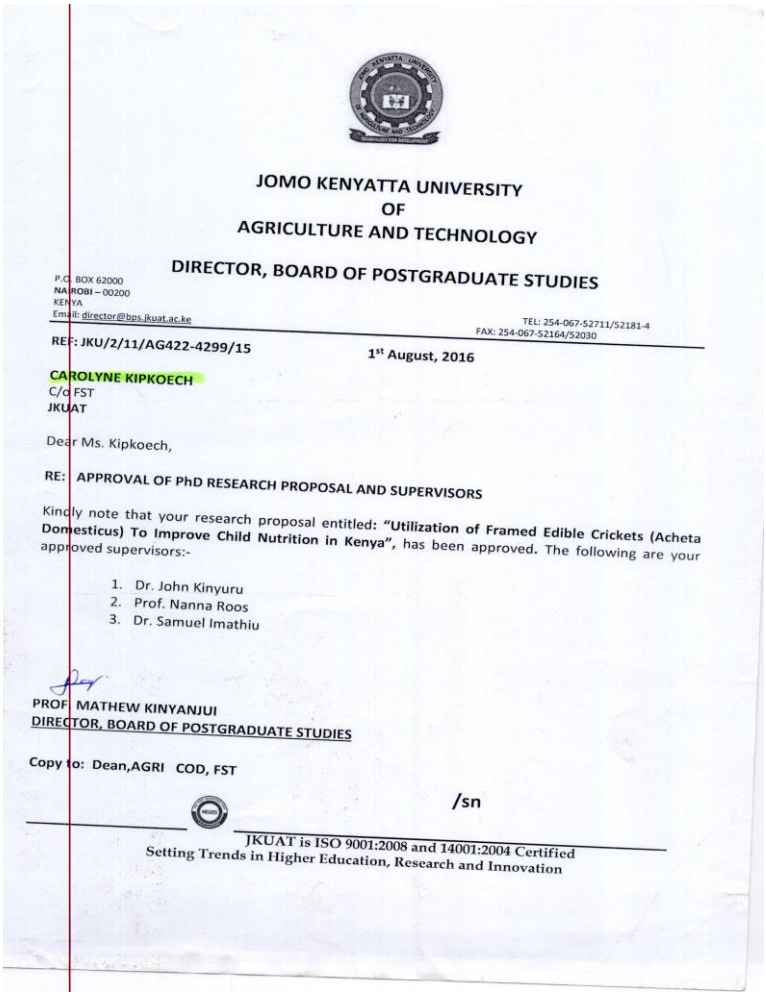
(School): _____ *(Date of data collection)* _____ / _____ / _____

(No of data collection: / 1 / 2 /

(Anthropometry)

1.1 *(Weight (record three measurements))*		
1.1.1 I__I__I•I__I__I kg	1.1.2 I__I__I•I__I__I kg	1.1.3 I__I__I•I__I__I kg
1.2 *(Length (record three measurements))*		
1.1.1 I__I__I__I•I__I cm	1.2.1 I__I__I__I•I__I cm	1.2.3 I__I__I__I•I__I cm
1.4 (MUAC*(record three measurements))*		
1.4.1 I__I__I•I__I cm	1.4.2 I__I__I•I__I cm	1.4.3 I__I__I•I__I cm
1.5 *Head circumference (record three measurements)*		
1.5.1 I__I__I•I__I cm	1.5.2 I__I__I•I__I cm	1.5.3 I__I__I•I__I cm
1.6 *Triceps Skinfold Thickness (TSF) (record three measurements)*		
Bicep's		
1.6.1 I__I__I•I__I cm	1.6.2 I__I__I•I__I cm	1.6.3 I__I__I•I__I cm
1.7 *Subscapular, Skinfold Thickness (TSF) (record three measurements)*		
Suprialiac		
1.7.1 I__I__I•I__I cm	1.6.2 I__I__I•I__I cm	1.6.3 I__I__I•I__I cm

Appendix 5: University admission letter



JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY

DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke

TEL: 254-067-52711/52181-4
FAX: 254-067-52164/52030

REF: JKU/2/11/AG422-4299/15

1st August, 2016

CAROLYNE KIPKOECH
C/o FST
JKUAT

Dear Ms. Kipkoech,

RE: APPROVAL OF PhD RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your research proposal entitled: **"Utilization of Framed Edible Crickets (Acheta Domesticus) To Improve Child Nutrition in Kenya"**, has been approved. The following are your approved supervisors:-

1. Dr. John Kinyuru
2. Prof. Nanna Roos
3. Dr. Samuel Imathiu

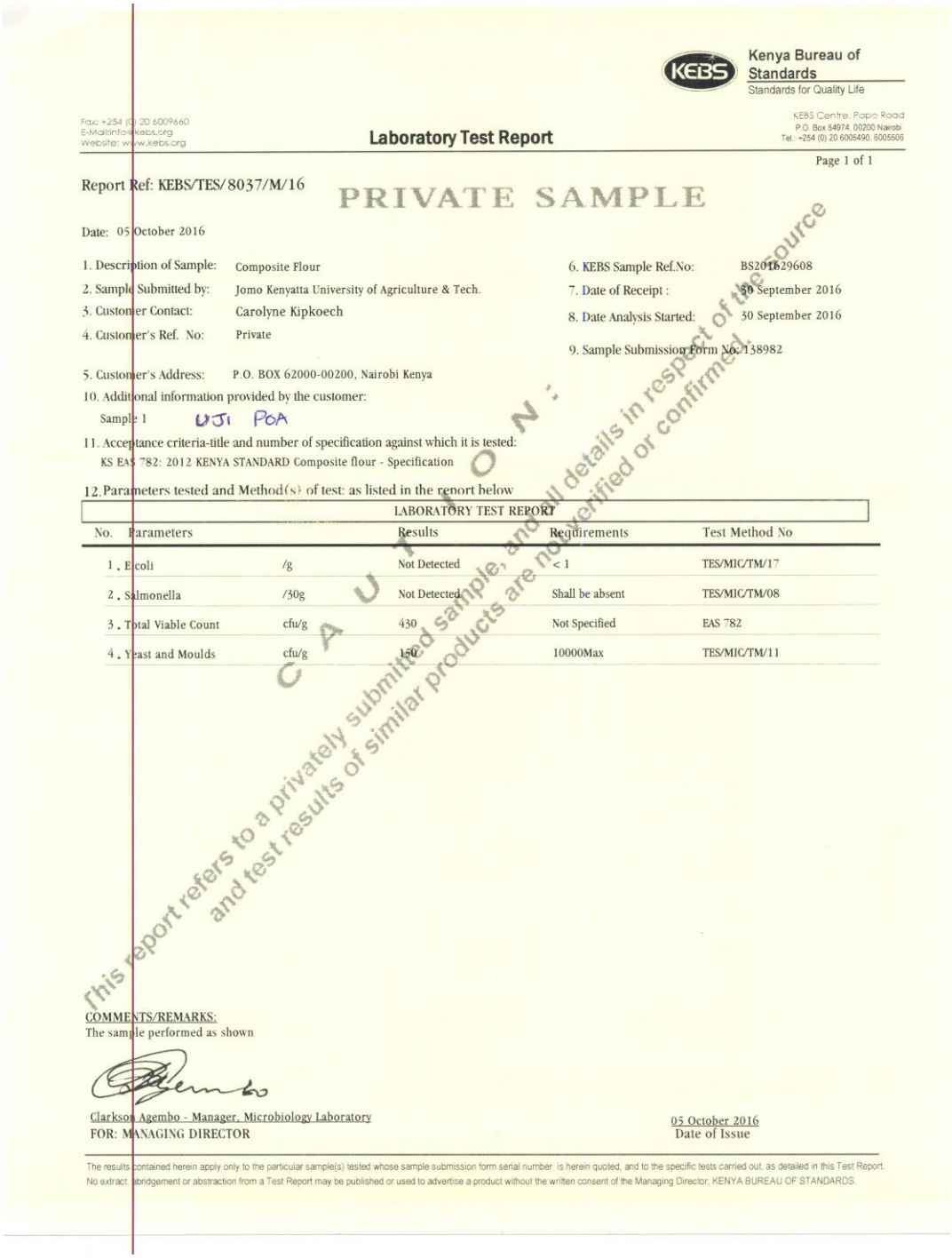
PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: Dean,AGRI COD, FST

/sn

JKUAT is ISO 9001:2008 and 14001:2004 Certified
Setting Trends in Higher Education, Research and Innovation

Appendix 6: Kenya Bureau Standards report (KEBS)



Kenya Bureau of Standards
Standards for Quality Life

Fax: +254 (0) 20 6009660
E-Mail:info@kebs.org
Website: www.kebs.org

KEBS Centre, Popo Road
P.O. Box 54974, 00200 Nairobi
Tel.: +254 (0) 20 6005490, 6005506

Laboratory Test Report

Page 1 of 1

Report Ref: KEBS/TES/8037/M/16

PRIVATE SAMPLE

Date: 05 October 2016

1. Description of Sample: Composite Flour
2. Sample Submitted by: Jomo Kenyatta University of Agriculture & Tech.
3. Customer Contact: Carolyne Kipkoech
4. Customer's Ref. No: Private
5. Customer's Address: P.O. BOX 62000-00200, Nairobi Kenya
6. KEBS Sample Ref.No: BS2016629608
7. Date of Receipt : 30 September 2016
8. Date Analysis Started: 30 September 2016
9. Sample Submission Form No. 138982

10. Additional information provided by the customer:
Sample 1 UJI POA

11. Acceptance criteria-title and number of specification against which it is tested:
KS EAS 782: 2012 KENYA STANDARD Composite flour - Specification

12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1.	E coli	/g	Not Detected	< 1	TES/MIC/TM/17
2.	Salmonella	/30g	Not Detected	Shall be absent	TES/MIC/TM/08
3.	Total Viable Count	cfu/g	430	Not Specified	EAS 782
4.	Yeast and Moulds	cfu/g	150	10000Max	TES/MIC/TM/11

CAUTION: This report refers to a privately submitted sample, and all details in respect of the source and test results of similar products are not verified or confirmed.

COMMENTS/REMARKS:
The sample performed as shown

Clarkson Agembo - Manager, Microbiology Laboratory
FOR: MANAGING DIRECTOR

05 October 2016
Date of Issue

The results contained herein apply only to the particular sample(s) tested whose sample submission form serial number is herein quoted, and to the specific tests carried out, as detailed in this Test Report. No extract, abridgement or abstraction from a Test Report may be published or used to advertise a product without the written consent of the Managing Director, KENYA BUREAU OF STANDARDS.

Kenya Bureau of Standards
Standards for Quality Life

Fax: +254 (0) 20 6009660
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Website: www.kebs.org

KEBS Centre, Popo Road
P.O. Box 54974, 00200 Nairobi
Tel.: +254 (0) 20 6005490, 6005506

Laboratory Test Report

Report Ref: KEBS/TES/8039/M/16

PRIVATE SAMPLE

Date: 05 October 2016

1. Description of Sample: Composite Flour
2. Sample Submitted by: Jomo Kenyatta University of Agriculture & Tech.
3. Customer Contact: Carolyne Kipkoech
4. Customer's Ref. No: Private
5. Customer's Address: P.O. BOX 62000-00200, Nairobi Kenya
6. KEBS Sample Ref.No: BS201629610
7. Date of Receipt : 30 September 2016
8. Date Analysis Started: 30 September 2016
9. Sample Submission Form No: 138982
10. Additional information provided by the customer:

Sample 2 UJI FINE

11. Acceptance criteria-title and number of specification against which it is tested:

KS EAS 782: 2012 KENYA STANDARD Composite flour - Specification

12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1.	E.coli	/g	Not Detected	< 1	TES/MIC/TM/17
2.	Salmonella	/30g	Not Detected	Shall be absent	TES/MIC/TM/08
3.	Total Viable Count	cfu/g	1380	Not Specified	EAS 782
4.	Yeast and Moulds	cfu/g	200	10000Max	TES/MIC/TM/11

COMMENTS/REMARKS:
The sample performed as shown

Clarkson Agembo - Manager, Microbiology Laboratory
FOR: MANAGING DIRECTOR

05 October 2016
Date of Issue

Kenya Bureau of Standards
Standards for Quality Life

Fax: +254 (0) 20 6009660
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Website: www.kebs.org

KEBS Centre, Popo Road
P.O. Box 54974, 00200 Nairobi
Tel.: +254 (0) 20 6005490, 6005506

Laboratory Test Report

Report Ref: KEBS/TES/8041/M/16

PRIVATE SAMPLE

Date: 05 October 2016

1. Description of Sample: Composite Flour
2. Sample Submitted by: Jomo Kenyatta University of Agriculture & Tech.
3. Customer Contact: Carolyne Kipkoech
4. Customer's Ref. No: Private
5. Customer's Address: P.O. BOX 62000-00200, Nairobi Kenya
6. KEBS Sample Ref.No: BS2016129612
7. Date of Receipt : 30 September 2016
8. Date Analysis Started: 30 September 2016
9. Sample Submission Form No: 138982
10. Additional information provided by the customer:
Sample 3 UJI YETU
11. Acceptance criteria-title and number of specification against which it is tested:
KS EAS 782: 2012 KENYA STANDARD Composite flour - Specification
12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1.	E.coli	/g	Not Detected	< 1	TES/MIC/TM/17
2.	Salmonella	/30g	Not Detected	Shall be absent	TES/MIC/TM/08
3.	Total Viable Count	cfu/g	550	Not Specified	EAS 782
4.	Yeast and Moulds	cfu/g	120	10000Max	TES/MIC/TM/11

COMMENTS/REMARKS:
The sample performed as shown

Clarkson Agembo - Manager, Microbiology Laboratory
FOR: MANAGING DIRECTOR

05 October 2016
Date of Issue

Appendix 7: Certificate of Ethical Clearance

Mount Kenya University

JANUARY 9, 2017

Ref. No. MKU/ERC/0274

CERTIFICATE OF ETHICAL CLEARANCE

This is to certify that the proposal titled **"USE OF FARMED EDIBLE CRICKETS (ACHETA DOMESTICUS) TO IMPROVE CHILD NUTRITION IN KENYA"**, whose Principal Investigator is Carolyne Kipkoech (AG422-4492/2015) has been reviewed by Mount Kenya University Ethics Review Committee (ERC), and found to adequately address all ethical concerns.

Mr Francis W. Makokha
Secretary, Mount Kenya University ERC

Sign: _____ **Date:** 9/01/2017

Dr Francis W. Muregi
Chairman, Mount Kenya University ERC

Sign: _____ **Date:** 09/01/2017

Mount Kenya University
Director, Research & Development
& Development
P. O. Box 342 - 01000, Thika

Appendix 8: Publications

Production and growth parameters of edible crickets: experiences from a farm in a high altitude, a cooler region of Kenya

J.N. Kinyuru, C. Kipkoech

https://doi.org/10.3920/JIFF2017.0081

Abstract References PDF

Abstract

The need for mass-production of crickets is increasing with continued awareness. Cricket farming has been introduced with considerable success among small-holder farmers in the warmer, low altitude Lake Victoria regions of Kenya. Efforts are however on-going to introduce the farming in cooler, higher altitude areas in the interest of expanding the enterprise and increase mass production. A pilot farm was established at a farm located 1,519 meters above sea level with a temperature range of 17-22 °C. Initial egg stock was incubated at the farm under room conditions in the month of November to December. Different agricultural side streams and farm weeds were tested as probable cricket feed. Hatch rate, duration of hatching, preferred food types, mortality, weight gain and nutrient content at different ages were assessed. The hatch rate averaged 60%, mortality for hatchlings (pinheads) was below 2% while a steady weight gain was observed over a 12 weeks growth period with highest maximum weight being 2.03 grams per cricket. All agricultural side streams were accepted by the crickets and the wandering Jew weed was among the preferred feed and a source of significant nutrient for the crickets. Protein content ranged from 36-60% while fat content was 12-25%. Cricket production can, therefore, be promoted in the higher altitude, cooler areas in order to promote industrial exploitation of the crickets in combating food and nutrition insecurity.

Keywords: edible insects, insect farming, nutrient composition, mass production

https://www.wageningenacademic.com/doi/pdf/10.3920/JIFF2017.0081 2/2

Edible Insects in Sustainable Food Systems pp 93-108

The Role of Edible Insects in Diets and Nutrition in East Africa

Authors John N. Kinyuru
Dorothy Nyangena, Edwin Kamau, Alex Ndiritu, Joyce Muniu, Carolyne Kipkoech, Johnson Weru, Nancy Ndung'u, Mercy Mmari

Chapter

First Online: 15 May 2018

Abstract

Insects have been used as food, medicine and in rituals by a number of communities in the East African region comprising of Kenya, Uganda, and Tanzania over centuries. Progressively, farmed edible insects mainly crickets and grasshoppers are gaining popularity within the region. However, the utilization of the edible insects is hampered by lack of storage and preservation facilities in the rural areas leading to high postharvest losses. Sun drying and roasting have been the main processing methods applied for decades by communities consuming edible insects such as the Luo from Kenya. Recently there has been the incorporation of insects as an ingredient in the processing of baked products and complementary foods. Culture, taboos, custom, and ethnic preferences have highly influenced the consumption of edible insects in East Africa. Edible insects such as grasshoppers, mayfly, and termites that are consumed in this region have been shown to be a source of both macro and micronutrients and other components such as chitin which has been linked to improved health and better management of chronic diseases. Therefore

edible insects promise to be a part of the solution to food and nutrition security within the East African region.

DOI http://sci-hub.tw/10.1007/9783-319-74011-9_6

Online ISBN 978-3-319-74011-9

African Journal of Agricultural Research

Use of house cricket to address food security in Kenya: "Nutrient and chitin composition of farmed crickets as influenced by age"

Carolyne Kipkoech, John N. Kinyuru, Samuel Imathiu, and Nanna Roos

House cricket is currently introduced for scaled-up production in farming systems in Kenya and other parts of the world, as an alternative source of animal proteins. The aim of this study was to assess the nutritional composition in farmed cricket as influenced by age in order to ascertain the optimal harvesting time for possible utilization of crickets in improving child nutrition in Kenya. Sampling was carried out between weeks 4 and 13. The moisture content was analysed by drying method, chitin by sodium hydroxide digestion, protein content by estimation of total nitrogen, crude fat by soxhlet extraction method, ash by muffle furnace incineration, available carbohydrates by subtraction, and energy by a calculation method. The crude protein mean ranged from 36.00 to 60.00 g/100 g, chitin 2.20 to 12.40 g/100 g, total lipids 12.00 to 25.00 g/100 g, over the 13 weeks period. Minerals concentration was optimum at week 9, with magnesium 1.30 to 11.30 mg/100 g, calcium 1.40 to 19.70 mg/100 g, and zinc 0.20 to 16.60 mg/100 g. Findings from this study indicate that farmed cricket would be best harvested between weeks 9 and 11 when the protein and mineral content is optimum. Nutrients available in farmed crickets show that farmed crickets can be used in child food ingredients to improve child nutrition.

Keywords: Farmed crickets, proximate, protein, fatty acid, omega 3, omega 6, minerals, child nutrition.

List of conferences

1. **Enhancing Legislation and Policy in the use of Insects as Food and Feed from March 2-3, 2016 in Kisumu/Kenya**

   Carolyne Kipkoech, Nanna Roos, Samwuel Imathiu, John Kinyuru.

   CRICKET-BASED DIET FOR IMPROVED CHILD NUTRITION IN KENYA

2. **Kenya Institute of Food Science and Technology conference June 1-2 2016 in Nairobi/Kenya**

   Carolyne Kipkoech, Samwuel Imathiu, John Kinyuru, Nanna Roos.

   PROXIMATE, FATTY ACID AND MINERAL COMPOSITION OF KENYAN FARMED CRICKETS

3. **Africa Nutrition and Epidemiology Conference October 9th -15th 2016 Marrakech/Morocco**

   Carolyne Kipkoech, Samwuel Imathiu, Nanna Roos, John Kinyuru.

   Nutrient composition of farmed crickets (*Acheta domesticus*) as influenced by age.

4. **Insect to feed the world conference (IFW 2018) May 15th – 18th 2018 Wuhan China**

   Carolyne Kipkoech, John Kinyuru, Samwuel Imathiu, Nanna Roos,

a) CAN CRICKET BASED PORRIDGE IMPROVE NUTRITIONAL STATUS OF SCHOOL CHILDREN IN KENYA? A RANDOMIZED CONTROLLED TRIAL
b) PREBIOTIC POTENTIAL OF CHITIN DERIVED FROM FARMED CRICKETS: A GATEWAY TO IMPROVED GUT HEALTH

5. THE 13Th JKUAT SCIENTIFIC AND TECHNOLOGY CONFERENCE: Commercialization of research outputs and innovations for sustainable development 15th -16th November 2018

Carolyne Kipkoech, John Kinyuru, Samwuel Imathiu, Nanna Roos,

CONSUMER ACCEPTABILITY AND SAFETY OF CRICKET POWDER

Appendix 9: Study Photos


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