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The effect of consuming instant-maize porridge fortified With Termites and *Moringa Oleifera* Leaf powders on the nutritional status of children aged three to five years old in Thulamela Municipality, South Africa (RCT)

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DECLARATION

I, Netshiheni Khavhatondwi Rinah (11602027), hereby declare that the thesis titled "Effect of consuming instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders on the nutritional status of children aged three to five years old in Thulamela Municipality, Limpopo Province, South Africa" for the Doctor of Philosophy in Public Health Nutrition at the University of Venda hereby submitted by me, has not been submitted for a degree at this or any other University and that it is my own work in design and execution and that all reference material contained therein has been duly acknowledged.

Signature: .... **Date:** 27 February 2023

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BAZ	Body mass index-for-age
BMI	Body mass index
EAR	Estimated average requirement
EDTA	Ethylenediamine tetra acetic acid
EER	Estimated energy requirement
FANTA	Food and Nutrition Technical Assistance
FAO	Food and Agricultural Organisation
FFQ	Food frequency questionnaire
H/A	Height-for-age
HAZ	Height-for-age Z-score
Hb	Haemoglobin
HPLC	High performance liquid chromatography
IDD	Iron deficiency disorder
LP	Limpopo Province
MO	*Moringa oleifera*
NFCS	National Food Consumption Survey
NFCS-FB	National Food Consumption Survey Fortification Base
NHANES	National Health and Nutrition Examination Survey
PEM	Protein-energy malnutrition
RCT	Randomised Control Trial
RDA	Recommended Dietary Allowance
RH	Relative humidity
RtHB	Road to Health Booklet
SA	South Africa
SABS	South African Bureau of Standards
SAM	Severe acute malnutrition
SANAS	South African National Accreditation System
SAVACG	South African Vitamin A Consultative Group
SD	Standard deviation
TAMBC	Total aerobic mesophilic bacterial count
TMYC	Total mould or yeast count
TSAT	Transferrin saturation

TVBC	Total viable bacterial count
UN	United Nations
UNICEF	United Nations Children's Fund
W/A	Weight-for-age
WAZ	Weight-for-age Z-score
WHO	World Health Organisation
WHZ	Weight-for-height

ABSTRACT

This chapter introduces the topic of the study, giving relevant background and highlighting the problem statement. It ends with the study's hypothesis, aim, specific objectives, significance, and conceptual framework.

Malnutrition is the cause of deaths of eight million children under five years worldwide. Undernutrition is the leading cause of over one-third of children's deaths, mainly from pneumonia and diarrhoea. Additionally, in 2015, zinc deficiency and anaemia prevalence in the Vhembe District preschool children in Limpopo Province was 42.6% and 28%, respectively. This study investigated the effect of consuming instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders on the nutritional status of children aged three to five years old. The research method used was quantitative. The study was conducted in Thulamela Municipality, one of the four municipalities in the Vhembe District. A randomised controlled trial was used, whereby participants were randomly assigned into either an experimental or control group. Twenty children from six preschools (three for the control and three for the experimental groups) participated in the study. The study was conducted in four phases, namely, Phase 1: the preparation of fortified instant-maize porridge and the assessment of safety and nutrient content of the product; Phase 2: a baseline study was done; Phase 3: participants were assigned to experimental and control groups. The consumption of 100% instant-maize porridge by the control group and fortified (80% maize, 15% termite powder and 5% *Moringa oleifera* powder) maize porridge by the experimental group for six months was done and Phase 4: post-intervention data collection and comparison of Phases 2 to 4 were made. The samples were prepared at the Department of Food Science & Technology at the University of Venda. Ethical certification was sought from the University of Venda Research Ethics Committee to conduct the study.

Keywords: Fortification, Instant-maize porridge, Iodine, Iron, Moringa, Nutritional status, Protein, Termites, Vitamins, Zinc.

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THESIS OUTLINE

An overview of the chapters presented in this thesis is as follows:

Chapter One: Introduction

This chapter introduces the topic of the study, giving relevant background and highlighting the problem statement. It ends with the study's hypothesis, aim, specific objectives, significance, and conceptual framework.

Chapter Two: Literature review

This chapter presents a detailed relevant literature review of the study, describing maize, *moringa* and termites and their nutritional significance in alleviating child malnutrition.

Chapter Three: Phase 1 (Manuscript 1)

In this phase, the fortified instant-maize porridge was prepared, and the safety and nutrient content of the product was assessed. This chapter also presents a journal manuscript titled: *"Biochemical properties and microbial activities of instant maize porridge as influenced by the addition of Moringa oleifera leaves and termite powders"* in the format of *Journal of Nutrition and Food Security*.

Chapter Four: Phase 2 (Manuscript 2)

A baseline study was done where anthropometric, biochemical and dietary assessments were conducted. This chapter also presents a journal manuscript titled: ***"Nutritional status among preschool children aged 3-5 years in Vhembe District, Limpopo Province, South Africa"*** in the format of *Journal of Nutrition and Food Science*.

Chapter Five: Phases 3 & 4 (Manuscript 3)

In this chapter, participants were assigned to experimental and control groups. The consumption of 100% instant-maize porridge by the control group and fortified (80% maize, 15% termite powder and 5% *Moringa oleifera* powder) maize porridge by the experimental group for six months was done here, along with post-intervention data collection and comparison of Phases 2 to 4. This chapter also presents a journal manuscript titled: ***"The Efficacy of consuming fortified maize porridge on preschool children aged 3 to 5 years in Vhembe District, Limpopo Province, South Africa"*** in the format of *Journal of Nutrition and Food Science*.

Chapter six: General Conclusion and recommendations

This section is the final thesis chapter, where the conclusion drawn from the study is outlined, and recommendations are made.

Chapter One: Introduction

1.1 Introduction

Malnutrition is a major health concern caused by a deficiency or excessiveness of micronutrients (vitamins and minerals) in a diet. Malnutrition affects the growth and development of children and increases their risk of death from common childhood illnesses (Black *et al*., 2010). In 2001, Mason *et al*. found that globally, 140 million children under five were vitamin A deficient. Furthermore, about 163 million children in developing countries have vitamin A deficiency (UNSCN, 2011), of which almost 100 million live in South Asia and sub-Saharan Africa (Mason *et al*., 2010). Although the extent of clinical vitamin A deficiency in South Africa is not as severe as in other sub-Saharan countries, one out of three children was identified as marginally vitamin A deficient in South Africa (SAVACG, 1995). Ten years later, The National Food Consumption Survey (2005) reported that two out of three children in South Africa had poor vitamin A status. Regardless of the high per capita income in South Africa, the prevalence of children who are stunted is almost the same as with children living in lower-income countries (WHO, 2015). Undernourished children stand a greater chance of being sick than well-nourished children. Although micronutrient deficiency is not visible to the naked eye, it can impact one's well-being significantly and is prevalent in South African children (Motadi *et al*., 2015). WHO (2015) supported the above findings by reporting that 17% and 24% of under-five children in South Africa have vitamin A deficiency and anaemia, respectively.

Netshiheni *et al*. (2019) outlined that the cause of malnutrition among South African children could be attributed to the high consumption of maize porridge, which is the staple food for most people in South Africa, as reported by Mushaphi *et al. (*2015). They also stated that maize contributes significantly to the dietary intake of most (80%) households in Limpopo Province. There, children's diets are predominately cereal-based (high in carbohydrates), with minimal intake of animal products. Mamabolo *et al*. (2006) reported that children in Central Limpopo Province received nearly 70% of their energy intake from carbohydrates, while fat contributed less than 20%. Regardless of all carbohydrates in maize grain, maize is not a good source of essential nutrients for human health. The bioavailability of minerals (vital for proper nutrition and health) in maize are often affected by phytic acid in the maize grain that interferes with the absorption of minerals (Hulse *et al*., 1980). Maize also has low amounts of lysine and poor protein digestibility, which plays a role in the nutritional composition of amino acids (Duodu *et al*., 2003). The high consumption of maize increased the prevalence of malnutrition in children under five years in South Africa; hence, there is a need to enhance the nutritional value of maize grain to improve the nutritional status of children, especially those from underprivileged households.

In 2003, the Department of Health in South Africa embarked on several nutrition intervention strategies, such as supplementation, food fortification, nutrition education, and dietary diversification (Ruel & Levine, 2010). One of these strategies was the mandatory fortification program of maize meal. Fortification is the practice of deliberately increasing the content of one or more micronutrients (vitamins and minerals) in food or condiments to improve the food supply's nutritional quality and provide a public health benefit with minimal health risk, particularly in rural places (Nestel *et al*., 2006). In addition, food fortification treats or prevents widespread nutrient intake shortfalls and associated deficiencies. It subsequently balances the total nutrient profile of a diet, restoring nutrients lost in processing or appealing to consumers looking to supplement their diet (Dwyer *et al*., 2015). This study, therefore, investigated the effect of consuming instant-maize porridge fortified with *Moringa oleifera* (*MO*) leaves and termite powders on the nutritional status of children aged three to five years in the Thulamela Municipality, Limpopo Province, South Africa.

1.2 Problem statement

Irrespective of nutrition intervention strategies employed to address child malnutrition, the prevalence remains high. Malnutrition, particularly micronutrient deficiency in children, can have long term, irreversible negative effects on neurological and cognitive outcomes. However, these deficiencies can be reversible while also increasing child mortality and mobility by 15% (Prado & Dewey, 2014; WHO, 2016). Children who are malnourished are susceptible to infections leading to a vicious cycle of malnutrition, if not treated (Muller *et al*., 2003). This means that, if malnourished children are not treated or continue having inadequate dietary intakes, they will grow to become malnourished adolescents and adults, which will then influence the nutritional status of the next generation, as the nutritional status of both the mother and father influences how the baby will turn out to be. In addition, inadequate dietary intake leads to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth and development in children (WHO, 2015). A sick person's nutrition is further aggravated by diarrhoea, malabsorption, loss of appetite, diversion of nutrients for the immune response, and urinary nitrogen loss, all of which lead to nutrient losses and further damage to the body's defence mechanisms. Zinc deficiency (42%) and anaemia (28%) among preschool children in Thulamela Municipality was observed by Motadi *et al*. in 2015 and 2017. The Vhembe Department of Health (2018) reported that the prevalence of malnutrition in children under the age of five years was higher in Thulamela as compared to other municipalities of the Vhembe District. This prevalence could also be attributed to the high consumption of maize, as it contributes significantly to the dietary intake of many households in Limpopo Province while being deficient in protein, vitamins and minerals. In the Vhembe District, however, there is an abundance of *MO* and edible termites which are known to be rich in minerals, vitamins and antioxidants, but are being under-utilised. Hence, this study shares insights into the effect of adding *MO* and termites to maize porridge (Salem *et al*., 2013).

1.3 The rationale of the study

Although many studies have been conducted on the prevalence of malnutrition in children under the age of five years, little has been done on intervention strategies to address child malnutrition using locally indigenous and available resources. Furthermore, there is no literature on any study conducted in South Africa where the iodine content of food was assessed.

1.4 Aim of the study

- The study aimed to investigate the effect of consuming instant-maize porridge fortified with termite and *MO* leaf powders on the nutritional status of children aged three to five years old in the Thulamela Municipality.

1.5 Objectives of the study

Objectives for Phase 1

- To prepare instant-maize porridge fortified with termite and *MO* leaf powders with factor levels: 100% (control) and 80% maize, 15% termites and 5% *MO* (experimental porridge)
- To determine the nutrient content of instant-maize porridge fortified with termite and *MO* leaf powder
- To determine the microbial activities of instant-maize porridge fortified with termite and *MO* leaf powder.

Objectives for Phase 2

- To assess the anthropometric status (height and weight) of children aged three to five years in the Thulamela Municipality.
- To assess the micronutrient status of children aged three to five years old in the Thulamela Municipality.
- To determine the dietary intake of children aged three to five years in the Thulamela Municipality.

Objectives for Phase 3

- To standardise the preparation method and portion sizes of the instant-maize porridge fortified with termite and *MO* leaf powder
- To feed children aged three to five years old for six months in Thulamela Municipality.

Objectives for Phase 4

- To compare baseline (Phase 2) to post-intervention data (Phase 4)
- To determine the effect of consuming instant-maize porridge fortified with termite and *MO* leaf powder.

1.6 Hypothesis

The hypothesis of the study was alternative. Hence, it was theorised that consumption of instant-maize porridge fortified with termite and *MO* leaf powder would improve the nutritional status of children aged three to five years old in Thulamela Municipality.

1.7 Significance of the study

The information derived from determining the effect of consuming instant-maize porridge fortified with termite and *MO* leaf powder on the nutritional status of children aged three to five years old could be useful in solving problems caused by poor nutrition, especially those arising from protein energy malnutrition (PEM) and micronutrient deficiency in children under the age of five years old in resource-poor communities. These findings might also assist in the promotion of commercial production of instant-maize porridge fortified with termite and *MO* leaf powder, as the findings are positive. Dissemination of the findings of the study may be of use in guiding policy makers in the Department of Health, Basic Education and Social Development in Limpopo Province to implement malnutrition alleviation programmes and may benefit the community at large.

1.8 Conceptual framework of the study

Figure1.1 presents the conceptual framework of the study. The study focused on improving the nutritional status of preschool children in Thulamela Municipality. Malnutrition is a serious global health problem brought about by inequalities in the economic systems and social injustices, resulting in incidences of underweight and stunting among children. In 2017 alone, 6.3 million children were reported to have died of malnutrition globally (World Health Education Service, 2018). Malnutrition is caused by several factors including poor feeding practices, inadequate dietary intake and diseases or infection (UNICEF, 2011). Termites and *MO* used in the present study to fortify instant-maize porridge are rich in essential micronutrients which are lacking in many households' diets and have been used in the production of other food products to increase the nutritional content. Therefore, this fortified instant maize porridge could be one of the sustainable strategies to alleviate malnutrition and improve the nutritional status of children under the age of five years.

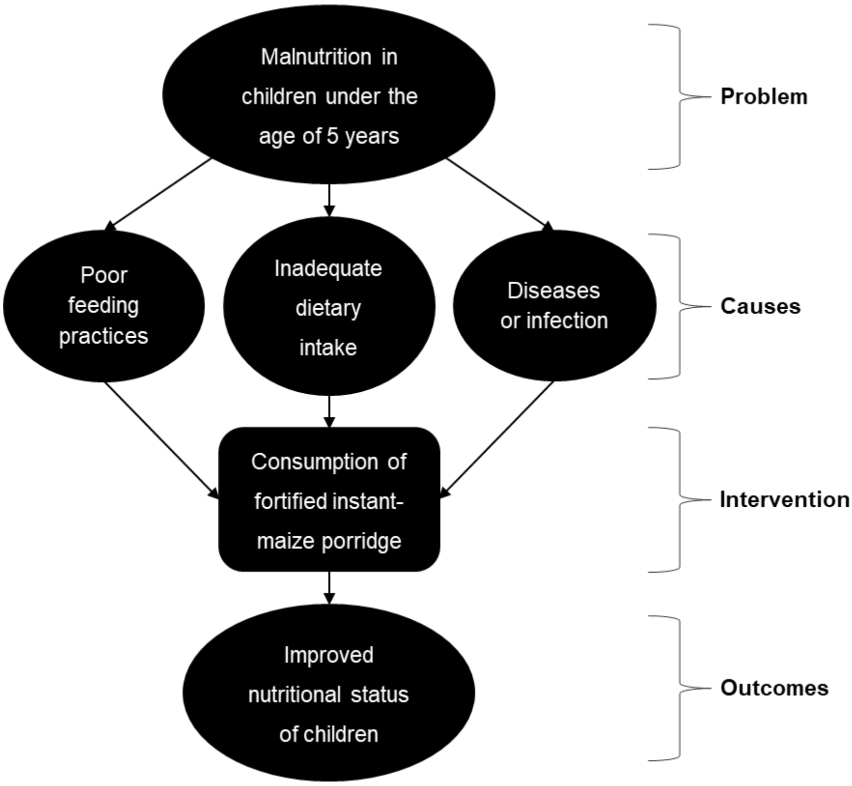

Figure1.1. Conceptual framework of the study (Adapted from UNICEF, 2011)

Chapter Two: Literature review

2.1 Introduction

This chapter presents a detailed relevant literature review of the study. It describes maize, moringa and termites and their nutritional significance in alleviating child malnutrition.

2.2 Prevalence of malnutrition

Child malnutrition remains a significant problem worldwide, even though there have been major improvements in health over past decades (WHO, 2016). In 2013, among under-five children, mortality rates were 32 per 1 000 live births, 20% were underweight and 37% were stunted. Such growth faltering often begins around 4 to 7 months of age and is accompanied by a high prevalence of anaemia and co-existing micronutrient deficiencies. Micronutrient deficiency constitute the most widespread form of malnutrition, with women and children being particularly vulnerable. It is a major impediment to socioeconomic development and contributes to a vicious cycle of underdevelopment, to the detriment of the already underprivileged groups. It has long ranging effects on health, learning ability and productivity. It leads to high social and public costs, reduced work capacity in populations due to high rates of illness and disability, as well as tragic loss of human potential. Poverty, lack of access to a variety of foods, lack of knowledge of optimal dietary practices and high incidence of infectious diseases are some of the factors which lead to micronutrient malnutrition. In growing children, the adverse effects of micronutrient deficiencies include poor growth and development, mental and neuromotor performance, immunocompetence, physical working capacity, overall reproductive performance as well as increased morbidity, mortality and risk of maternal death. More than two billion people in the world suffer from various micronutrient deficiencies.

The UNSCN (2011) further estimates that 22% of children in Asian countries are underweight. A similar trend was also estimated for African countries, where 20% of children are underweight (UNSCN, 2011). In Nigeria, the proportion of people suffering from such deficiencies and resultant diseases is at a critical level. For example, nearly 20 million Nigerians are estimated to suffer from iodine deficiency disorder (IDD), with the prevalence of goitre put at 20%; while the prevalence of nutritional anaemia, because of iron deficiency, is put at 25% among mothers and children and accounts for the very high maternal mortality in the country. Overcoming micronutrient malnutrition is, therefore, a precondition for ensuring rapid and appropriate national development.

About 23.1% (18% stunted; 5.1% severely stunted) of children in South Africa suffer from chronic malnutrition according to the National Food Consumption Survey Fortification Baseline

(NFCS-FB, 2005). Netshiheni *et al*. (2019) explained that the cause of malnutrition among South African children may be attributed to the high consumption of maize porridge, which is the staple food for most people in South Africa, as reported by Mushaphi *et al. (*2015). Maize was also reported to be a staple food for most households (80%) in Limpopo Province. There, children's diets are predominately cereal-based (high in carbohydrates), with minimal intake of animal products. Mamabolo *et al*. (2006) reported that children in Central Limpopo Province received nearly 70% of their energy intake from carbohydrates, while fat contributed less than 20%. Regardless of all carbohydrates in maize grain, maize is not a good source of essential nutrients for human health. The bioavailability of minerals (vital for proper nutrition and health) in maize are often affected by phytic acid in the maize grain that interferes with the absorption of minerals (Hulse *et al*., 1980). Maize also has low amounts of lysine and poor protein digestibility, which plays a role in the nutritional composition of amino acids (Duodu *et al*., 2003). The high consumption of maize increased the prevalence of malnutrition in children under five years in South Africa; hence, there is a need to enhance the nutritional value of maize grain to improve the nutritional status of children, especially those from underprivileged households.

2.3 Production of maize, termite and *Moringa oleifera*

The production and origin of maize, *MO* and termites are described below.

2.3.1 Maize

Maize (Figure 2.1) originated from a wild grass 7 000 years ago in central Mexico and was transfigured into a source of food by Native Americans (FAOSTAT, 2013). The United States, China and Brazil are reported to produce the highest amount (563 of the world's 717 million metric tons/year) of maize globally. Most of the micronutrients are contained in the outer layer of the maize kernel; eliminating these layers in the milling process destroys essential nutrients. Different kinds of maize are grown worldwide, with one essential variety being the colour (FAO, 2005). In Africa, yellow maize is related to low economic-social status, thus less preferred; it is regarded as food which only poor people consume. White maize is preferred over other varieties because people in these countries are more comfortable with eating white products. However, people who prefer white maize-porridge take in low amounts of β-carotene and cryptoxanthin, vitamin A precursors, which have been reported to be high in orange and yellow maize (FAO, 2015). Maize contains high amounts of energy that is utilised by humans, animals and as fuel (Gwirtz & Garcia-Casal, 2014; Ongol *et al*., 2013; Brockway, 2001). It contains good amounts of dietary fibre, while containing low amounts of fat and sodium (Sangwan *et al*., 2014).

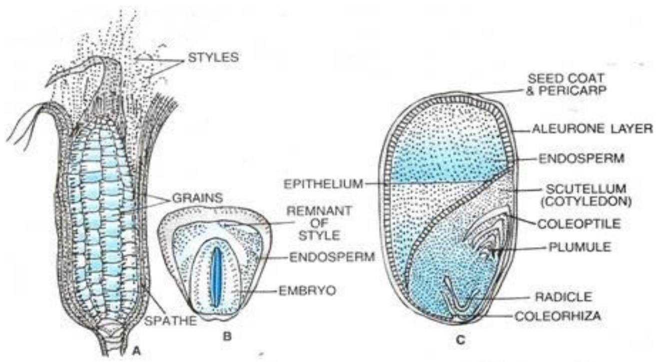

Figure 2.1. Maize (*Zea mays*) A–Cob, B–The entire grain, C–Maize grain (FAO, 2012)

Maize grains are nutritious and assist with healthy growth as they are composed of 72% starch, 10% protein, 4-8% oil, 8.5% fibre, 3% sugar and 1.7% ash (Hussain *et al*., 2003). Maize is considered an important source of carbohydrate in most developing countries. It also facilitates speedy passage of faeces through the intestine. Furthermore, it preserves the digestive tract and promotes the function of the gallbladder, reducing stomach acidity and development of certain cancers.

2.3.2 Termites (Isoptera)

Insects are used for dietary and medicinal purposes by different population groups in the world (Defoliart, 2002). Insects are utilised in about 130 countries as an essential element of the diet. An increase in the interest in insects-based food products has been observed even in the United States in current years (Dossey *et al*., 2016). Termites belong to the family *Isoptera* and play a major role in the tropical ecosystem (Figure 2.2). Termites are also used as food in sub-Saharan Africa, Asia, Australia, and Latin America—and have been for centuries. According to FAO (2010), the number of people who commonly consume termites in Asia and Africa was reported to be above 2.5 billion; this is because of their good nutritional and therapeutic properties, which could be used to eradicate malnutrition in developing countries. Termites play a major role in terms of dietary purposes in East Africa (Ayieko, 2007). In Western Kenya, termites are consumed by different ethnic groups as part of a meal or as a complete meal with tapioca, bread and roasted corn or simply eaten as a snack. Some mothers mill the dried termites into flour and sprinkle it on babies' porridge (Christensen *et al.,* 2006). Some people consume termites raw directly as they emerge from their holes in the ground (Ayieko *et al*., 2010).

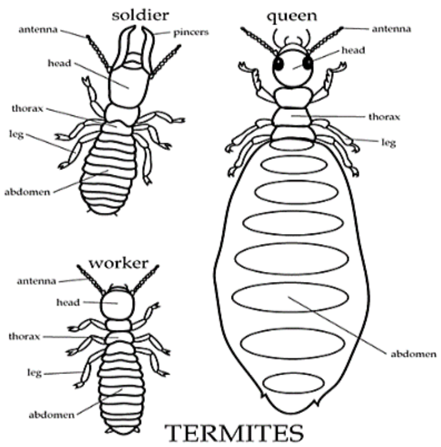

Figure 1.2. Structure of termites (Defoliart, 2002)

Termites have been reported to be a food source of great nutritional value. They are high in protein and essential amino acids such as tryptophan, micronutrients and essential fatty acids (Jongema, 2014) which are generally minimal in cereal-based foods (Defloliart, 2002).

2.3.3 Moringa oleifera (*MO*)

Moringa oleifera (*MO*) is called the 'miracle plant' or the 'tree of life' in some parts of the world. This name derives from its uses associated with its therapeutic and nutritional purpose. It originated in India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). *MO* (Figure 2.3) has been utilised for thousands of years in Rome, Greece and Egypt, where early supreme rulers of the day used *MO* leaves and its fruit in their diet to keep themselves mentally alert and healthy. *Moringa* abides under the umbrella *Moringaceae* (Nadkarni, 2006). The family *Moringaceae* is a monotypic family of single genera with around 33 species of which four are accepted, four are synonym and 25 are unassessed. Out of these, 13 species, native of old-world tropics, are documented. It has been used in Asia, particularly in India as a source of food for about 5 000 years (Anwar *et al*., 2005; Anwar & Bhanger, 2015; D'Souza & Kulkarni, 2013). Formerly, *Moringa* leaf extract was given to Maurian soldiers of India on the warfront. They believed that this elixir drink gave them strength and soothed them from the trauma and agony caused by war (Mossa, 2005). Different species of *Moringa* have gained interest because of their excellent economic potential. Of all the *Moringa* species, *MO* is the mostly utilised species because of its nutritious and therapeutic properties that have been acknowledged for many years in different parts of the world (D'Souza & Kulkarni, 2013). Mossa (2005) reported that *M. stenopetala, M. peregrine and M. concanensis* contain therapeutic and nutritional properties as *MO* (Mossa, 2005).

Figure 2.2. The *Moringa oleifera* tree

Source: Foidl et al (2001)

MO leaves should be advocated for expecting and lactating mothers because they contain high amounts of iron (Fe). *MO* is called the 'drumstick tree' or the 'horse radish tree', by some people (Anwar & Bhanger, 2015). In other places, it is called *kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna* or Ben oil tree (Anwar & Bhanger, 2015; Prabhu *et al*., 2011). *MO* contains high amounts of essential micronutrients: amino acids, β-carotene, antioxidants, protein, inflammatory nutrients with omega 3 fatty acids (Fahey, 2005; Hsu *et al*., 2006; Kasolo *et al*., 2010) and is highly recommended for expectant mothers. Fresh *MO* leaves (weight per weight) contain calcium (Ca) which is four times higher than that found in milk; the content of vitamin C is reported to be seven times higher than that found in oranges. The potassium (K) is three times higher compared to what is found in bananas; three times higher than the Fe of spinach and the amount of vitamin A was reported to be four times higher than that of carrots. *MO* leaves contain seventeen times higher protein content compared to that found in milk. The vitamin C content in dried leaves is reported to be twelve times higher than that of oranges, while the K is reported to be fifteen times higher than compared to that found in bananas, twenty-five times the Fe of spinach, ten times the amount of vitamin A in carrots, and nine times the protein in milk (Kamal, 2008; Fuglie, 2014). Moreover, *MO* is also recommended to supplement diets low in minerals as the pods are rich in minerals (Aslam *et al*., 2005).

2.3.4 Maize porridge fortification with termites

Insects link bio-diversity preservation and human nutrition in a way that many other food sources do not. Termites have substantial amounts of micronutrients, protein, fat and carbohydrates more than equal to amounts in beef or fish and a higher energy value than soybeans, maize, beef, fish, lentils, or other beans (FAO, 2012; Adepoju & Omotayo, 2014). Termites are low in anti-nutrients and it is recommended that they be included in producing adequate, nutrient-dense complementary foods. When termite powder was added to maize, it significantly increased the mineral value of all fortified weaning food at ($p < 0.05$). It was also observed that when 20% of termites were added to maize flour, the nutritional value increased compared to the blend where 10% of termites were added to maize meal flour (Jongema, 2014). When termite powders were added to maize, it resulted in a significant increase in β-carotene, niacin, vitamin B6, and B12 content. It was also observed that as the level of inclusion increased, the amount of vitamin A increased significantly. Vitamin B6 content of the formulated diets was found to be higher than what FAO/WHO recommended (Adepoju & Omatayo, 2014).

2.3.5 Maize porridge fortification with *Moringa oleifera* leaves powder

Cereal porridge is a food preferred for weaning infants and a food consumed by adults for breakfast. It was observed that as *MO* leaf powder was added to maize porridge, it improved the nutritional composition and increased its vitamin A content (Olorode *et al*., 2013). The inclusion of *MO* leaf powder to maize also increased protein, Ca, Fe and phosphorus (P) contents, according to a study conducted by Abioye and Aka (2015) & Olorode *et al*. (2013). The inclusion of *MO* leaf powder in maize was observed to influence the nutritional value and functional properties differently according to different authors (Olorode *et al*., 2013). Abioye and Aka (2015) reported that as 15% of *MO* leaf powder was added to white maize, it increased the protein value by 94%. Olorode *et al*. (2013) also confirmed that as 15% of MO leaf powder was added to yellow maize it increased the protein value by 44%. A reduction in swelling power of maize porridge fortified with 15% *MO* leaf powder was observed. The start-off protein, starch and lipid composition of the maize could have influenced the difference in swelling power.

Based on the study conducted by Olorode *et al*. (2013), it was observed that porridge fortified with 10% *MO* leaf powder can be accepted by consumers, but more panellists than what was reported by the above authors for sensory evaluation may be required. For example, it was observed that when 2.5% of *MO* leaf powder was added to stiff dough, it was acceptable to consumers and increased the nutritional composition (Karim *et al*., 2015). Abioye and Aka (2015) and Olorode *et al*. (2013) recommended a higher percentage of *MO* leaf powder to be added to stiff dough than what Karim *et al*. (2015) reported. When *MO* leaf powder is added to most foods, they tend to change the colour to dark green, which could be due to the high content of chlorophyll contained in the leaves (Karim *et al*., 2013). According to the study done by Arise *et al*. (2014), it was observed that when *MO* flower powder was added to maize and millet, it enhanced the nutritional content of complementary foods made from the two. It was also observed that when 20% *MO* flower powder was added to complementary foods made from maize and millet, it was accepted by consumers. It was reported that *MO* leaf powder was more acceptable in terms of colour and texture than *MO* flower powder when both were added to maize in large quantity. Shiriki *et al*. (2015) recently researched the nutritional value of weaning food produced from peanut, maize and soya bean fortified with *MO* leaf using albino rats. Higher protein content was observed in diets composited with *MO* than in diets with no *MO* leaves and with commercially sold weaning food fortified with other food items (Shiriki *et al*., 2015). When an experiment was done on rats, it was found that the protein efficiency ratio (PER), net protein ratio (NPR) and feed conversion efficiency (FCE) of the weaning food was higher when 10% of *MO* leaves were added. Moreover, the addition of *MO* leaves to maize and millet improved the apparent digestibility (AD) as compared to Nestle brand "*Cerelac*", which is known to be a favoured weaning food (Shiriki *et al*., 2015). However, a decrease in the quality of protein parameters (PER, NPR, FCE and AD) was reported when 15% of *MO* was added to the diets. Shiriki *et al*. (2015) reported that the quantity of *MO* leaves ingested by the experimental rats was low, due to the reportedly bitter taste of *MO* leaves.

Chapter Three: Phase 1

Vitamin, mineral content and microbial activities of instant maize porridge as influenced by the addition of *Moringa oleifera* leaves and termite powders

Abstract

In this chapter, the fortified instant-maize porridge was prepared, and the safety and nutrient content of the product was assessed.

Background: Fortification of cereal-grain products was first introduced in 1941 to reduce malnutrition. Iron and three vitamins were added to flour and bread to increase the dietary intake of micronutrients and reduce malnutrition. Later, the South African Department of Health embarked on mandatory food fortification of staples such as maize, as a strategy to reduce malnutrition, thus enhancing the nutritional and well-being status of people, particularly in rural places. However, the prevalence of malnutrition remains high in South Africa, irrespective of nutrition intervention strategies such as nutrition education, food fortification and vitamin A supplementation.

Aim: To investigate the effect of *Moringa oleifera* (*MO*) leaves and termite powders on the nutritional properties of an instant-maize porridge.

Methods: Samples were prepared in the Department of Food Science and Technology, School of Agriculture, University of Venda, South Africa. *MO* leaves and termite powders were composited to make an instant-maize porridge, at different ratios using a completely randomised design. The control porridge was composed of 100% maize and the experimental of 80% maize, 5% cooked *MO* leaves and 15% termite. The minerals were determined using a polarised Zeeman atomic absorption spectrophotometer model ZA3000 from HITACHI. Vitamins were determined using the AOAC's standard method (2010). The PCR method was used for microbial analyses. Data from two replicates was analysed using SPSS version 24.

Results: Vitamin A and folic acid content significantly ($p < 0.05$) increased from 107 and 173 in the control to 139 µ/100 g and 226 µ/100 g in the fortified porridge respectively. The iodine and potassium content of the fortified porridge increased compared to the control porridge. Microbial activities of the fortified sample were found to be within what the South African National Accreditation System (SANAS) and South African Bureau of Standards (SABS) recommend.

Conclusion: The addition of *MO* leaves and termite powders to maize porridge has led to a substantial increase in the nutritional value of maize porridge. The study gives insight into the effect of incorporating *MO* leaves and termite powders with maize porridge.

Contribution: The study gives insight into the effect of incorporating *MO* leaves and termite powders with maize porridge.

Keywords: Instant-maize porridge, Malnutrition, Minerals, Microbial activities, Moringa, Termites, Vitamins

3.1. Background of the study

Child malnutrition remains a significant problem worldwide, even though there have been major improvements in health over past decades. In 2013, mortality rates were 32 per 1 000 live births among children under five years, of whom 20% were underweight and 37% were stunted. Such growth faltering often begins around 4-7 months of age and is accompanied by a high prevalence of anaemia and co-existing micronutrient deficiencies. Micronutrient deficiencies constitute the most widespread form of malnutrition, with women and children being particularly vulnerable. Growing children are the ones mostly affected with micronutrient deficiencies resulting in poor growth and development, mental and neuromotor performance, immunocompetence, physical working capacity, overall reproductive performance as well as increased morbidity, mortality and risk of maternal death (WHO, 2020).

Despite several advances and improvements in child health, malnutrition remains one of the main public health concerns of the 21st century, particularly in poor countries. According to a study done by SANHANES (2016), the prevalence of stunting in children under five was found to be 39.4% in 2010, while in 2013 it was 38.4%. This shows that there was no significant improvement in the nutritional status of children from 2010 to 2013 in South Africa. Labadarios *et al*. (2015) even reported an increase in stunting among children under the age of five years in South Africa. Amongst the nine provinces in South Africa, Limpopo was found to one with the highest prevalence of malnutrition in children under the age of five years. The rate of stunted children there was found to be higher than the national average of 23.1%. Mamabolo *et al.* (2006) observed that 35% and 48% of children at one and three years respectively were stunted in the central region of Limpopo. Mamabolo *et al.* (2006) and the NFCS (2007) further revealed that chronic malnutrition is still a major problem there. Regardless of nutrition intervention strategies employed to address child malnutrition, the prevalence, therefore, remains high. This frequency could also be attributed to the high consumption of maize (Figure 3.1), as it contributes significantly to the dietary intake of many households in the Vhembe District, Limpopo Province but is deficient of protein, vitamins and minerals. In addition, Netshiheni *et al*. (2019) emphasised that maize porridge has been reported to be consumed by 80% of the population in South Africa, contributing significantly to the dietary intake of households in Limpopo as reported by Mushaphi *et al.* in 2015. However, the bio-availability of minerals which are vital for proper nutrition and health is often affected by phytic acid, which is contained in the maize grain.

Figure 3.1. Maize (*Zea mays*)

Source: (https://www.google.com/search?q=maize+pictures&tbm=isch&chips=q:maize+pictures,g_1:yellow:DoSeKY8i3ac%3D&rlz=1C1OKWM_enZA967ZA969&hl=en&sa=X&ved=2ahUKEwjD5e_yqbjzAhXYBGMBHYcHD2kQ4lYoAXoECAEQFA&biw=1263&bih=520 (Accessed 07 May 2022)

Phytic acid interferes with the absorption of minerals (Hulse *et al*., 1980), such as: Fe, Zn and Ca. Inadequate intake of these essential minerals is closely associated with Anaemia *osteomalacia,* Zn deficiency and rickets, respectively (Widdowson, 2002). Moreover, the number of pre-scholars with Zn deficiency and anaemia was reported to be 42.6% and 28% respectively in the Vhembe District in 2014 (Motadi *et al*., 2015). In contrast, *MO* and termites have been reported to be high in essential micronutrients and are a good source of protein. Therefore, incorporating *MO* and termite powders with maize porridge could be useful in reducing the prevalence of undernutrition, improving the health and nutritional status of children under the age of five. These two locally available resources have been reported to be high in proteins, minerals and essential amino acid required for proper health and nutrition. However, the utilisation of these two locally available food items is low. Hence, this study shares insight into the effect of adding *MO* and termites to maize porridge.

3.2 Materials and methods

Experimental site

The samples were prepared in the Department of Food Science and Technology, School of Agriculture, University of Venda, Thohoyandou, South Africa.

Sample collection

Purposive sampling was used for the selection of samples. Maize meal, sugar, stainless pots, stainless steel and wooden spoons were purchased from a supermarket; *MO* leaves were purchased from a farm at *Tshifudi* village and termites were purchased from street vendors, in Thohoyandou. The samples were transported to the Department of Food Science and Technology and stored at room temperature (25˚C) until they were analysed.

Experimental design

The inclusion of *MO* leaves and termite powders in instant-maize porridge, at different treatments was considered. The experiment was set up as a completely randomised design. The factor levels were: T_0-control (100% maize, 0% *MO* leaves and 0% termites); T_1 (80% maize, 5% blanched *MO* leaves and 15% termite powders). The addition of *MO* leaves and termite powders (independent variables) at different treatments increased the nutritional content of an instant-maize porridge. Each experiment was done in triplicate and the statistical model was: $Yij = M + Ti + Eij$, where:

Yij = Observation

M = Overall mean

Ti = Effect of i^{th}treatment on instant maize porridge

Eij = Random error.

Sample preparation

Maize meal was cooked as *Phuthu* to make porridge at 92 ºC for 35 minutes, cooled at temperatures between 25–30 ºC for 30 minutes and oven dried at 50 ºC for eight hours (Gutierrez *et al*., 2007). It was then milled using a hammer mill and packaged in a polyethylene bag. A stainless-steel metal was used to fabricate the hammer mill. *MO* leaves were destalked, washed and cooked at 92 ºC for 15 minutes, cooled at temperatures between 25–30ºC for 15 minutes, oven dried at 50 ºC for two hours (Aslam *et al*., 2005) and milled to powder using a hammer mill and packaged in a polyethylene bag. Termites were purchased from street vendors around Thohoyandou, cooked at 95 ºC for 30 minutes; oven dried for 12 hours at 50 ºC (Ayieko *et al*., 2010), milled using a hammer mill and packed in a polyethylene bag. The processing diagram of fortified instant maize porridge is shown in Figure 3.2.

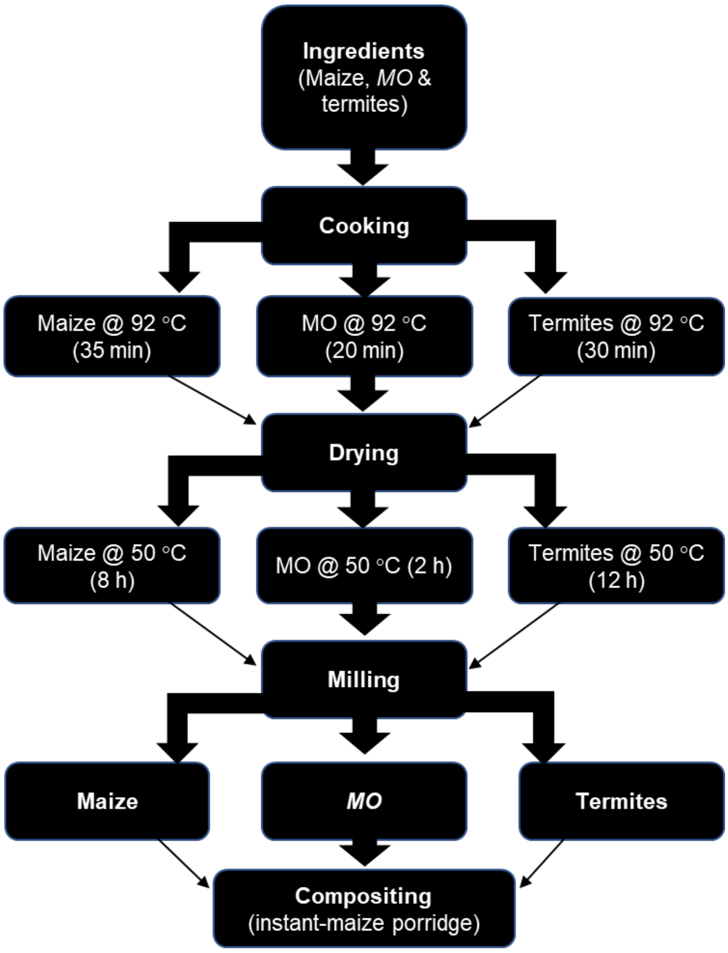

Figure 3.2. Processing diagram of fortified instant maize porridge development (Gutierrez *et al.*, 2007)

Potassium, iodine and folic acid analysis of instant maize porridge

The samples (5.0 g) were weighed into clean porcelain crucibles and subjected to dry ashing in a muffle furnace (Model: SHIMADEN SR1) at 550 °C for five hours. The resultant ash was dissolved in 5.0 ml of HNO3/HCl (1:2) and heated gently on a heating metal until the brown fumes disappeared. Then they were transferred into conical flasks and 5.0ml of deionised water was added into each flask and heated until a colourless solution was obtained. The mineral solution was filtered into a 25.0 ml volumetric flask through double filter papers and was made up to the mark with deionised water. The solutions were analysed in triplicate for potassium and iodine, using the polarised Zeeman atomic absorption spectrophotometer of the model ZA3000, HITACHI [23].

Folic acid, vitamin A and vitamin C analysis of instant maize porridge

Folic acid and vitamin A content were determined using the procedure described by the standard method of AOAC (2010). A sample of 5.0 g was soaked in 20 ml of acetone overnight and filtered into a separating funnel to which 10 ml of Polyethylene terephthalate (PET) ether was added. The resultant solution was shaken vigorously and allowed to stand for some time with the lid open. The top layers were collected, and the absorbance was taken at 452 nm using the UV–Vis spectrophotometer (Model: UV mini-1240 SHIMADZU, Serial no: A109347). Ascorbic acid/vitamin C content was determined using the 2.6-dichloroindophenol titrimetric method as described by the standard method of AOAC (2005).

Microbial Activities

Salmonella and *Listeria monocytogenes* were analysed using the PCR method to amplify specific fragments of bacterial DNA, which are stable and unaffected by growth environment. The fragments that were amplified related to the genetic sequences unique to Salmonella or Listeria monocytogenes, thus providing a highly reliable indicator of whichever organism was present. *Coliform Count and E. Coli* were analysed using a coliform count to determine the amount of coliforms such as *E. coli* and others in the samples by plating a small amount of product onto a specific medium and then counting the number of coliform-forming colonies. *E. coli* and *E. coli 0157:H7* were also analysed using PCR technology to determine if *E. coli* was present in the samples. Two different testing protocols were used to identify samples containing all *E. coli* species and samples containing the *E. coli* 0157:H7 strain. A portion of the samples was diluted and plated onto a selective medium. The number of colonies that formed was counted and from this, the amount of *Staphylococcus aureus* in the sample were determined. Yeast and mould were determined by diluting the samples and plating onto a selective medium. Total viable count was also establshed whereby samples were mixed with a specific medium allowing the bacteria to multiply and counting the bacterial colonies that formed.

Colour analysis of instant-maize porridge

Colour of raw and prepared samples was determined using Lovibond LC 100 Spectrocolorimeter (Mart'inez-Romeo *et al*., 2015), following the recording of individual L^{*}, a^{*} and b^{*}, c^{*} and h° parameters.

Texture analysis of instant-maize porridge

The texture analysis was done using TA-XT *plus* Texture Analyser. To make the porridges, boiling water (95 ºC) was added to the samples with manual stirring. Texture was measured after the porridge (50 ml achieved room temperature (25 ºC). A Perspex cylinder probe (SMS P/20p) of 20 mm diameter was used. The penetration of probe into the product was 8.0 mm and the test speed was 2.0 mm/s with the same post-test speed.

Ethical clearance

Ethical clearance was sought from the University of Venda Research Ethics Committee to conduct the study.

Statistical analysis

All analyses were carried out in triplicate. Results from this study were analysed using statistical Package of the Social Sciences (SPSS version 25). Data was subjected to analysis of variance and means for vitamins A and C and mineral property content separated using Duncan's multiple range test at $p < 0.05$. Each experiment was done in triplicate and the statistical model was: $Yij = M + Ti + Eij$.

Where: Yij = Observation

M = Overall mean

Ti = Effect of i^{th} treatment on instant-maize porridge

Eij = Random error

test at $p < 0.05$ (Duncan, 1955).

Results

Potassium, iodine and folic acid content of instant maize porridge

Table 3.1 shows the mineral composition of fortified and control samples. When compared to the control sample, the fortified sample contained significant amounts of potassium, iodine, and folic acid. The fortified sample's potassium (K) content increased from 1 100.20 to 2 399.90 g/100 g.

Table 3.1: Mineral and vitamin content of instant-maize porridge

Composition	Control	Fortified maize porridge
Potassium (µg/100g)	1100.20 ± 0.39a	2399.90 ± 0.03b
Iodine (µg/kg)	10.00 ± 0.02 a	47.00 ± 0.001b
Folic acid (µg/100g)	173.00 ± 0.04a	226.00 ± 0.02b
Vitamin A (µg/100g)	107.00 ± 0.04a	149.00 ± 0.02b
Potassium (µg/100g)	1100.20 ± 0.39a	2399.90 ± 0.03b

ANOVA Test: Independent variables

The fortified sample's iodine content increased from 10 g/l in the control to 47 g/l in the fortified porridge and the folic acid content of the fortified sample increased from 173 to 226 g/100 g when compared to the control sample in this study.

Vitamin content of instant maize porridge

The average levels of vitamins A and C are shown in Table 3.1. The fortified sample had the highest vitamin A content (149 g/100 g) when compared to the control sample (107 g/100 g). The fortified sample's vitamin C content increased from 6 to 20 mg/100 g.

Microbial activity of instant-maize porridge fortified with MO leaves and termite powders

Table 3.2 shows the microbial activities of fortified and unfortified maize porridges. In coliform and total viable bacteria counts, there was no significant difference between the fortified and unfortified samples at $p < 0.05$. Listeria monocytogenes and Salmonella were also found to be absent in both fortified and unfortified samples. The presence of E. Coli was not detected in the unfortified sample and was <10 in the fortified sample. The fortified sample did not contain Salmonella or Staphylococcus aureus, but the unfortified sample contained 20 staphylococcus counts. Mould increased from 0 (unfortified) to <10 (fortified). The yeast count in the unfortified sample increased from 6.3 to <10 in the fortified sample. The total viable bacterial count (TVBC) increased from 06 (unfortified) to <10 in the fortified sample.

Table 3.2: Identification of microorganisms in instant-maize porridge

Identification	Control	Fortified Maize porridge
Clostridium perfringens	No growth	No growth
Coliforms	05	<10
E. Coli	No growth	<10
Listeria monocytogenes	Absent	Absent
Mould	No growth	<10
Salmonella spp	Absent	Absent
Staphylococcus aureus	20	No growth
Total Viable count	06	<10
Yeast	4.3	<10

SANAS Test methods

Colour and texture attributes of instant-maize porridge

The colour and texture characteristics of instant-maize porridge are shown in Table 3.3. The L*, b*, and c* values of the control porridge were higher at $p<0.05$ than those of the fortified instant-maize porridge, and the control porridge's lightness was higher than that of the fortified porridge. With the addition of *MO* leaves and termite powder, the L* value of fortified (34.70) instant-maize porridge decreased when compared to the control (61.61). At $p<0.05$, the texture of the control sample (37.67) was significantly harder than the fortified sample (30.17).

Table 3.3: Colour and texture attributes of instant-maize porridge fortified with leaves and termite powder (n = 06)

Values	Control	Fortified porridge
L^*	61.61 ± 0.86^b	34.70 ± 1.73^a
a^*	-1.46 ± 0.42^a	1.93 ± 0.55^c
b^*	10.03 ± 0.38^a	13.70 ± 1.47^c
c^*	10.07 ± 0.58^a	16.70 ± 0.91^c
h°	191.10 ± 12.56^b	83.47 ± 1.26^a
Texture	37.67 ± 2.15^d	30.17 ± 4.90^c

ANOVA Test

3.3 Discussions

When compared to the control sample, the fortified sample contained significant amounts of potassium, iodine, and folic acid. The fortified sample's potassium (K) content increased from 1 100.20 to 2 399.90 g/100 g. The results of this study are similar with those of Karim et al. (2013), who found that after adding *MO* leaf powder to the fortified Amala (stiff dough), the K content of the fortified Amala increased. This increase could be due to what Surbhi et al. reported in 2017, that *MO* is high in potassium, which has health benefits beyond traditional nutrients for normal body functioning and disease prevention. Potassium aids the body in maintaining normal water balance in cells, transmitting nerve impulses, balancing acids, and alkalis, and stimulating normal intestinal movement (Gordon, 1999). The fortified sample's iodine content increased from 10 g/l in the control to 47 g/l in the fortified porridge in this study. Maize has been found to have a high iodine content (100.963.50 mg/l) among cereals. This could also be explained by Prasanthi *et al*. (2017)'s finding that processing methods can alter the mineral content of food. According to Jideani and Dietricks (2014), *MO* differs in nutrient composition depending on where it is grown; the nutritional composition of the *MO* tree grown in Nigeria may differ from that of the *MO* tree grown in South Africa.

In the present study, the folic acid content of the fortified sample increased from 173 to 226 g/100 g when compared to the control sample. Folic acid is the fully oxidised monoglutamate form of the vitamin, which can be found in supplements and fortified foods but is uncommon in nature. Vitamin B12 (cobalamin) is synthesised by only a few microorganisms, and animal foods are the primary source of the vitamin (FAO/WHO, 2020). Deficiency in folate and cobalamin is a worldwide problem. Despite a lack of data, it is estimated that over 20% of women of reproductive age in low- and middle-income countries are folate deficient (Rogers *et al*., 2018), and Sukumar *et a*l. (2016) found that global prevalence of vitamin B12 insufficiency during pregnancy was 19–29%, with higher rates on the Indian subcontinent and in the United States. Folate and vitamin B12 are needed for Deoxyribose nucleic acid (DNA) and Ribose nucleic acid (RNA) synthesis, as well as serving as cofactors in the conversion of homocysteine to methionine, which is needed for neurotransmitter and phospholipid synthesis. Elevated plasma homocysteine levels are linked to a higher risk of negative pregnancy outcomes (stillbirths, preeclampsia, very low birth weight, preterm delivery, and neural tube defects) in women who are deficient in folate and vitamin B12 (Bergen et al., 2012; Mujawar, Patil & Daver, 2011; Rogers *et al*., 2018). Low folate and vitamin B12 levels have also been linked to megaloblastic anaemia and cognitive impairment in the elderly (Morris, Jacques, Rosenberg, & Selhub, 2007). Because folate and vitamin B12 are required for normal embryogenesis, researchers are paying more attention to young women's folate and vitamin B12 status during the periconceptional period and during pregnancy. Vegans, vegetarians, the elderly with impaired absorption, and people living in countries where animal products are not consumed, either for religious reasons or due to poverty, are all at risk of vitamin B12 deficiency (Allen, 2009; Pawlak, Lester, & Babatunde, 2014). Saini *et al.* (2016) found that the relative bio-availability of folic acid from *MO* leaves was extremely high (approximately 82 %) in a rat model, suggesting that *MO* leaves could be a potential source of dietary folate. Folic acid is a biochemical co-factor that serves as acceptors and donors of single-carbon units in a variety of reactions involved in amino acid and nucleotide metabolism. During various stages of life, such as pregnancy, lactation, and childhood, folic acid requirements have been shown to increase. Folic acid deficiency, which can occur during pregnancy, has been linked to megaloblastic anaemia. This fortified instant maize porridge may therefore be recommended for people with megaloblastic anaemia.

Vitamin content of instant maize porridge

The fortified sample had the highest vitamin A content (149 g/100 g) when compared to the control sample (107 g/100 g). Arise *et al*. (2014) and Olorode *et al*. (2013) found a 15-fold increase in vitamin A content when *MO* leaf powder was added to ogi (A fermented cereal porridge from West Africa). The effect of *MO* leaf powder on food nutrient content and functional properties varies depending on the author. According to fortification principles, maize meal is fortified with protected and stabilised vitamin A palmitate to improve the stability of the added vitamin. Because the protected particles are distributed unevenly throughout the maize meal, the precision of the analyses may be harmed (Blake, 2007). This could lead to maize meal segregation, resulting in vitamin A levels that differ from those reported by other researchers. This variation could be the result of faulty equipment or different processing methods, resulting in varying nutritional quality (Asante *et al*., 2015). According to Asante *et* *al*. (2015), the high temperature used during the drying of the ingredients in this study could have caused this variation because vitamin A is heat sensitive. Fresh *MO* contains four times the vitamin A (carotene) found in carrots, according to Olushola (2006) (6 780 mg vs. 1 890 mg/100g). Vitamin A is required for the growth and metabolism of all body cells. It is required to produce rhodopsin (visual purple), a complex compound containing retinol and protein. Rhodopsin is a pigment found in the retina, a membrane at the back of the eye that is necessary for low-light vision. Vitamin A is also required for the maintenance of healthy skin and surface tissues, especially moist mucous membranes such as the cornea of the eye and the lining of the respiratory tract. Deficiency causes stunted growth, night blindness, and decreased resistance to infection due to the state of the mucous lining of the respiratory tract. When the tear glands become blocked, the membranes in front of the eyes become dry and inflamed; this is known as 'xeropthalmia.' Corneal ulceration and blindness can result from a severe and long-term deficiency. Olushola (2006) 's Recommended Daily Allowances (RDAs) for 1-3 year-olds are met in this study's vitamin A content.

The fortified sample's vitamin C content increased from 6 to 20 mg/100 g. When Bothwell (2007) compared four Moringa cultivars, it was discovered that *MO* had the highest amount of ascorbic acid (Vitamin C) compared to the other three. The increase in vitamin C content is consistent with the findings of Saskin and Martin (2008), and it could be due to a substitution effect, as Moringa leaves are known to contain high levels of vitamins A, C, and B. (Fugile, 2001). Fresh *MO* leaves contain seven times the vitamin C found in oranges (220 mg vs. 20 mg/100 g), according to Olushola (2006), while *MO* leaf powder contains 17.30 mg/100 g, according to Fulgie (2001). Collagen, the main protein of connective tissue, is the packaging material that separates, protects, and supports various organs, and vitamin C is required for its formation. It also helps with iron absorption from the intestine. Only man, monkeys, and guinea pigs require a dietary source of vitamin C, as most other animals synthesise ascorbic acid from glucose in their body cells. Scurvy is a condition caused by a lack of vitamin C, with the main symptoms being bruising and spontaneous hemorrhaging under the skin. Gums turn black and spongy, and wounds and fractures don't heal properly. The failure to form connective tissue is the root of all these symptoms. Anaemia can also be caused by a lack of iron absorption and the inability to form red blood cells.

In coliform and total viable bacteria counts, there was no significant difference between the fortified and unfortified samples at $p < 0.05$. Listeria monocytogenes and Salmonella were also found to be absent in both fortified and unfortified samples. This could indicate that eating the fortified instant maize porridge carries fewer or no risks. The presence of E. Coli was not detected in the unfortified sample and was <10 in the fortified sample. The slight increase in E-coli count could be due to chemical reaction of the composition of different ingredients used to produce the fortified porridge. This is in line with a study by Ejibo *et al*. (2020), which found that the microbial activity of spiced ogi increased as compared to unfortified ogi. This, however, contradicts the findings of John *et al*. (2017). According to John *et al*. (2017), the total bacterial count in untreated samples was higher than in treated samples. The fortified sample did not contain Salmonella or Staphylococcus aureus, but the unfortified sample contained 20 staphylococcus counts. *Shigellashinga* and six Gram-positive bacteria like *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus-B-haemolytica, Bacillus subtilis, Sarcinalutea,* and *Bacillus megaterium* have antibacterial activity in methanolic, ethanolic, and chloroform extracts of leaves (Surbhi *et al*., 2017). This is also consistent with Masurekar's *et al*. (2014), which found that leaf extracts were effective against fungus strains like *Alternaria species (sp), Colletotrichum sp, Curvularia sp,* and *Fusarium sp*. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found to be inhibited by fresh leaf juice and aqueous extracts from the seeds (Caceres *et al*., 1991). One of the antibacterial compounds found in Moringa is pterygospermin (Horwath *et al*., 2011). The activity of the plant is comparable to the synthetic antibiotic kanamycin (Zade *et al*., 2013).

Mould increased from 0 (unfortified) to <10 (fortified). The yeast count in the unfortified sample increased from 6.3 to <10 in the fortified one. The presence of yeasts in a product, according to Enwa *et al*. (2011), is a good sign because yeast stimulates lactic acid bacteria, which are a source of soluble nitrogen compounds and vitamin B. Furthermore, microbial combinations of lactic acid bacteria and yeasts may have a significant impact on the nutritional content and stimulate the product's organoleptic properties (Dada & Muller, 1983). According to a study published in 2020 that looked at the microbial quality of flour samples over the course of six months, the microbial load of samples packed in PET/aluminium bags did not change and remained acceptable for either total aerobic mesophilic bacterial count (TAMBC) and total mould or yeast count (TMYC). When stored at 65 % RH (Relative humidity), samples packed in paper bags remained acceptable for six months, whereas those stored at 85 % RH increased and exceeded the acceptable limits after 1.5 months. It was also discovered that relative humidity and type of packaging were the most important factors influencing TAMBC and TMYC, with relative humidity being more important for mould and yeast growth than for aerobic bacteria growth. The importance of the interaction between RH and packaging also confirmed that the type of packaging had a significant impact on RH influence. The total viable bacterial count (TVBC) increased from 06 (unfortified) to 10 in the fortified sample. This could be due to the availability of some easily metabolisable nutritional components found in grains and legumes, which are required for microbe growth (Adebayo, Ogunsina & Gbadamosi, 2013). According to Nsofor *et al.* (2014), the TVBC in this study is lower than 10 cfu/ml staphylococcal count. Furthermore, several factors can influence microbial growth in maize grain and other cereals. Locations, where grains are planted, harvested, handled, and processed, are examples of such factors. Temba (2020) went on to say that water activity (a^w) in the powder of the ingredients can also influence microbial growth, with the higher the a^w, the more likely that microbes will thrive. This correlation, however, may differ from species to species.

Colour and texture attributes of instant-maize porridge

The L*, b*, and c* values of the control porridge were higher than those of the fortified instant-maize porridge, and the control porridge's lightness was higher than that of the fortified porridge. The control and fortified samples' L*, a*, b*, c*, and h values, on the other hand, were significantly different at $p < 0.05$. With the addition of *MO* leaves and termite powder, the L* value of fortified (34.70) instant-maize porridge decreased when compared to the control (61.61). It is possible that this is due to termite prodigiosin. Prodigiosin is a natural red pigment produced by termites (Song *et al*., 2006). It is a tripyrrol-structured alkaloid secondary metabolite that is unique. This pigment has been shown to have antibacterial, antifungal, antimalarial, and antineoplastic properties (Khanafari *et al*., 2006). After adding *MO* leaves and termite powder, the greenness of the control (-1.46) instant-maize porridge was found to be lower than that of the fortified (1.93) instant-maize porridge (Table 2.3). The addition of *MO* leaves and termite powder to instant-maize porridge increased the b* values from 10.03 to 13.70. The inclusion of *MO* leaves and termite powder in instant-maize porridge resulted in a colour darker than the common maize porridge, due to the presence of chlorophyll and carotenoid pigments in *MO* leaves (Muhammad & Waraporn, 2017). Significant amounts of carotenoids (-carotene) and chlorophyll have been found in *MO* leaves, both of which have been shown to have potent antioxidant properties (Owusu, 2008). Photosynthesis, the conversion of light energy into chemical energy in organic compounds, is enabled by plant pigments (Chlorophyll A, Chlorophyll B, and carotenoids). Carotene, a carotenoid, is also known as pro-vitamin A because it is converted to vitamin A, which protects human eyes (Owusu, 2008).

At $p < 0.05$, the texture of the control sample (37.67) was significantly harder than the enhanced sample (30.17). The addition of *MO* and termite powder improved the textural properties of the fortified sample, making it softer than the control sample. This could be due to the lower starch content in fortified samples, which was reduced when *MO* leaves and termite powder were added. *MO* leaves, like termites, are known to be low in starch (Rajaratnam *et al*., 2010). Furthermore, differences in the amylose-amylopectin ratio in maize, *MO* leaves and termites could account for the softer texture in enhanced samples. As a result, fortified porridge in this study is recommended, as it would be easily digestible by infants and young children.

3.4 Conclusion

The South African Department of Health began food fortification, specifically mandatory maize fortification, as one of the nutrition intervention strategies to reduce malnutrition and improve people's nutritional and well-being status, particularly in rural areas. Furthermore, food fortification has been used to treat or prevent widespread nutrient intake shortfalls and associated deficiencies, as well as to balance a diet's total nutrient profile, restore nutrients lost during processing, and appeal to consumers seeking to supplement their diet. The addition of *MO* leaves and termite powders to maize porridge, which is a staple food for the majority of South Africans (80 %) and is consumed almost every day for breakfast as thin or thick porridge with meat or vegetables by both children and adults, has resulted in a significant increase in the nutritional value of maize porridge. The composition of these three ingredients is crucial, because it could be used as one of the long-term strategies to combat malnutrition in Africa as a whole, where maize is a staple.

3.5 Recommendations

Future research should be considered on the efficacy of consuming instant-maize porridge fortified with *MO* and termites for the nutritional status of children under five years.

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3.7 Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

3.8 Authors contributions

The principal investigator (Netshiheni KR) collected and analysed the data. Nesamvuni CN and Mushaphi LF directed and supervised the research process. All authors contributed to designing the proposal, drafting the manuscript, and approving the final version as manuscript for publication.

3.9 Data availability statement

Data is available from the corresponding author on request due to privacy or other restrictions.

3.10 Disclaimer statement

All views in the submitted article are the authors own and not an official position of the institution or funder.

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Chapter Four: Phase 2

Nutritional status among preschool children aged 3 to 5 years in Thulamela Municipality, Vhembe District, Limpopo Province, South Africa

Abstract

Background: Limpopo Province (LP) is one of the economically poorest provinces in South Africa, with an estimated population size of 5.9 million. The poor status of the province is reflected in the findings of past studies on the nutritional status of children, where a prevalence of 42.6% for zinc deficiency and 28% for anaemia in preschool children was reported.

Aim: This study aimed to assess the nutritional status of children aged 3 to 5 years old in the Thulamela Municipality, Vhembe District.

Methodology: The study population consisted of children aged 3 to 5 years attending preschools in Thulamela Municipality, Vhembe District. Systematic sampling was used for the selection of preschools. Simple random sampling was used for the selection of study participants from each preschool to arrive at a study population size of 120. Anthropometric assessments were performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry (Stewart *et al.*, 2011). Biochemical tests were done according to the LANCET Laboratory procedures. Dietary assessment was also conducted. Data was exported into Statistical Package for Social Sciences (IBM SPSS version 26) and categorised as frequencies, mean, standard deviation, median and interquartile range (IQR). The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ).

Results: About 120 children from six preschools participated in the study. There were more boys (57.5%) than girls (42.5%). The majority (69.2%) of the respondents were mothers of the children, while only one child was cared for by the father (0.8%). Almost half of the parents were single (48.3%) and 30.8% were married. Most had attended Grades 11-12 (45.8%) followed by tertiary education (26.7%). Over half of the children (60%/) were dewormed and 56.7% were given vitamin A supplementation. Almost all children (99.2%) had the Road to Health booklet (RtHB). The weight-for-height z-scores (WHZ) ranged from -4.94 to 4.50, with mean (SD) of 0.38 (1.60). The BMI-for-age z-scores (BAZ) ranged from -4.99 to 4.43, with mean (SD) of 0.41 (1.57). Just over 10% of the children had adequate vitamin A status (113 to 647 units). Iron had mean (SD) of 16.7 (6.0) ranging from 6.7 to 29.4 (µg/dl) and seemed normally distributed. The majority (90.0%) were in the mild under-weight to normal to possible growth problem range on the other hand, 12.5% were overweight and three (1.3%?) were obese. Seven children were stunted, and one was severely stunted. Most of the children (108,9%) had low iodine excretion and only 10% had normal iodine excretion. All children in this study consumed a diet high in cereals and low in fruits and vegetables.

Conclusion: The biochemical results in the present study confirmed that there is still micronutrient deficiencies among preschool children in the Thulamela Municipality, Vhembe District. The micronutrient deficiencies observed among preschool children could be due to inadequate intakes of essential micronutrients such as vitamin A, zinc, iron, iodine and folate in their diet.

Keywords: Dietary patterns, Micronutrient status, Stunting and Wasting

4.1. Background of the study

The prevalence of malnutrition has been and still is a public health problem affecting children under five years of age worldwide and requires to be prioritised (Bailey, West & Black, 2015). Malnutrition refers to both over- and undernutrition (Robinson *et al*., 1986). In South Africa, malnutrition is present both as overnutrition and undernutrition (Bradshaw *et al*., 2006:9). However, in the present study, malnutrition refers to undernutrition, which is the focus of this research. Malnutrition in children under five years constitutes the most widespread form of malnutrition resulting in poor growth and development, mental and neuromotor performance, immunocompetence, physical working capacity, overall reproductive performance as well as increased morbidity, mortality, and risk of maternal death (WHO, 2020). Despite several advances and improvements in child health; malnutrition remains one of the main public health concerns of the 21st century, particularly in poor countries. The prevalence of stunting in children under five was found to be 39.4 % in 2010, while in 2013 it was reported as 38.4% (SANHANES, 2016). The SANHANES (2016) report shows that there was no improvement in the nutritional status of children from 2010 to 2013 in South Africa. This study therefore assessed the nutritional status of children aged three to five years old in Thulamela Municipality, Vhembe District.

The National Food Consumption Survey-Fortification Baseline [NFCS-FB-I, 2005] reported that 38.7% and 63.6% of South African children were anaemic and vitamin A deficient respectively, while in 2012, the South Africa National Health and Nutrition Examination Survey (SANHANES-1) found that 11% and 43.6% of children were vitamin A and iron deficient (SANHANES-1 2012). Even though Mushaphi *et al*. (2015) reported a low prevalence of vitamin A deficiency (7.7%) and iron deficiency (3.5%) in children under the age of five years in Limpopo, another chronic malnutrition trend was observed where 28% and 42.6% of preschool children there were anaemic and zinc deficient respectively (Motadi *et al*., 2015). The deficiency of micronutrients in children under the age of five years is associated with poor dietary intakes, especially in South Africa, because cereals are used for complementary feeding and as a staple food by most (80%) people (Bailey *et al*., 2015; Berti, Faber & Smuts 2014; Mushaphi *et al*., 2017). Hence, the South African Department of Health embarked on mandatory food fortification of staples such as maize, as a strategy to address micronutrient deficiencies, thus enhancing the nutritional and well-being status of people, focused more on rural places. However, the prevalence of micronutrient deficiencies remains high in South Africa, irrespective of nutrition intervention strategies such as nutrition education, food fortification, deworming, and vitamin A supplementation.

The high prevalence of micronutrient deficiency in South Africa can be reflected by what the NFCS (1999) revealed that South African children aged one to nine years had median energy intakes that were below 67% of estimated energy requirement (EER). Several studies conducted in Africa and Asia further supported that preschool children mostly consume cereal-based diets, which leads to high intake of carbohydrates (Faber *et al*., 2001; Uppal *et al*., 2005; Iram & Butt, 2006; Manu & Khetarpaul, 2006). Smuts *et al*. (2008) also observed that more than half of the children aged zero to 71 months in rural districts of KwaZulu-Natal and the Eastern Cape seldom or never consumed meat products. The NFCS (1999) observed a similar trend where maize and brown bread were found to be amongst the most consumed food items in children aged one to nine years. Dannhauser *et al*. (2000) and Faber *et al*. (2001) also reported that maize is consumed by children predominantly in the Free State and rural KwaZulu-Natal respectively.

4.2. Methods and materials

A baseline study was conducted, whereby anthropometric, biochemical, dietary assessment and screening for eligibility of children aged 3-5 years old was done. This was before any intervention was introduced to assess the current growth, nutrient intake, and the nutritional status of the children. The baseline study involves study design, identifying a study population and area, sampling methods, measurements to be taken, measuring techniques as well as validity and reliability factors.

4.2.1. Study design

A cross-sectional study was used to assess the nutritional status of children. The quantitative method was used to gather and analyse information. A quantitative method is a technique used to gather numerical data and anything that is measurable.

4.2.2. Study area

Thulamela Municipality is Vhembe's most eastern sub-district or municipality and borders the Kruger National Park in the east. Thohoyandou is the main town of this sub-district and houses the local municipality. With an area of 5 834 km^2, Thulamela is Vhembe's smallest municipality, geographically. However, its population of 618 642 people in 2017 made it Limpopo's most populated municipality—also ranking Thulamela as the fourth most populated of all South Africa's municipalities. There is a population density of 106 persons per km^2, the highest in Vhembe District, with more than 85% of the inhabitants living in rural areas. Within the municipality, 85.7% of the dwellings are formal structures and 14.3% informal. Thulamela Municipality has an economic growth rate of 0.62%, the second lowest in Vhembe District. Agriculture is the district's main economic activity with agricultural households accounting for

45.9% of the area's total 156 594 households. Females head more than half (54.4%) of all households.

4.2.3. Study population

The study population was made up of children aged 3 to 5 years attending preschools in Thulamela Municipality. The municipality has 12 296 preschool children from 224 preschools. The list of preschools was obtained from the Department of Basic Education. Preschool children are more vulnerable to micronutrient deficiency which can have a long term, irreversible negative effect on neurological and cognitive outcomes (Prado & Dewey, 2014). If these deficiencies, however, are detected and treated in the early stages, the outcomes can be reversed, decreasing child mortality and mobility by 15% (WHO, 2015); treatment can also help in increasing the IQ score of children under the age of five years. Preschool children are usually readily accessible at preschools, and they are often representative because children from both affluent and low-income households attend pre-schools.

4.2.4. Inclusion

All children aged 3-5 years old within the selected preschools who are not allergic to one of the ingredients used to produce the fortified maize porridge in the present study had an equal chance to participate in the study.

4.2.5. Exclusion criteria

Children with known immune suppression diseases, known allergies to maize, *MO* or termites, as well as those visiting Thulamela Municipality during the data collection were excluded from the study.

4.2.6. Sampling

A simple random sampling was used for the selection of six preschools (three for control and three for the experimental group) in Thulamela Municipality. The sample size was calculated using Solvin's formula:

$$n = \frac{N}{1 + N\, x\, (e)2}$$

$$n = \frac{12296}{1 + 12296\, x\, (0.05)2}$$

$$n = \frac{12296}{1 + 12296\, x\, 0.0025}$$

$$n = \frac{12296}{1 + 30.74}$$

$$n = 387$$

Where: n = Number of samples

N = Total population

e = Error margin

The original sample size was 387 children aged 3 to 5 years; however, only 120 children participated, due to budgetary reasons. As the study was conducted during COVID19 season, most parents or guardians withdrew their children from the study, leaving only the 120. This number is still statistically significant. A systematic sampling was used for the selection children in selected preschools.

4.2.7. Subject recruitment

The researcher sought for permission from the Provincial Department of Basic Education and preschools' principals to conduct the study. The researcher visited preschools three times for recruitment purposes:

Visit one -The researcher distributed letters to preschool principals outlining the purpose and procedure of the study and setting dates on which to meet parents of the preschoolers.

Visit two - The researcher met parents of the preschoolers and explained the purpose and procedure of the study as well as the material from the information sheet (Appendix A). The researcher gave consent forms (Appendix B) for parents to read.

Visit three - During this visit, a session was conducted with parents who needed more clarity and wanted to ask questions about the research before providing consent. Signed consent forms were collected from parents at this time.

4.2.8. Measurements/assessments

This section involved anthropometric, biochemical and dietary assessment of the children aged 3-5 years old.

4.2.8.1. Instrument development

A questionnaire (Appendix C) was developed following the objectives of the study and relevant literature. This was presented to promoters for scrutiny and inputs; peer review provided yet another avenue for improving it. It was shown to the Faculty of Health Sciences Research Committee and University of Venda Higher Degree Committee for approval. The instrument was pre-tested. It consisted of three sections: Section A (socio-demographic information); Section B (anthropometry) and Section C (dietary). The questionnaire was developed by the researcher in English and translated to Tshivenda by the University of Venda's Tshivenda Dictionary Unit.

4.2.8.2. Pre-test of instrument

According to Polit and Hungler (2015), a pre-test is a small-scale version or trial run done to ensure validity of an instrument in preparation for a major study. It is conducted to identify possible weakness in the research instrument. This exercise is carried out to assist in areas such as estimating the time needed to complete the questionnaire, determining the ease with which the interviewer asks the questions and how the respondents understand them or identifying questions that are too difficult or ambiguous in terms of language or conceptualisation.

4.2.8.3. Anthropometric measurements

Anthropometric assessments were performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry (Stewart *et al*., 2011). Weight (kg) and height (cm) were measured following the procedures presented below.

i. Weight

A solar scale was used to avoid error in the schools where there is no electricity. The children were weighed without shoes and wearing light clothing. The subjects stood still in the middle of the scale's platform without touching anything, with body weight equally distributed on both feet (Norton & Olds, 1996). The scale was accurate within 0.1 kg. The zero weight on the scale's horizontal beam was periodically examined. The scale was checked continuously against an electronic scale. Weight was taken twice and the average was calculated and recorded on section B of the questionnaire to ensure accuracy.

ii. Height

Height measurements were taken using a portable height meter fitted with a metal tape measure that determines to the nearest cm. Height of children was taken without shoes and socks (bare feet) with feet flat on the floor, heels close together and against the wall. Heels, shoulder blades, and buttocks were against the wall. The shoulders were in a relaxed position, arms at the sides and the head in the Frankfort horizontal plane (looking straight ahead) (Norton & Olds, 2006). The measure was taken twice, and the average was calculated to ensure accuracy. The calculated measure was recorded on section B of the questionnaire.

The children's weight and height measurements were entered into the World Health Organization (WHO) Anthro-plus version 1.0.2 software for the calculation of weight-for-age (WAZ), height- for- age (HAZ), weight-for-height (WHZ), and body mass index (BMI)-for-age (BAZ) z scores (Table 4.1). Logistic regression was computed to assess biochemical predictors of nutritional status (HAZ, WAZ and WHZ).

Table 4.1: Z-scores classification and interpretation (WHO, 2009)

Z-score classification	Interpretation		
	WAZ	**HAZ**	**WHZ**
-3 SDs or less	Severely underweight	Severely stunted	SAM (Severe Acute Malnutrition)
Between -3 and -2 SDs	Underweight	Stunted	Wasted
Between -2 and -1 SDs	Mild underweight	Mild stunted	Mild wasted
Between -1 to +1 SDs	Normal WAZ	Normal height	Normal WHZ
Between +1 and +2 SDs	Possible growth problem	Normal height	Possible risk of overweight
Between +2 and +3 SDs	Overweight	Normal height	Overweight
+3SDs or more	Obese	Above normal	Obese

4.2.8.4. Biochemical assessment

Biochemical assessment involves the measuring of a nutrient or a metabolite in blood, faeces, or urine, or measuring a variety of other components in blood and other tissues that have a relationship to nutritional status (WHO, 2015). Biochemical tests were done in this study as they provide the most objective and quantitative data on nutritional and micronutrient status. Biochemical tests are also useful indicators of nutrient level in the tissue or fluid sampled than with other indicators (Benjamin, 2000). In this study blood samples were collected and used as one of the indicators of nutritional levels (see the procedure below).

i. Procedure for blood specimen collection

A professional nurse from LANCET was responsible for the blood collection. They gathered the tubes and supplies needed for drawing blood, positioned the participant in a chair and washed their hands. They selected a suitable site for venepuncture, by placing the tourniquet 10 cm above the selected puncture site on the participant and put on non-latex gloves and palpated for a vein. When a vein was selected, the nurse cleansed the area in a circular motion and allowed the area to air dry. They then asked the participant to make a fist, while avoiding "pumping the fist." The nurse grasped the participant's arm firmly using their thumb to draw the skin taut and anchor the vein, then swiftly inserted the needle through the skin into the lumen of the vein and collected 5 ml of blood into the tube. The needle formed a 15-30 degree angle with the arm surface. When the last tube was filled, the nurse removed the tourniquet. They removed the needle from the participant's arm using a swift backward motion and placed gauze immediately on the puncture site. They asked the participant to apply and hold adequate pressure to avoid formation of a hematoma. After applying pressure for 1-2 minutes, the nurse taped a fresh piece of gauze or plaster onto the puncture site and disposed of contaminated materials/supplies in designated containers (WHO, 2017). Vacutainer blood collection tubes were used during blood sample collections. The blood was stored in a cooler box inside the LANCET laboratory until all children's blood was drawn and analysed the same day.

ii. Iron analysis

Collected blood specimens were analysed for iron content, serum ferritin levels, serum transferrin and the % of saturation transferrin using standardised procedures at LANCET laboratories (Table 4.2).

Table4.2: Interpretation of Iron of children under five years (WHO, 2011; SANHANES-1, 2012)

Classification	Iron
Mild anaemia	10.9–10.0 g/Dl
Moderate anaemia	9.9–7.0 g/Dl
Severe anaemia	<7 g/Dl

Serum ferritin measurement was used to check iron status of children. The cut-off point of serum ferritin <12 ng/mL was classified as iron deficiency, <12 ng/mL and Hb ≥11 g/dL was classified as iron depletion while <12 ng/mL and Hb <11 g/dL was classified as iron deficiency anaemia. The cut-off points for serum ferritin are indicated in Table 4.3.

Table 4.3: Interpretation of iron status (WHO, 2011; SANHANES-1, 2012)

Classification	Serum ferritin
Iron deficiency	<12 ng/Ml
Iron depletion	<12 ng/mL and Hb ≥11 g/dL
Iron deficiency anaemia	<12 ng/mL and Hb <11 g/dL

Serum transferrin measurements were used to assess iron deficiency anaemia of children. The cut-off point of serum transferrin of >2.0 g/L was classified as normal; 1.5-2.0 g/L was classified as mild while <1.0 g/L was classified as severe. The cut-off points for serum transferrin are indicated in Table 4.4.

Table 4.4: Interpretation of serum transferrin (Gibson, 2005)

Classification	Serum transferrin
Normal	> 2.0 g/L
Mild	1.5 - 2.0 g/L
Severe	< 1.0 g/L

The cut-off point of saturation transferrin between 10%-20% was classified as normal, <15% was classified as low while >20% was classified as high. Table 4.5 indicated the cut-off points for saturation transferrin.

Table 4.5: Interpretation of saturation transferrin (Gibson, 2005)

Classification	Saturation transferrin %
Normal	10–20%
Low	<10%
High	>20%

iii. Zinc analysis

Serum zinc was measured using 125I-radioimmunoassay (Table 4.6). Enzyme-linked immunosorbent assays were used to measure serum transferrin saturation (TSAT) and ferritin (Ramco Laboratories, Inc., Stafford, TX, USA).

Table 4.6: Interpretation of zinc status in children

Zinc indicator	Classification	Reference
Serum zinc deficiency	<9.9 µmo/dl	IZINCG, 2007
Normal	65 µg/dl	Gibson, 2005

iv. Iodine analysis

Urine samples were collected from preschool children using two litre specimen containers with screw caps. The samples were kept in a cooler box with freezer blocks and aliquots (3 x 1.5 ml) and stored at -20 °C until analysis. Urinary iodine concentrations were determined using 24-hour urine procedure from LANCET. The cut-offs were used to categorise the study population by iodine status (Table 4.7).

Table 4.7: Epidemiological criteria for interpretation of iodine status in school-aged children based on median and/or range of urinary iodine concentration values (WHO/UNICEF/ICCIDD, 2007)

Median	Iodine intake	Iodine nutrition status
School age children <20µg/L	Insufficient	Severe iodine deficiency
20–49µg/L	Insufficient	Moderate iodine deficiency
50–99µg/L	Insufficient	Mild iodine deficiency
100–199µg/L	Adequate	Optimal
≥300µg/L	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

v. Vitamin A analysis

A blood sample (9 mL; vacuette Z Serum Sep Clot Activator 5-mL tube for serum) from each child was obtained. Serum was isolated by centrifugation of whole blood using a portable centrifuge, aliquoted into Eppendorf safe-lock tubes, and stored at -20 °C until all children's samples were collected (five hours), then analysed. Serum retinol was determined by a reversed-phase high performance liquid chromatography (HPLC) method, which is based on a method previously described by Catignani and Bieri, (1983) (see Table 4.8).

Table 4.8: Interpretation of Vitamin A status in children

Classification	Classification of Serum retinol levels (NFCS-FB, 2005)	Laboratory levels (Drs Du Buisson, Kramer Inc./Ing.)
Vitamin A deficiency	<10 µg/dl	<100 µg /L
Marginal vitamin A status	10–19.9 µg/dl	100–199.9 µg/L
Adequate status	20–29.9 µg/dl	200–299.9 µg/L
Normal/well-nourished status	>30 µg/dl	>300 µg/L

vi. Protein analysis

Visceral protein was measured using 125I-radioimmunoassay. Enzyme-linked immunosorbent assays were used to measure serum transferrin saturation (TSAT) and ferritin (Ramco Laboratories, Inc., Stafford, TX, USA) and interpreted (Table 4.9).

Table 4.9: Interpretation of serum protein status in children

Protein indicator	Classification	Half-life	Reference
Albumin	3.5–5.5 g/dl	18–20 days	Nutrition assessment
	35–55 g/l		
Transferrin	2.6–4.3 g/l	8–9 days	Wang, 1994
Pre-albumin	0.2–0.4 g/l	2–3 days	Wang, 1994

4.2.8.5. Dietary assessment

Dietary assessment involves measuring the quantity of foods consumed by an individual during one to several days or assessing the pattern of food use over several months (USDA, 2000). Conducting dietary assessment was important in this study, as it assisted in determining the foods commonly consumed by the participants. A 24-hour recall was used to develop a food frequency questionnaire (FFQ). The FFQ was used to collect data on frequency of food consumed and portion size. Collected data was recorded on section C of the questionnaire.

4.2.9. Fieldworkers

Nutrition graduates with a few years of work experience were recruited as fieldworkers as they were already exposed to nutritional assessment and different techniques used to assess nutritional status. Professional nurses were provided by LANCET Laboratories for blood collection. The responsibilities of nutritionists included:

- Managing the data collection process
- Preparing equipment for fieldwork
- Arranging transport to the field
- Organising lunch packs for fieldworkers.

The responsibilities of nurses included:

- Drawing blood of preschool children and storing in vacutainer blood collection tubes.

The researcher was responsible for:

- Making appointments with preschool principals
- Training field workers on how to administer a questionnaire
- Anthropometric and dietary assessment
- Standardising method of data collection.

4.2.10. Validity

Validity is the extent to which the research findings accurately represent what is really happening in the situation (Welman *et al.,* 2005). Content validity was used in the study and ensured by:

- Developing a questionnaire following the objectives of the study and relevant literature
- The instrument was presented to promoters for scrutiny, and inputs and peer review provided yet another avenue for improvement
- The instrument was presented to the Faculty of Health Sciences Research Committee and University of Venda Higher Degrees Committee for approval
- The instrument was also pre-tested and the researcher interviewed participants using the local language.

4.2.11. Reliability

Reliability is a matter of whether a particular technique applied repeatedly to the same object would yield the same results each time (Woods, 2006). The weighing scale was calibrated before use to ensure reliability and to provide accurate measurements. All analysis was carried out in triplicate and the average number was recorded down. A pre-test study was conducted to ensure clarity where the wording or phrasing may not be understood. Respondents were allowed to ask for clarification.

4.2.12. Statistical methods

Data was exported into Statistical Package for Social Sciences (IBM SPSS) and categorised as frequencies, mean, standard deviation, median and IQR. The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ).

4.2.13. Institutional approval

The proposal was submitted to the Faculty of Health Sciences, the University Higher Degrees Committee, and the Limpopo Department of Basic Education to seek permission to conduct the study. After permission was granted, the preschools were visited, and the researcher explained to the principals to obtain permission for including preschool children in the study.

4.2.14. Ethical considerations

Ethical clearance was obtained from the University of Venda Research Ethics Committee (Appendix E). The study was performed in accordance with the principles of the declaration of Helsinki (2013), Good Clinical Practices, and the laws of South Africa. The study was explained to preschool principals and the researcher sought their permission to conduct the study. Preschool principals were requested to call a meeting for the parents of preschool children. The researcher subsequently explained the purpose and procedure of the study to parents or legal guardians and gave them the consent forms to read, sign and return. Preschool children were also requested to give verbal assent. Parents or guardians who needed more clarity were requested to attend an information session before they completed the informed consent form. The researcher provided parents or legal guardians with an information sheet and explained the research and assessments, as well as the consent requirements if he/she allowed his/her child to participate in the study (Appendix A).

Although preschool children are vulnerable, this study involved no risks. The parents of the pre-scholars and the preschool children had the right to withdraw from the study at any point without being disadvantaged in any way. The preschool children did not benefit directly from participating in the study but received a food and small bag of fruits at each one of the two data collection points, as a token of appreciation for their time and commitment. Data obtained from the study was stored on a computer database in such a manner that it ensured the participants' confidentiality (use of codes instead of names).

4.3. Results

4.3.1. Socio-demographic characteristics of children under five years

About 120 preschool children (57.5%) were boys and 51 (42.5%) were girls) from six primary schools participated in the study. Their characteristics are summarised in Table 4.10. The majority (69.2%) of the respondents were mothers of the children, 30% were caregivers while only one child was cared for by the father (0.8%). Almost half of the mothers were single (48.3%) and 30.8% were married. UNICEF (2006: Online) reported that most single mothers have poor emotional and psychological status, as well as an unsupportive caregiver-child interaction which has a negative influence on child growth (Dannhauser, 2013). In terms of level of education of the parents, most had attended Grades 11–12 (45.8%), followed by tertiary education (26.7%). Most of the households did not have a child under five (43.3%) followed by one child (32.5%), two children (16.7%), and the remaining had three or more children under five (7.5%). In terms of number of members of households older than five years, most of the households had more than four members (71.7%), followed by four members (19.2%). Three quarters of the parents were not working. Lack of money due to not having a job can lead to inadequate dietary intake which results in malnutrition, especially in children, as their nutritional requirements are high (Dannhauser, 2013). In most of the households, the mother is responsible for the household income (29.2%) followed by the grandparents (28.3%). Source of employment was mainly other (51.7%) followed by social grant (30%) and retail (12.5%). In terms of monthly income, the most earned was between R1 000-R2 000 (39.2%) followed by R2001-R5000 (24.2%). Almost 90% of the parents were receiving a child grant (88.3%) and more than half had any other form of grant (55%). Most had a traditional house (97.5%), with a tap indoors (98.3%) and pit latrine (85%). The majority had no special diet (95.8%). In terms of the type of maize meal eaten, most eat Magnifisan (40%) followed by Super Maize Meal (30.8%) and Tafelberg (20.8%). Over half had done deworming (54.2%). Also, more than half had taken vitamin A (56.7%). More than 30% had suffered illness in the past 15 days (31.7%). Almost all had the RtHB (99.2%). All of them had their blood and urine taken.

Table 4.10: Socio-demographic characteristics at baseline

Characteristic	N = 120	%
Relationship with the child		
Mother	83	69.2
Father	1	0.8
Caregiver	36	30
Marital status of parent		
Single	58	48.3
Married	37	30.8
Widowed	6	5
Living together	19	15.8
Parent level of education		
Never attended school	2	1.7
Grades 1 – 4	8	6.7
Grades 5 – 7	7	5.8
Grades 8 – 10	11	9.2
Grades 11 – 12	55	45.8
Tertiary	32	26.7
(Missing)	5	4.2
Children in the household under 5		
1 Child	39	32.5
2 Children	20	16.7
3 Children	3	2.5
4 Children	2	1.7
More than 4 children	4	3.3
None	52	43.3
Members older than 5 in the household		
2 Members	2	1.7
3 Members	9	7.5
4 Members	23	19.2
More than 4 members	86	71.7
Is the parent working		
Yes	30	25
No	90	75
Person responsible for house income		
Mother	35	29.2
Father	27	22.5
Grandparents	34	28.3
Mother and father	6	5
Other	18	15
Type of employment		
Health worker	2	1.7
Educator	4	3.3

Characteristic	N = 120	%
Police	1	0.8
Retail	15	12.5
Social grant	36	30
Other	62	51.7
Monthly income		
Less than R500	3	2.5
R501 - R1 000	18	15
R1 001 - R2 000	47	39.2
R2 001 - R5 000	29	24.2
R5 001 - R10 000	8	6.7
R10 000 and above	7	5.8
Unknown	8	6.7
Child grant		
Yes	106	88.3
No	14	11.7
Any other form of grant		
Yes	66	55
No	54	45
Type of house		
Traditional house	117	97.5
Brick and mortar (modern house)	1	0.8
Shack	2	1.7
Source of water		
Tap in house	118	98.3
Communal tap	2	1.7
Type of toilet		
Flush toilet in house	11	9.2
Flush toilet outside	6	5
Pit latrine	102	85
Bush	1	0.8
Special diet		
Allergic	4	3.3
Weight loss	1	0.8
None	115	95.8
Type of maize meal		
Ace	3	2.5
White Star	3	2.5
Super Maize Meal	37	30.8
Tafelberg	25	20.8
Magnifisan	48	40
Home-made	4	3.3
Deworming		
Yes	65	54.2

Characteristic	N = 120	%
No	55	45.8
Vitamin A		
Yes	68	56.7
No	52	43.3
Illness suffered in the last 15 days		
Yes	38	31.7
No	82	68.3
RtHB		
Yes	119	99.2
No	1	0.8
Blood taken		
Yes	120	100
Urine taken		
Yes	120	100

4.3.2. Cooking energy used

The cooking energy used by the households of the 120 children is summarised in Table 4.11. Over 80% of the households use firewood coal (82.5%), more than two-thirds use electricity (67.5%), only 6.7% use gas, 1.7% use solar energy, while none of the households use paraffin, gel or cow dung.

Table 4.11: Cooking energy used by the households of the 120 under-five children enrolled in the study

Cooking energy used	N=120	%
FIREWOOD COAL		
Yes	99	82.5
No	21	17.5
COAL		
Yes	2	1.7
No	118	98.3
ELECTRICITY		
Yes	81	67.5
No	39	32.5
GAS		
Yes	8	6.7
No	112	93.3
PARAFFIN		
No	120	100
GEL		
No	120	100
COW DUNG		

Cooking energy used	N=120	%
No	120	100
SOLAR		
Yes	2	1.7
No	118	98.3

4.3.3. Food preparation

The food preparation space used by the 120 households is summarised in Table 4.12. Almost 60% of the households use an open fire outside the house to prepare their food (57.5%), about 40% use open fire inside the house (38.3%), 10.8% use a gas stove, 3.3% use a paraffin stove, 57.5% use an electric stove, none of the households use coal or a gel stove, 14.2% use a microwave oven, and only one household uses Wonder Box or other method.

Table 4.12:Food preparation

Food Preparation	N	%
OPEN FIRE OUTSIDE		
Yes	69	57.5
No	51	42.5
OPEN FIRE INSIDE		
Yes	46	38.3
No	74	61.7
GAS STOVE		
Yes	13	10.8
No	107	89.2
PARAFFIN STOVE		
Yes	4	3.3
No	116	96.7
ELECTRIC STOVE		
Yes	69	57.5
No	51	42.5
COAL STOVE		
No	120	100
GEL STOVE		
No	120	100
MICROWAVE OVEN		
Yes	17	14.2
No	103	85.8
WONDER BOX		
Yes	1	0.8
No	119	99.2
OTHER		
Yes	1	0.8

Food Preparation	N	%
No	119	99.2

4.3.4. Household food production

As shown in Table 4.13, analysis of household food production of the 120 households showed that 56.7% had a garden, almost a quarter had an orchard (23.3%), more than a fifth had a field in the household (22.5%), and 5% had a smallholder farm.

Table 4.13: Household food production

Household food production	N	%
GARDEN		
Yes	68	56.7
No	52	43.3
ORCHARD		
Yes	28	23.3
No	92	76.7
FIELD IN HOUSEHOLD		
Yes	27	22.5
No	93	77.5
FIELD AWAY FROM HOUSEHOLD		
Yes	1	0.8
No	119	99.2
SMALL HOLDER FARM		
Yes	6	5
No	114	95
LARGE FARM		
No	120	100

4.3.5. Illness

The illnesses experienced by the 120 children are summarised in Table 4.14. The most common illnesses include flu (90.8%) and throat problems (87.5%). Other less common illnesses include headache (8.3%), vomiting (4.2%), chicken pox (3.3%), worm infection (3.3%), pneumonia (2.5%), gastro-intestinal problems (2.5%), hepatitis (1.7%), earache (1.7%), stomachache (1.7%), mouth sores (1.7%), piles (1.7%), asthma (0.8%), fever (0.8%), diarrhoea (0.8%), ulcer (0.8%), and wound (0.8%). None of the children had a history of Tuberculosis (TB), COVID-19, ear discharge, tooth ache, appendicitis, allergy, tumour, itching, epilepsy or malaria.

Table 4.14: Illnesses experienced by the 120 under-five children enrolled in the study

Illness	N=120	%
FLU		
Yes	11	9.2
No	109	90.8
THROAT PROBLEMS		
Yes	15	12.5
No	105	87.5
ASTHMA		
Yes	1	0.8
No	119	99.2
PNEUMONIA		
Yes	3	2.5
No	117	97.5
TB		
No	120	100
COVID-19		
No	120	100
STOMACHACHE		
Yes	2	1.7
No	118	98.3
FEVER		
Yes	1	0.8
No	119	99.2
HEADACHE		
Yes	10	8.3
No	110	91.7
VOMITING		
Yes	5	4.2
No	115	95.8
DIARRHOEA		
Yes	1	0.8
No	119	99.2
CHICKEN POX		
Yes	4	3.3
No	116	96.7
WORM INFECTION		
Yes	3	2.5
No	117	97.5
HEPATITIS		
Yes	2	1.7
No	118	98.3
EARACHE		
Yes	2	1.7

Illness	N=120	%
No	118	98.3
EAR DISCHARGE		
No	120	100
EAR PROBLEM		
Yes	1	0.8
No	119	99.2
TOOTHACHE		
No	120	100
GASTROINTESTINAL PROBLEM		
Yes	3	2.5
No	117	97.5
MOUTH SORES		
Yes	2	1.7
No	118	98.3
APPENDICITIS		
No	120	100
ULCER		
Yes	1	0.8
No	119	99.2
PILES		
Yes	2	1.7
No	118	98.3
WOUND		
Yes	1	0.8
No	119	99.2
ALLERGY		
No	120	100
TUMOUR		
No	120	100
ITCHING		
No	120	100
EPILEPSY		
No	120	100
MALARIA		
No	120	100

4.3.6. Child characteristics and anthropometrics

Out of 120 children, 69 (57.5%) were boys and 51 (42.5%) were girls. Their ages in months ranged from 33.5 months to 66.1 months, with mean age (standard deviation [SD]) of 50.5 (7.3) months. The weight in kilograms (kg) ranged from 10.6 kg to 30.5 kg, with mean (SD) of

17.6 (3.3) kg. Their height in centimeters (cm) ranged from 81 cm to 112.5 cm, with mean (SD) of 101.4 (5.4) cm.

The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ). The weight-for-height z-scores (WHZ) ranged from -4.94 to 4.50, with mean (SD) of 0.38 (1.60). The height-for-age z-scores (HAZ) ranged from -4.61 to 2.73, with mean (SD) of -0.64 (0.99). The weight-for-age z-scores (WAZ) ranged from -3.72 to 3.50, with mean (SD) of -0.12 (1.28). The BMI-for-age z-scores (BAZ) ranged from -4.99 to 4.43, with mean (SD) of 0.41 (1.57).

4.3.7. Classification of children by anthropometrics

i. WAZ

The majority (108, 90.0%) were in the mild under-weight to normal to possible growth problem range; more than 60% of the children had normal WAZ range (75, 62.5%). Seven children were underweight: three underweight and four severely underweight. In addition, five children were overweight or obese: four overweight and one obese (see Figure 4.1).

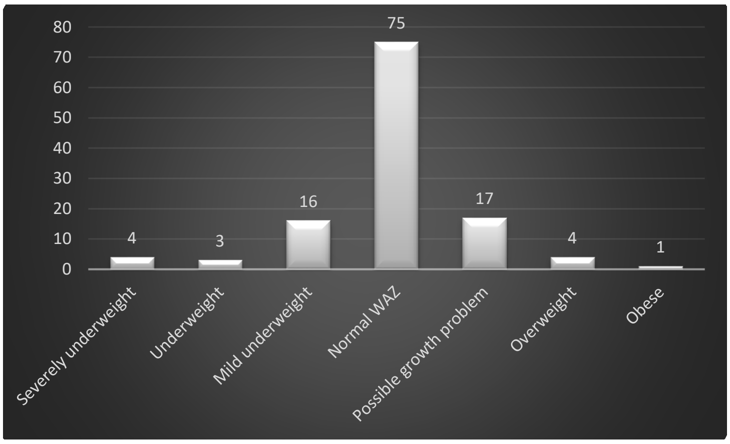

Figure 4.1. WAZ scores of study participants

ii. HAZ

The majority (112, 93.3%) were in the mild stunted to normal height range; more than two-thirds of the children had normal height range (82, 68.3%). Seven children were stunted and one was severely stunted (see Figure 4.2).

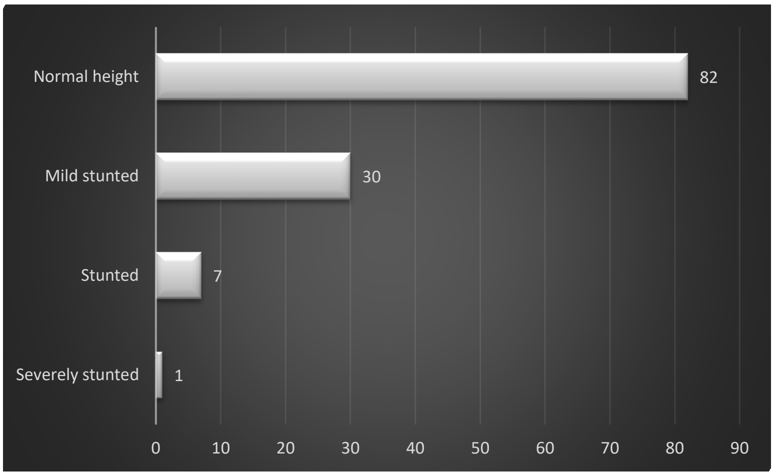

Figure 4.2. HAZ scores for study participants

iii. WHZ

The majority (95, 79.1%) were in the mild wasted to normal to possible risk of overweight range; about half of the children were having normal WHZ range (58, 48.3%). Three children were wasted and four had SAM. On the other hand, (15, 12.5%) were overweight and three were obese (Figure 4.3).

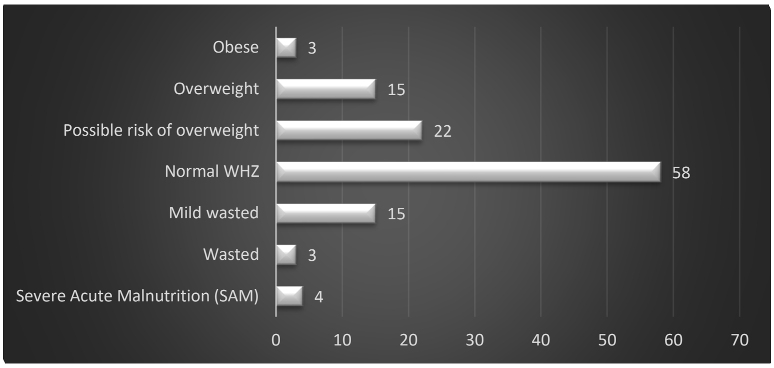

Figure 4.3. WHZ scores of study participants

4.3.8. Analysis of biochemical Results

The biochemical results for the 120 children are shown in Table 4.15. Vitamin A had mean (SD) of 72.3 (36.6); however, its distribution is skewed with median of 60.1 and ranging from 40 to 253.9. Zinc had mean (SD) of 21.4 (11.2); also skewed with median of 18.6 and ranging from 5.7 to 80.3. Iron had mean (SD) of 16.7 (6.0) and seemed normally distributed and ranging from 6.7 to 29.4. This was like serum ferritin with mean (SD) of 48.2 (21.2) and ranging from 11.6 to 124. Serum transferrin was also normally distributed with mean (SD) of 36.5 (5.7) and ranging from 25.9 to 80.9. Also normally distributed were saturation and protein with mean (SD) of 23.4 (8.8) and 69.6 (6.2), respectively. The rest of the biochemical elements were skewed, meaning they had outlying or extreme values, that is, 24-Iodine excretion, 24-creatinine, and iodine urine; their median (Interquartile range [IQR]) were 31.4 (19.1, 47.0), 1.3 (0.8, 1.8), and 73.8 (58.3, 110.5), respectively.

Table4.15: Biochemical results at baseline

Biochemical element (N=120)	Mean (SD)	Median (IQR)	Range (Min, Max)
VITAMIN A (µg/L)	72.3 (36.6)	60.1 (47.4, 85.7)	(40,253.9)
ZINC (µg/dl)	21.4 (11.2)	18.6 (16.4, 21.5)	(5.7,80.3)
IRON (ng/mL)	16.7 (6.0)	16.7 (11.6, 22.0)	(6.7,29.4)
SERUM FERRITIN (ng/mL)	48.2 (21.2)	45.0 (35.2, 61.3)	(11.6,124)
SERUM TRANSFERRIN (g/L)	36.5 (5.7)	36.4 (33.8, 38.6)	(25.9,80.9)
SATURATION (%)	23.4 (8.8)	21.9 (15.7, 31.4)	(9.3,49.2)
PROTEIN (g/l)	69.6 (6.2)	70.0 (68.0, 72.5)	(29.1,84)
24-IODINE EXCRETION (µg/L)	40.6 (35.0)	31.4 (19.1, 47.0)	(9.7,238.3)
24-CREATININE (g/d)	2.4 (9.0)	1.3 (0.8, 1.8)	(0.2,95.9)
IODINE URINE (µg/L)	98.6 (117.0)	73.8 (58.3, 110.5)	(0.72,1119.5)

SD: Standard Deviation, IQR: Interquartile Range

The researcher also categorised the children as having deficiency, normal and excess levels of these biochemical elements (see Table 4.16).

i. ***Vitamin A***

According to the Lancet Laboratories, normal vitamin A is in the range (113–647) ug/dl. Therefore, just over 10% of the children had adequate vitamin A status.

ii. ***Zinc (10.7–18.4) ug/dl***

Four children (3.3%) had zinc deficiency, 55 (45.8%) had normal zinc levels, and 61 (50.8%) had excess zinc levels.

iii. ***Iron levels (113–647) umol/l***

According to the Lancet Laboratories, normal iron is in the range (113–647) umol/l. Therefore, 10% of the children had iron deficiency.

iv. ***Serum ferritin levels (13–400 ng/ml)***

Three children had serum ferritin deficiency (2.5%), while the rest had normal serum ferritin levels (117, 97.5%).

v. *Serum transferrin levels (26–47 umol/l)*

One child had serum transferrin deficiency, the majority (117, 97.5%) had normal levels, and two children had excess levels.

vi. *Saturation (%) (20–55%)*

Forty percent (n=48) of the children had low saturation, while the rest (60%) had normal saturation of 20 to 55%.

vii. *Protein (60–80 ug/dl)*

Three children (2.5%) had low protein, the majority (116, 96.7%) had normal protein, and one child had high protein levels.

viii. *24-hour creatinine (0.98–6.04 mol/24h)*

Fifty children (41.7%) had creatinine deficiency, the majority (68, 56.7%) had normal creatinine, and two children had excessive creatinine levels.

ix. *Iodine excretion (75–851 ug/day)*

Most of the children had low iodine excretion (108, 90%) and only 12 (10%) had normal iodine excretion.

Table 4.16: Biochemical results in categories at baseline

Biochemical element	Categories	N=120	%
VITAMIN A (µg/L)	Deficiency	106	88.3
	Normal	14	11.7
ZINC (µg/dl)	Deficiency	4	3.3
	Normal	55	45.8
	Excess	61	50.8
IRON (ng/mL)	Deficiency	12	10.0
	Normal	108	90.0
SERUM FERRITIN (ng/mL)	Deficiency	3	2.5
	Normal	117	97.5
SERUM TRANSFERRIN (g/L)	Deficiency	1	0.8
	Normal	117	97.5
	Excess	2	1.7
SATURATION (%)	Deficiency	48	40.0
	Normal	72	60.0
PROTEIN (g/l)	Deficiency	3	2.5
	Normal	116	96.7
	Excess	1	0.8
24-IODINE EXCRETION (µg/L)	Normal	12	10.0

Biochemical element	Categories	N=120	%
24-CREATININE (g/d)	Severe	108	90.0
	Deficiency	50	41.7
	Normal	68	56.7
	Excess	2	1.7

4.3.9. Dietary patterns of preschool children

Data on dietary patterns of preschool children are summarised in Table 4.17 as well as medians and IQR. About 36.6% of the children had energy intakes below the RDA. More than 70% of the children had poor vitamin A intake. About 10% of the children also had poor iron intake. The majority (96.7%) of the children had normal protein intakes.

Table 4.17: Dietary intakes of nutrients by preschool children as determined by the QFFQ (median, IQR) and percentages below 67% DRI

Nutrient	FFQ	N (%) FFQ deficiency
Energy (kJ)	5 779.44 (2 570.25)	54 (45)
Total protein (g)	43.04 (16.18)	3 (2.5)
Plant protein (g)	26.44 (12.35)	
Animal protein (g)	13.51 (8.53)	
Total fat (g)	34.06 (17.44)	
SFA (g)	8.45 (5.49)	
MUFA (g)	9.95 (6.19)	
PUFA (g)	11.18 (6.89)	
Carbohydrate (g)	206.93 (84.91)	1 (0.6)
Total sugars (g)	24.01 (19.51)	
Added sugar (g)	27.85 (16.37)	
Total dietary fibre (g)	16.56 (7.11)	31 (19.1)
Calcium (mg)	257.47 (155.19)	118 (72.8)
Fe (mg)	7.67 (3.85)	13 (8.0)
Magnesium (mg)	244.47 (104.52)	1 (0.6)
Phosphorus (mg)	726.06 (331.79)	1 (0.6)
Zinc (mg)	5.08 (2.26)	1 (0.6)
Vitamin A (µg)	206.74 (237.14)	80 (49.4)
Thiamin (mg)	0.79 (0.32)	1 (0.6)
Riboflavin (mg)	0.91 (0.52)	8 (4.9)
Niacin (mg)	9.58 (5.18)	2 (1.2)
Vitamin B_6 (mg)	0.69 (0.31)	4 (2.5)
Folate (µg)	130.85 (76.87)	40 (24.7)
Vitamin B_{12} (µg)	2.39 (2.50)	13 (8.0)
Vitamin C (mg)	44.37 (31.34)	0 (0)
Vitamin D (µg)	3.66 (3.15)	73 (45.1)

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates; P/S ratio = polyunsaturated fat to saturated fat ratio.

Furthermore, means for vitamin A, folate and calcium intakes were below the RDA. Mean nutrient intakes measured by the 24-HR were lower compared to those measured by the Food Frequency Questionnaire. The macronutrients' mean contributions to energy intakes were observed to be within the ranges made by the WHO, except for the mono-unsaturated fatty acids (see Table 4.18).

Table4.18: Mean percentage energy contribution of macronutrients of preschool children

Macronutrient	Reference ranges	FFQ
Protein	10–15%	12.18 ±1.67
Fat	15–30%	22.07 ± 5.68
SFA	<10%	5.77 ± 1.89
MUFA	>10%	6.59 ± 2.16
PUFA	6–10%	7.34 ± 2.20
CHO	65–75%	65.74 ± 5.98

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates.

Table 4.19 shows foods consumed by preschool children. The commonly consumed foods include maize meal, bananas, sweets, Simba chips, potatoes, bread and milk. The data stating that fruits and vegetables are not at the top of the list is supported by the low vitamin intakes found in these children. The low-fat intake can be explained by the finding that margarine comes far down the frequency list.

Table 4.19: Commonly consumed foods by preschool children

FFQ	24-HRQ
Sugar	Maize meal
Maize meal	Sugar
Sweets	Tea
Crisps	Bread
Potatoes	Chicken
Bread	Milk
Cabbage	*Amaranthus thunbergii*
Amaranthus thunbergia	Pumpkin leaves *(Cucurbita)*
Pumpkin leaves *(Cucurbita)*	Cabbage
Fish	Tomato and onion
Mafula	Mangoes
Mangoes	Mafula
Tea	Banana
Banana	Oranges
Oranges	Naartjies
Naartjies	Crisps
Rice	Eggs
Tomato and onion	Soup
Cookies	Beef
Apple	Potatoes
Eggs	Mabela
Spinach	Bread, white
Atchar	Rice

FFQ	24-HRQ
Chicken	Spinach
Cold drink	Margarine
Samp	Sweets
Beef	Fish
Milk	Apple
Vetkoek	Pumpkin
Margarine	Beetroot
Squash	Vetkoek
Macaroni	Squash
Soya	Beans
Beans	Avocado
Soup	Cookies

4.4. Discussion of results

4.4.1. Socio-demographics characteristics of children under five years

About 120 preschool children, 57.5% boys and 42.5% girls, from six primary schools in Limpopo Province, Vhembe District participated in the present study (Table 4.11). Limpopo province is known to be one of the poorer regions in South Africa, with a population of almost six million (STATSSA, 2022). In the present study, the majority (69.2%) of the respondents were mothers of the children, 30% were caregivers while only one child was cared for by the father (0.8%). The following poverty indicators were observed: about 75% of mothers/guardians were unemployed and 88% were receiving either a child or pension grant. The unemployment results in the present study are higher than what has been reported by other authors in previous years, and this could be one of the COVID-19 effects as most people lost their jobs since 2020 because of the lockdown. Furthermore, 97.5% of the residents in Limpopo still used traditional houses; this finding may imply a higher degree of poverty with poorer infrastructure in the rural areas of Limpopo. The poor infrastructure results observed in the present study are comparable to what Mamabolo *et al*. reported in 2006 and this shows that there has been no significant change in rural areas of Limpopo with regard to infrastructure.

In terms of number of members of household older than five years, most of the households had more than four members (71.7%), followed by four members (19.2%). In 2006, Mamabolo *et al*. (2006) reported that most households in Limpopo had large household sizes of more than four members. In addition, it has been evaluated and reported that household size may lead to competition for available food. Almost half of the parents were single (48.3%), 30.8% were married, 15.8% were living together and the remaining 5% were widowed. Most parents had attended Grades 11–12 (45.8%) followed by tertiary education (26.7%). A trend contradicting the results in the present study was observed in South Africa, where it was found that 25% of caregivers of children aged 1–9 years had no schooling (NFCS, 1999). In addition, Wamani *et al*. (2006) reported that 21% of mothers with children aged 0–23 months in rural Uganda had no schooling. On the other hand, Matthews *et al*. (2009) reported a percentage (39%) of caregivers who had no schooling in underdeveloped areas of North Western Nigeria.

Mushaphi *et al*. 2015 reported that the education level of mothers/caregivers contributes significantly to the quality of health and nutrition of a child. Mushaphi *et al*. 2015 further explained that educated mothers/caregivers process information on nutrition, acquire skills and display positive caring behaviours more than less educated mothers/caregivers, and this may reflect in the child's nutritional status. In addition, the International Food Policy Research Institute (IFPRI, 2012) also agrees that an improved educational level of mothers/caregivers can improve the child's nutritional status. This can be supported by a 43% of total reduction in child malnutrition which was observed from 1970 to 1995, after mothers/caregivers were educated on nutrition. Chen & Li (2008) and Semba *et al*. (2008) also observed the similar trend that mothers/caregivers without formal education or only primary education were less likely to contribute positively to the nutritional status of their children, compared to mothers/caregivers who had secondary or college education. In the present study, three quarters of the parents were not working and 90% of those parents were receiving child grant— this could be because most mothers were students who were still studying either at high school or a tertiary institution, which resulted in lower family income.

According to the study conducted by Mushaphi *et al*. (2015), a higher number (74%) of the households who depended mainly on child support grants was recorded. In contrast Smuts *et al*. (2008) reported a much lower number of households that depended on child grants in rural districts of the Eastern Cape (37%) and KwaZulu-Natal (14%). In this study, most of the households were headed by mothers (29.2%). In terms of monthly income, the most earned was between R1 000–R2 000 (39.2%) followed by R2 001–R5 000 (24.2%). The income ranges of R1 001 to R2 000 in the present study are higher than what was reported by Mushaphi *et al*. (2015), who showed that 60% of the households' income was R1 000 or less per month in Limpopo Province. These findings are almost comparable to those of the NFCS (1999), which reported that 58% and 49% of households' income in South Africa, specifically in Limpopo Province, was below R1 000 per month. Even though the income reported in the present study is a bit higher compared to previous years, the difference is not huge. In a study done in rural localities of Northwest Ethiopia, preschool children who belonged to families with low income were reported to be at a greater risk of being wasted, underweight and stunted (Edris, 2007). In this study, most mothers/caregivers (90%) were unemployed and depended on child support grants for their main source of income, which could have a negative impact on the nutritional status of the children.

4.4.2. Cooking energy used

The cooking energy used by the households of the 120 children is summarised in Table 0.11. Over 80% of the households use firewood coal (82.5%), more than two-thirds use electricity (67.5%). The results in the present study are almost the same as what Mushaphi *et al*. (2015) reported where more than 90% of the households relied on firewood for cooking in Limpopo. In 2008, Smuts *et al.* reported that 86% of the people in Eastern Cape and 71% in Kwazulu Natal were also using firewood for cooking. In contrast, the NFCS (1999) recorded that 49% of households in Limpopo province with children aged one to nine years were using firewood/coal for cooking. Even though a higher percentage of households using firewood for cooking in Limpopo is reported than what NFCS reported in 1999, the same households also use electricity—but mainly for lights and other electrical appliances—in the present study. Mamabolo *et al*. (2006) reported using firewood for cooking as one of the poverty indicators, meaning that even though there are some improvements in terms of lifestyle in Limpopo, poverty exists.

4.4.3. Household food production

As shown in Table 4.13, analysis of household food production of the 120 households showed that 56.7% had a garden, almost a quarter had an orchard (23.3%), more than a fifth had a field in the household (22.5%), and 5% had a smallholder farm. Even though most of the participants had either a home garden or orchard, producing food was still a challenge because of lack of water for irrigation and inadequate knowledge about modern farming. A lack of rural infrastructure, limited access to modern inputs and irrigating infrastructure, storage facilities, limited knowledge about modern farming techniques and access to credit all lead to low food production, limited participation in markets and a lack of investment (FAO, 2009). It is important that the government ensures that most rural people have access to land for food production, modern agricultural technologies, and credit, as that would help significantly in improving food security at a household level, which in turn will result in adequate dietary intake and improved nutritional status of children in families (Mushaphi *et al*., 2015; Ajani, 2008).

Home gardens and small animal farming projects may also help in improving household food security and increase household income when a surplus is produced (vegetables and animals are sold, which can reduce poverty by generating income that will assist the family to buy other basic supplies). Promoting the production of horticultural crops with a high carotene content, such as carrots, pawpaw and leafy vegetables, increases access to and consumption of them, leading to improved vitamin A status of children (Babu, 2000; Faber *et al*., 2002b). However, home gardens and small animal farming should be integrated with nutrition education to ensure that the small farmers also consume the food they produce before they sell everything. Several studies have shown that the home garden, coupled with nutrition education, increased the intake of vitamin A-rich foods, leading to improved micronutrient status of children (Chakravarty, 2000; Faber *et al*., 2002, Jones *et al*., 2005; Ruel & Levin, 2000).

4.4.4. Illness

The illnesses experienced by the 120 children are summarised in Table 4.14. The most common illnesses include flu (90.8%) and throat problems (87.5%). The high number of children who experienced flu in the present study could be due to the study being conducted in the second part of the year, which is cold compared to other seasons. Other less common illnesses include vomiting (4.2%), gastro-intestinal problems (2.5%) and about 0.8% of the children were affected with diarrhoea. The use of medications can lead to symptoms such as nausea, vomiting and diarrhoea, which result in loss of appetite and decreased food intake. Diarrhoea in most cases results in malabsorption of nutrients. Malabsorption may be a result of small-bowel bacterial overgrowth, due to gastrointestinal dysmotility or hypochlorhydria, and may also predispose the child to malabsorption (Miller, 2003:S134). None of the children had a history of TB, COVID-19, ear discharge, toothache, appendicitis, allergy, tumour, itching, epilepsy or malaria. Dannhauser & Veldman (2013) reported that illnesses can contribute to the development of malnutrition in children. Dannhauser & Veldman (2013) further explained that an illness can influence the food intake of the ill person, which in turn influences the nutritional and health status of the individual. Children are even more vulnerable to malnutrition, due to their less developed immune system and their relatively high nutritional needs for proper growth and development. In addition, increased energy expenditure is regarded as one of the main causes of weight loss in individuals who are ill, as their requirement for energy is approximately 10% more compared to those who are not. Inability to meet these increased nutritional needs results in malnutrition (Semba & Tang, 1999:182).

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4.4.5. Child characteristics and anthropometrics

Out of 120 children, 69 (57.5%) were boys and 51 (42.5%) were girls. Their ages in months ranged from 33.5 months to 66.1 months, with mean age (standard deviation [SD]) of 50.5 (7.3) months. The weight in kilograms (kg) ranged from 10.6 kg to 30.5 kg, with mean (SD) of 17.6 (3.3) kg. Their height in centimeters (cm) ranged from 81 cm to 112.5 cm, with mean (SD) of 101.4 (5.4) cm. The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ). The weight-for-height z-scores (WHZ) ranged from -4.94 to 4.50, with mean (SD) of 0.38 (1.60). The height-for-age z-scores (HAZ) ranged from -4.61 to 2.73, with mean (SD) of -0.64 (0.99). The weight-for-age z-scores (WAZ) ranged from -3.72 to 3.50, with mean (SD) of -0.12 (1.28). The BMI-for-age z-scores (BAZ) ranged from -4.99 to 4.43, with mean (SD) of 0.41 (1.57).

4.4.6. Classification of children by anthropometrics

i. WAZ

The majority (90.0%) were in the mild under-weight to normal to possible growth problem range; more than 60% of the children had normal WAZ range (75,62.5%). Seven children were underweight: three underweight and four severely underweight. In addition, five children were overweight or obese: four overweight and one obese (Figure 4.1). The number of children who are underweight in the present study is lower that what Dannhauser & Veldman

(2013) reported where 78.6% of the children in the were classified as underweight (14.3% severely underweight, 35.7% moderately underweight and 28.6% mildly underweight).

ii. HAZ

The majority (112, 93.3%) were in the mild stunted to normal height range; more than two-thirds of the children had normal height range (82, 68.3%). Children were stunted and one was severely stunted (Figure 4.2). Similar data was reported by Dannhauser & Veldman (2023) who relayed that a total of 78.5% of the children in the Free State were severely stunted (21.4%, 35.7% moderately and 21.4% mildly). Seven The low prevalence rate of stunting in children in the current study could be correlated to education level of a mother/guardian. It was observed that mothers with higher education level or who are literate have fewer children, which can enable them to provide better care. They also have access to health information, leading them to adopt improved behaviour related to maternal and child health care and feeding and eating practices, which ultimately influences the nutritional status of children. In addition, women with no education are more likely to embrace the traditional status quo and be less open to changes for better health and family practices, which may influence the way they feed their children (Mushaphi *et al.*, 2015). The results in the present study are almost similar to what Mushaphi et al. (2015) reported that literate mothers are less likely to raise malnourished children.

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iii. WHZ

The majority (95, 79.1%) were in the mild wasted to normal to possible risk of overweight range; about half of the children had normal WHZ range (58, 48.3%). Three children were wasted and four had SAM. On the other hand, 15 (12.5%) were overweight and three were obese. A total of 85.7% (35.7% + 50%) of the children were reported to have normal BMZ at the start of the intervention.

4.4.7. Analysis of biochemical results

The biochemical results for the 120 children are shown in Table 4.15. Vitamin A had mean (SD) of 72.3 (36.6); however, its distribution is skewed with median of 60.1 and ranging from 40 to 253.9. Zinc had mean (SD) of 21.4 (11.2); also skewed with median of 18.6 and ranging from 5.7 to 80.3. Iron had mean (SD) of 16.7 (6.0) and seemed normally distributed and ranging from 6.7 to 29.4. This was like serum ferritin with mean (SD) of 48.2 (21.2) and ranging from 11.6 to 124. Serum transferrin was also normally distributed with mean (SD) of 36.5 (5.7) and ranging from 25.9 to 80.9. Also normally distributed were saturation and protein with mean (SD) of 23.4 (8.8) and 69.6 (6.2), respectively. The rest of the biochemical elements were skewed, meaning they had outlying or extreme values, that is, 24-Iodine excretion, 24-

creatinine, and iodine urine; their median (Interquartile range [IQR]) were 31.4 (19.1, 47.0), 1.3 (0.8, 1.8), and 73.8 (58.3, 110.5), respectively.

The researcher also categorised the children as having deficiency, normal and excess levels of these biochemical elements (Table 4.16):

i. Vitamin A

According to the Lancet Laboratories, normal vitamin A is in the range (113 to 647) ug/dl. Therefore, just over 10% of the children had adequate vitamin A status. In the present study, more or less one third of the children had marginal vitamin A status (100 to 199.9 µg/L), signifying a severe public health problem (≥ 20%) of marginal vitamin A according to the WHO (1996) classification. A similar trend was observed in the SAVACG (1995) study, where 33% of children aged six to 71 months in South Africa were found to have marginal vitamin A deficiency. On the contrary, the NCFS-FB (2005) revealed a higher prevalence of marginal vitamin A deficiency amongst children aged one to nine years in South Africa (49%) and Limpopo (63.2%). However, two studies that were done on preschool children in rural Vietnam reported a much lower prevalence of vitamin A deficiency (11.3%) (Nhien *et al*., 2008) and marginal vitamin A deficiency (12%) (Khan *et al*., 2007). The poor vitamin A intake could be attributed to the high consumption of maize which is a staple food and consumed daily in Limpopo. According to research conducted by Netshiheni *et al*. (2019), maize is rich in carbohydrates; however, it is a poor source of vitamins and minerals that are required for proper health. Netshiheni *et al*. (2019) indicated that most indigenous foods (green leafy vegetables and insects) found in Limpopo Province are rich in micronutrients such as vitamin A, vitamin C, iron, zinc and calcium. However, the consumption of these indigenous foods is low.

ii. Zinc (10.7 – 18.4) ug/dl, iron levels (113 to 647) umol/l and saturation (%) (20-55%)

In this study, four children (3.3%) had zinc deficiency, 55 (45.8%) had normal zinc levels, and 61 (50.8%) had excess levels: According to Lancet Laboratories, normal iron is in the range 113 to 647 umol/l. Therefore, 10% of the children had iron deficiency. Forty percent (n=48) of the children had low saturation while the rest (72, 60%) had normal saturation of 20% to 55%. Mugware *et al*. (2022) reported that about a quarter (24%) and 13% of children in Limpopo province had iron depletion and iron deficiency respectively. According to a study conducted by Mushaphi *et al*. (2015), it was observed that many (80%) children in rural villages of Limpopo had normal values for serum iron. In contrast, Keskin *et al*. (2005) reported that iron deficiency was common in boys who were attending primary school in urban Turkey and who came from poor households. According to Litchford (2008), high serum iron values in children may reflect day-to-day variations or may indicate iron overload. The interpretation of serum iron should thus be done with caution because it may be elevated later in the day even in healthy individuals. In the present study, blood samples were collected during the early morning hours to minimise the day-to-day variations. The lower number of children with iron deficiency in the study may be due to their participation in a de-worming programme. In addition, about 90% of children were given fortified bread and maize-meal porridge, which may have contributed to adequate iron status in the current study. The low iron deficiency status of children in this study could be due to more than 95% of mothers/caregivers including animal-based food in the diet of their children, which may also improve haem iron intake.

iii. Protein (60-80 ug/dl)

Three children (2.5%) had low protein and the majority (116, 96.7%) had normal protein levels. The normal protein levels could be supported by the fact that more than 95% of the children were consuming protein-rich foods at preschool and at home. The findings in the present study could be attributed to more than 95% of mothers/caregivers including protein foods on the child's plate; in addition, these children were given animal-based food (chicken feet, hearts, or giblets), which also improved haem iron intake.

iv. Iodine excretion (75-851 ug/day)

Most of the children had low iodine excretion (108, 90%) and only 10% (n=12) had normal iodine excretion. The low iodine excretion results reported in the present study contradicts what Labadarios *et al*. (2008) reported—that salt iodation programme has reduced the number of children with iodine deficiency disorder.

4.4.8. Dietary patterns of preschool children

Data on dietary patterns of preschool children is summarised in Table 0.17 as well as medians and IQR. About 36.6% of the children had energy intakes below the RDA. The data in the present study is comparable to what Manu & Khetarpaul (2006) observed—that the energy intake of preschool children in India was below the RDA recommended by the Indian Council of Medical Research. The NFCS (1999) revealed that South African children aged one to nine years had median energy intakes that were below 67% of estimated energy requirement (EER). A similar observation was made by Mamabolo *et al*. (2006), who reported that children aged three years in central Limpopo Province had energy intakes that were mostly below 67% of the dietary reference intake (DRI). Several studies conducted in Africa and Asia have revealed that preschool children mostly consume cereal-based diets, which in turn leads to high intake of carbohydrates (Faber *et al*., 2001; Uppal *et al*., 2005; Iram & Butt, 2006; Manu & Khetarpaul, 2006). Mushaphi *et al*. (2015) reported that most children in Limpopo were found to be consuming cereal-based diets, which resulted in high median carbohydrate intakes that were above the estimated average requirement (EAR) recommended for their age.

More than 70% of the children had poor vitamin A intake. About 10% of the children also had poor iron intake. These could be attributed to the high consumption of maize, which is a staple food and consumed daily in Limpopo. Even though maize is rich in carbohydrates, due to high level of phytic acids, its bioavailability and bioaccessibility of vitamins and minerals required for proper health is poor. Netshiheni *et al*. (2019) indicated that most indigenous foods (green leafy vegetables and insects) found in Limpopo are rich in micronutrients such as vitamin A, vitamin C and calcium. However, the consumption of these indigenous foods is low. Indigenous foods are likely to be a more sustainable as well as long-term solution to the elimination of micronutrient deficiencies (Netshiheni *et al*., 2019; Mushaphi *et al.*, 2015; Hong, 2003). There is a need to protect and promote the consumption of indigenous foods to improve the overall health and nutritional status of children—particularly in poor regions like Limpopo (Netshiheni *et al*., 2019; Venter *et al*., 2007). In most countries, important improvements in the micronutrient status of the population can be achieved by fortifying staple foods (Aphane *et al*., 2003).

Even though poor Vitamin A and iron intake were observed in the present study, there is a significant change in terms of vitamin A and iron intake as compared to previous studies. This could be due to the mandatory micronutrient fortification of staple foods, which has increased the micronutrient intake of most children in the country.

Furthermore, mean nutrient intakes measured by the 24-HR were lower compared to those measured by the Food Frequency Questionnaire. The macronutrients' mean contributions to energy intakes were observed to be within the ranges made by the WHO, except for the mono-unsaturated fatty acids (Table 4.18).

Table 4.19 shows foods consumed by preschool children. In this study, the commonly consumed foods include maize meal, bananas, sweets, Simba chips, potatoes, bread and milk. The data in the current study shows that fruits and vegetables are not at the top of the list, which is supported by the low vitamin A and iron intake found in these children. The low-fat intake can be explained by the finding that margarine comes far down the frequency list. In the present study, all mothers/caregivers reported that they were usually including starchy foods such as maize porridge, bread or samp on their children's plate daily. The NFCS (1999) observed a similar trend where maize and brown bread were found to be amongst the most consumed food items in children aged one to nine years. Dannhauser *et al*. (2000) and Faber *et al*. (2001) also reported that maize is consumed by children predominantly in the Free State and rural KwaZulu-Natal respectively. Moreover, Manu & Khetarpaul (2006) also supported that wheat is mostly consumed by rural Indian preschoolers. Maize-meal porridge is a staple food for most (80%) people in South Africa; hence, most children were given starchy foods with each meal.

In the current study, most (96%) of the mothers/caregivers as well as preschools reported that they included protein-rich foods, such as eggs, meat, beans, fish or mopani worms on the child's plate at least four times a week. According to the 24-hour recall in this study, the protein-rich foods commonly consumed by children were eggs, chicken feet and heart and beef or chicken liver. Contrasting the present study, most children under the age of five years in developing countries such as South Africa (NFCS, 1999), Tanzania (Tatala *et al*., 2004), Pakistan (Muller & Krawinkel, 2005), and rural Vietnam (Iram & Butt, 2006) consumed diets with very low amounts of meat and meat products. Compared to previous studies, the consumption of protein rich foods in this study has improved. Recently, Smuts *et al*. (2008) observed that more than half of the children aged zero to 71 months in rural districts of KwaZulu-Natal and the Eastern Cape either seldom or never consumed meat products.

In the present study, 59% of the children consumed indigenous vegetables such as small pig weed (*Amaranthus thunbergii*) and pumpkin leaves (*Cucurbita moschata*). A study conducted by Mushapi *et al*. (2015) stated that 69.2% to 100% of the children in Limpopo consumed indigenous vegetables such as pumpkin leaves (*Cucurbita moschata*), wild-jute plant (*Corchorus hirstirus* or *Corchorus olitorius*), night shade leaves (*Solanum retroflexum dun.*), small pig weed (*Amaranthus thunbergii*) and spider flower (*Cleome gynandra*). A similar report was given by other authors, where more than 70% of people in rural communities of Malawi and South Africa were found to consume indigenous vegetables, with *Amaranthus hybridus and Biddens Pilosa* at the top of the list (Babu, 2000; Faber *et al*., 2010; Nesamvuni *et al*., 2001; Van Rensburg *et al*., 2007a; Van Rensburg *et al*., 2007b).

In the current study, indigenous fruits that were available and mostly consumed by children at preschool and home included *Mafula*, mango, naartjies and oranges—at least one a day. Mushaphi *et al*. (2015) reported that about 90% of children in Limpopo consumed indigenous fruit such as mango and guavas. Smuts *et al*. (2008) reported that tropical fruits such as mango and paw-paw were consumed more regularly in rural districts of KwaZulu-Natal than those of the Eastern Cape. The more regular consumption of tropical fruits observed in rural districts of KwaZulu-Natal and in the present study may be due to availability and accessibility. Tropical fruit such as mango is available to almost all households in the Vhembe region of Limpopo Province; hence the high consumption of mango. Contrarily, the NFCS (1999) revealed that most mothers/caregivers included less fruit in their children's diet due to seasonal availability. A similar observation was made by Iram & Butt (2006) in India and by Dannhauser *et al*. (2000)

in informal settlements of the Free State, where consumption of fruit was low and dependent on seasonal availability.

4.5 Conclusion

In conclusion, the socio-demographic data of the present study were comparable to that found in other studies in rural or developing areas. The data of previous studies and of this one shows that a high number of people in developing countries/areas still do not have access to basic services, as demonstrated by the number of people using firewood as the main source of fuel, depending on social grants and having an income of less than R2 000 per month. In addition, the prevalence of malnutrition in African countries is still a challenge, as shown by the majority (108, 90.0%) of children who were in the mild under-weight to normal to possible growth problem range and mild stunted to normal height range (112, 93.3%), regardless of the nutrition intervention strategies employed to address the issue. Moreover, about 80% of the children had inadequate vitamin A intake and 10% had iron deficiency, which could be attributed to the high consumption of cereal-based foods eaten daily in Limpopo province.

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4.7. Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

4.8. Authors' contributions

K.R. Netshiheni was the project leader responsible for project design, data collection, analysis, and manuscript drafting. C.N. Nesamvuni and L.F. Mushaphi supervised the project and proofread the manuscript.

4.9. Funding information

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4.10. Data availability

The data that supports the findings of this study are available from the corresponding author, K.R.N, upon reasonable request.

4.11. Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

4.12. REFERENCES

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Chapter Five: Phases 3 and 4

In this chapter, the important observations from the results regarding the effect of consuming maize porridge fortified with *Moringa oleifera* and termites on the nutritional status of children aged three to five years old in the Vhembe District was discussed. Where possible, the results were compared with available literature and discussed. Unfortunately, very few other studies have been undertaken in South Africa on the effect of consuming maize porridge fortified with *Moringa oleifera* and termites on the nutritional status of children aged three to five years old, which makes it difficult to compare the findings. The sample was selected from six preschools, with three preschools constituting the experimental group and three preschools the control group. At baseline post-intervention, the sample consisted of 120 children.

The effect of consuming instant-maize porridge fortified with termites and *Moringa oleifera* leaves powders on the nutritional status of children aged three to five years old in Thulamela Municipality, South Africa (RCT)

Background: Maize porridge is used for complementary feeding and is consumed in many African countries, including South Africa. However, the nutritional value of maize is poor.

Aim: This study shared insight on the efficacy of consuming instant maize porridge fortified with *MO* and termite powder on the nutritional status of children aged three to five years old in the Thulamela Municipality, Vhembe District.

Methodology: The study population was children aged three to five years attending preschools in Thulamela Municipality. Systematic sampling was used for the selection of preschools. A randomised controlled trial (RCT) was used, whereby participants were randomly assigned into an experimental and a control group; the research method was quantitative. This study was conducted in four phases, namely, Phase 1: the fortified instant-maize porridge was prepared and the safety and nutrient content of the product were assessed; Phase 2: a baseline study was done; Phase 3: participants were assigned to experimental and control groups. The consumption of 100% instant-maize porridge by the control group and fortified (80% maize, 15% termites and 5% MO) maize porridge by the experimental group for six months was done here and Phase 4: post-intervention data collection and comparing Phases 2–4 was done. Anthropometric assessments were performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry. Biochemical tests were done according to LANCET Laboratory procedures. Dietary assessment was also conducted. Data was exported into Statistical Package for Social Sciences (IBM SPSS) and categorised as frequencies, mean, standard deviation, median and IQR. The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ).

Results: About 120 children from six preschools participated in the study. More than half—69 (57.5%) were boys—and 51 (42.5%) were girls. The majority of the respondents (69.2%) were mothers of the children, while only one child was cared for by the father (0.8%). Almost half of the parents were single (48.3%) and 30.8% were married. Most had attended Grades 11–12 (45.8%) followed by tertiary education (26.7%). Over half had done deworming (54.2%) and 56.7% had taken vitamin A. Almost all had the Road to Health booklet (RtHB) (99.2%). All of them had blood and urine taken for biochemical tests.

Conclusion: The biochemical results in the present study confirmed that there was an improvement in nutritional status of the experimental group after six month's intervention. In addition, maize porridge fortified with *MO* leaves and termite powder can have a potentially significant effect in reducing anaemia and protein energy malnutrition. Therefore, the instant maize porridge fortified with MO leaves and termite powder can potentially improve the iron and the Vitamin A status of children by reducing anemia and protein energy malnutrition.

Keywords: Fortification, Instant-maize porridge, Moringa, Nutritional status, Protein, Termites.

5.1. Introduction

The prevalence of malnutrition is still a major health problem for the public, affecting children under five years of age worldwide, requiring priority (Bailey, West & Black 2015). Despite several advances and improvements in child health, malnutrition remains one of the main health concerns of the 21st century, particularly in poor countries. The previous statement can be supported by 39.4 % of children under five who were reported to be stunted in 2010 and another 38.4 % of children who were also reported to be stunted in 2013 by SANHANES (2016). The previous years' reports on stunting show that there was no significant improvement in the nutritional status of children from 2010 to 2013 in South Africa. Another malnutrition case was reported by Mushaphi *et al*. (2015), where it was observed that 7.7% and 3.5% of children under the age of five years in Limpopo Province were vitamin A and iron deficient respectively. In agreement with what Mushaphi *et al*. (2015) reported, Motadi *et al.* (2015) also observed a malnutrition case where the prevalence of zinc deficiency and anaemia in preschool children of Vhembe District in Limpopo was 42.6% and 28% respectively in 2014. Iron and zinc deficiency in children is associated with poor growth development, alteration in neurological function, immunological response and behaviour changes (Baltimore, 2009; Bothwell, 2007). In addition, some researchers in Africa and Asia also confirmed that preschool children mostly consume cereal-based diets, which in turn leads to high intake of carbohydrates (Faber *et al*., 2001; Uppal *et al.*, 2005; Iram & Butt, 2006; Manu & Khetarpaul, 2006). A similar trend was observed by the NFCS (1999) where maize and brown bread were found to be amongst the most consumed food items in children aged one to nine years in South Africa (SA).

The prevalence of malnutrition in children under five in SA and Limpopo proves that regardless of the mandatory food fortification of staples such as maize, bread etc. by the National Department of Health, it remains high in South Africa, particularly in Limpopo—and that could be due to high consumption of maize porridge, which contributes significantly to the diet of 80% of households there. Irrespective of the high starch content in maize, it does not provide adequate nutrients for proper health and nutrition. Moreover, the bioavailability of essential nutrients such as iron and zinc, which are vital for proper nutrition and health, is often affected by the presence of phytic acid in the maize grain, as it inhibits the absorption of minerals (Hulse*et al*., 1980), such as iron, zinc and calcium. Therefore, there is a need for further research on fortifying maize with indigenous food items which are known to be rich in nutrients; this could be one of the sustainable strategies to alleviate malnutrition—particularly in rural areas. In addition, the impact of fortifying instant maize porridge with *MO* leaves and termites is important, due to its low bioavailability of zinc and iron and maize is also a poor source of vitamins A and C. In addition, *MO* contains essential nutrients such as vitamins, minerals, amino acids, β-carotene, antioxidants, inflammatory nutrients and omega 3 fatty acids (Fahey, 2005; Hsu *et al*., 2006; Kasolo*et al*., 2010). Termites are rich in protein and essential amino acids such as tryptophan, which is generally limited in the food insects (Defloliart, 2014). They are undeniably a rich source of iron and their inclusion in the daily diet could improve iron status and help prevent anaemia and protein energy malnutrition (PEM) in children and women in developing countries. This study investigated the effects of consuming maize porridge fortified with *MO* and edible insects on the nutritional status of children aged three to five years old in Thulamela Municipality, Vhembe District, Limpopo Province, South Africa.

5.2. Methods and materials

A post-intervention study was conducted, whereby anthropometric, biochemical, dietary assessment and screening for eligibility of children aged three to five years old was done. This was after intervention was introduced to assess the growth, nutrient intake and nutritional status of the children after consuming a fortified maize porridge. This phase involves study design, allocating participants to experimental and control groups, sampling methods, measurements to be taken, measuring techniques as well as validity and reliability factors.

5.2.1. Study design

A randomised controlled trial (RCT) was used, whereby participants were randomly assigned into an experimental and control group. As the study progresses, the only expected difference between the control and experimental groups in a randomised controlled trial is the outcome variable being studied. The research method was quantitative.

5.2.2. Study area

Thulamela Municipality is Vhembe's most eastern sub-district or municipality and borders the Kruger National Park in the east. Thohoyandou is the main town of this sub-district and houses the local municipality. With an area of 5 834 km^2, Thulamela is Vhembe's smallest municipality, geographically; however, its population of 618 642 people in 2017 made it Limpopo Province's most populated municipality, also ranking Thulamela as the fourth most populated of all South Africa's municipalities. Across the municipality, there is a population density of 106 persons per km^2, the highest in Vhembe District; however, more than 85% of the inhabitants live in rural areas. Within the municipality, 85.7% of the dwellings are formal structures and 14.3% informal. Thulamela Municipality has an economic growth rate of 0.62%, the second lowest in Vhembe District. Agriculture is the district's main economic activity with agricultural households accounting for 45.9% of the area's total 156 594 households. Females head more than half (54.4%) of all households.

5.2.3. Study population

The study population comprised children aged three to five years attending preschools in Thulamela Municipality. This municipality has 12 296 preschool children from 224 preschools. The list of preschools was obtained from the Department of Basic Education. Preschool children are more vulnerable to micronutrient deficiency, which can have a long term, irreversible, negative effect on neurological and cognitive outcomes (Prado & Dewey, 2014). If these deficiencies, are detected and treated in the early stages, the outcomes can be reversed, decreasing child mortality and mobility by 15% (WHO, 2015); treatment can also help in increasing the IQ score of children under the age of five years. Preschool children are usually readily accessible at preschools, and they are often representative because children from both affluent and low-income households attend them.

5.2.4. Eligibility criteria

Screening of preschool children was done. Preschool children were screened before the implementation of any intervention to check if they have immune suppression diseases, worms or allergies as these can affect the nutritional status of children (WHO, 2015). According to Food and Nutrition Technical Assistance (FANTA, 2004), immune suppression diseases affect nutrition through increasing resting energy expenditure, reductions in food intake, nutrient malabsorption and loss, and complex metabolic alterations that culminate in weight loss and wasting. Weight loss and wasting are associated with increased risk of opportunistic infections (WHO, 2015). The immune system can be impaired because of diseases, thus contributing to malnutrition (FANTA, 2004). Albendazole or mebendazole was administered to children for a week for those who were not dewormed. Children were screened for any known allergy or to see if they were allergic to some of the ingredients used to produce the product, and if they had received any supplement such as vitamin A six months before the intervention as these could affect the results.

5.2.5. Inclusion

All children aged three to five years old within the selected preschools and whose parents consented to all three assessment occasions had an equal chance to participate in the study.

5.2.6. Exclusion criteria

Children with known immune suppression diseases, known allergies to maize, *MO* or termites, as well as those visiting Thulamela Municipality during the data collection were excluded from the study.

5.2.7. Sampling

A simple random sampling was used for the selection of 22 preschools (11 for control and 11 for the experimental group) in Thulamela Municipality. The sample size was calculated using Solvin's-Formula:

$$n = \frac{N}{1 + N\,x\,(e)2}$$

$$n = \frac{12296}{1 + 12296\,x\,(0.05)2}$$

$$n = \frac{12296}{1 + 12296\,x\,0.0025}$$

$$n = \frac{12296}{1 + 30.74}$$

$$n = 387$$

Where: n = Number of samples
N = Total population
e = Error margin

The original sample size was 387 children aged three to five years; however, only 120 of the actual size was used due to budgetary reasons; also the study was conducted during COVID 19, so most parents or guardians had to withdraw their children from the study and only 120 children were left; 120 participants are still statistically significant. A systematic sampling was used for the selection of children in designated preschools.

5.2.8. Subject recruitment

The researcher sought for permission to conduct the study from the Provincial Department of Basic Education (Appendix C) and preschools' principals. The researcher visited preschools three times for recruitment purposes.

Visit one—The researcher distributed letters to preschool principals outlining the purpose and procedure of the study and setting dates on which to meet parents of the preschoolers.
Visit two—The researcher met parents of the preschoolers and explained the purpose and procedure of the study, as well as the material from the information sheet (Appendix A). The researcher gave consent forms (Appendix B) for parents to read.
Visit three—During this visit, a session was conducted with parents who needed more clarity and wanted to ask questions about the research before providing consent. Signed consent forms were collected from them at this time.

5.2.9. Measurements/Assessments

This section involved anthropometric, biochemical and dietary assessment of the children aged three to five years old.

5.2.9.1. Instrument development

A questionnaire was developed following the objectives of the study and relevant literature. The instrument was presented to promoters for scrutiny and inputs; peer review provided yet another avenue for improving it. It was presented to the Faculty of Health Sciences Research Committee and University of Venda Higher Degree Committee for approval. The instrument was pre-tested. It consisted of three sections: section A (socio-demographic information); section B (Anthropometry) and section C (Dietary). The questionnaire was developed by the researcher in English and translated to Tshivenda by the University of Venda Tshivenda Dictionary Unit.

5.2.10. Pre-test of instrument

According to Polit & Hungler (2015), a pre-test is a small-scale version or trial run done to ensure the validity of an instrument in preparation for a major study. It is conducted to identify possible weakness in the research instrument. This exercise is carried out to assist in areas such as estimating the time needed to complete the questionnaire, determining the ease with which the interviewer asks the questions and how the respondents understand them—or to identify questions that are too difficult or ambiguous in terms of language or conceptualisation.

5.2.11. Intervention (consumption of fortified and unfortified instant maize porridge)

Participants were assigned to experimental and control groups. Two factors were considered—the consumption of 100% instant-maize porridge by the control group and fortified (80% maize, 15% termites and 5% *MO*) maize porridge by the experimental group - for a period of six months. The experimental group was fed 170 g (1/2 cup) of instant-maize porridge fortified with *MO* leaves and termite powders and the control group was fed with 170 g (1/2 cup) of placebo; both groups, once a day in the morning, twice a week for six months. According to the Nutrition Information Centre of the University of Stellenbosch, it is recommended that children under the age of five years should consume 170 g of cooked cereal once a day (NICUS, 2005).

5.2.12. Plate waste

Plate waste was assessed (Figure 5.1) to measure the amount of porridge unconsumed by the preschool children out of the 170 g served. Plate waste refers to the volume or percentage of the served food that is discarded (Williams & Walton, 2011). There are two main measurement methods (weighing or visual estimation) used to measure plate waste; in the present study, visual estimation was used. Visual estimation uses a scale to measure approximately what proportion of food remains. A 7-point scale—all, one mouthful eaten, ¾, ½, ¼, one mouthful left, none—was used in this study. A recent innovation, digital photography, was used to record the food waste, which minimised disruptions and allow unhurried estimates of portion sizes later (Williamson *et al*., 2003).

Figure 5.1. Plate waste being conducted

5.2.13. Anthropometric measurements

Anthropometric assessments were performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry (Stewart *et al*., 2011). Weight and height were measured following the procedures presented below.

i. Weight

A solar scale was used to avoid error in schools where there is no electricity. The children were weighed without shoes and wearing light clothing. The subjects stood still in the middle of the scale's platform without touching anything and with body weight equally distributed on both feet (Norton & Olds, 1996). The scale was accurate within 0.1 kg. The zero weight on the scale's horizontal beam was periodically examined. The scale was checked continuously against an electronic scale. Weight was taken twice and the average calculated and recorded on section B of the questionnaire. This was done to ensure accuracy.

ii. Height

Heights were taken using a portable height meter fitted with a metal tape measure that determines to the nearest cm. Height of children was taken without shoes and socks (bare feet) with feet flat on the floor, heels close together and against the wall. Heels, shoulder blades, and buttocks were against the wall. The shoulders were in a relaxed position, arms at the sides and the head in the Frankfort horizontal plane (looking straight ahead) (Norton & Olds, 2006). The measure was taken twice, and the average was calculated to ensure accuracy. The calculated measure was recorded on section B of the questionnaire.

The children's weight and height measurements were entered into World Health Organization Anthro-plus version 1.0.2 software for the calculation of weight-for-age (WAZ), height- for- age (HAZ), weight-for-height (WHZ), and body mass index (BMI)-for-age (BAZ) z scores (Table 5.1). Logistic regressions were computed to assess biochemical predictors of nutritional status (HAZ, WAZ, WHZ, and BAZ).

Table5.1: Z-scores classification and interpretation (WHO, 2009)

Z-score classification	Interpretation		
	WAZ	HAZ	WHZ
-3 SDs or less	Severely underweight	Severely stunted	SAM (Severe Acute Malnutrition)
Between -3 and -2 SDs	Underweight	Stunted	Wasted
Between -2 and -1 SDs	Mild underweight	Mild stunted	Mild wasted
Between -1 to +1 SDs	Normal WAZ	Normal height	Normal WHZ
Between +1 and +2 SDs	Possible growth problem	Normal height	Possible risk of overweight
Between +2 and +3 SDs	Overweight	Normal height	Overweight
+3SDs or more	Obese	Above normal	Obese

iii. Biochemical assessment

Biochemical assessment involves the measuring of a nutrient or metabolite in blood, faeces, or urine, or measuring a variety of other components in blood and other tissues that have a relationship to nutritional status (WHO, 2015). Biochemical tests were done in this study as they provide the most objective and quantitative data on nutritional and micronutrient status. Biochemical tests are also useful indicators of nutrient level in the tissue or fluid sampled than with other indicators (Benjamin, 2000). In this study, blood samples were collected and used as one of the indicators of nutritional levels (see the procedure below).

iv. Procedure for blood specimen collection

A professional nurse from LANCET was responsible for the blood collection. They gathered the tubes and supplies needed for drawing blood, positioned the participant in a chair and washed their hands. The nurse selected a suitable site for venepuncture, by placing the tourniquet 10 cm above the selected puncture site on the participant and put on non-latex gloves and palpated for a vein. When a vein was selected, they cleansed the area in a circular motion and allowed the area to air dry. The nurse then asked the participant to make a fist, while avoiding "pumping the fist." The nurse grasped the participant's arm firmly using their thumb to draw the skin taut and anchor the vein, then swiftly inserted the needle through the skin into the lumen of the vein and collected 5 ml of blood into the tubes. The needle formed a 15–30-degree angle with the arm surface. When the last tube was filled, the nurse removed the tourniquet. They removed the needle from the participant's arm using a swift backward motion and placed gauze immediately on the puncture site. The nurse asked the participant to apply and hold adequate pressure to avoid formation of a hematoma. After applying pressure for 1–2 minutes, the nurse taped a fresh piece of gauze or plaster onto the puncture site and disposed of contaminated materials/supplies in designated containers (WHO, 2017). Vacutainer blood collection tubes were used during blood sample collections. The blood was stored in a cooler box inside the LANCET laboratory until all children's blood was drawn and analysed the same day.

v. Iron analysis

Blood specimens collected were analysed for haemoglobin (Hb) content, serum ferritin levels, serum transferrin and the % of saturation transferrin using standardised procedures at LANCET laboratories (Table 5.2).

Table5.2: Interpretation of haemoglobin of children under five years

(WHO, 2011; SANHANES-1, 2012)

Classification	Haemoglobin
Mild anaemia	10.9–10.0 g/Dl
Moderate anaemia	9.9–7.0 g/Dl
Severe anaemia	< 7 g/dL

Serum ferritin measurement was used to check iron status of children. The cut-off point of serum ferritin <12 ng/mL was classified as iron deficiency, <12 ng/mL and Hb ≥11 g/dL was classified as iron depletion while <12 ng/mL and Hb <11 g/dL was classified as iron deficiency anaemia. The cut-off points for serum ferritin are indicated in Table 5.3.

Table5.3: Interpretation of iron status (WHO, 2011; SANHANES-1, 2012)

Classification	Serum ferritin
Iron deficiency	<12 ng/Ml
Iron depletion	<12 ng/mL and Hb ≥ 11 g/dL
Iron deficiency anaemia	<12 ng/mL and Hb < 11 g/dL

Serum transferrin measurements were used to assess iron deficiency anaemia of children. The cut-off point of serum transferrin of >2.0 g/L was classified as normal; 1.5–2.0 g/L was classified as mild while <1.0 g/L was classified as severe. The cut-off points for serum transferrin are indicated in Table 5.4.

Table5.4: Interpretation of serum transferrin (Gibson, 2005)

Classification	Serum transferrin
Normal	> 2.0 g/L
Mild	1.5 – 2.0 g/L
Severe	< 1.0 g/L

The cut-off point of saturation transferrin between 10%–20% was classified as normal, <15% was classified as low, while >20% was classified as high. Table 5.5 indicated the cut-off points for saturation transferrin.

Table5.5: Interpretation of saturation transferrin (Gibson, 2005)

Classification	Saturation transferrin %
Normal	10–20%
Low	<10%
High	>20%

vi. Zinc analysis

Serum zinc was measured using 125I-radioimmunoassay (Table 5.6). Enzyme-linked immunosorbent assays were used to measure serum transferrin saturation (TSAT) and ferritin (Ramco Laboratories, Inc., Stafford, TX, USA).

Table 5.6: Interpretation of zinc status in children

Zinc indicator	Classification	Reference
Serum Zinc deficiency	<9.9 µmo/dl	IZINCG, 2007
Normal	65 µg/dl	Gibson, 2005

vii. Iodine analysis

Urine samples were collected from preschool children using two litre specimen containers with screw caps. The samples were kept in a cooler box with freezer blocks and aliquots (3 x 1.5

ml) and stored at -20 °C until analysis. Urinary iodine concentrations were determined using 24-hour urine procedure from LANCET. The cut-offs were used to categorise the study population by iodine status (Table 5.7).

Table 5.7: Epidemiological criteria for interpretation of iodine status in school-aged children based on median and/or range of urinary iodine concentration values (WHO/UNICEF/ICCIDD, 2007)

Median	Iodine intake	Iodine nutrition status
School age children <20µg/L	Insufficient	Severe iodine deficiency
20–49µg/L	Insufficient	Moderate iodine deficiency
50–99µg/L	Insufficient	Mild iodine deficiency
100–199µg/L	Adequate	Optimal
≥300µg/L	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

viii. Vitamin A analysis

A blood sample (9 mL; vacuette Z Serum Sep Clot Activator 5-mL tube for serum) from each child was obtained. Serum was isolated by centrifugation of whole blood using a portable centrifuge, aliquoted into Eppendorf safe-lock tubes, and stored at -20 °C until all children's samples were collected (five hours) and analysed. Serum retinol was determined by a reversed-phase high performance liquid chromatography (HPLC) method, which is based on a method previously described by Catignani & Bieri, (1983) (see Table 5.8).

Table5.8: Interpretation of vitamin A status in children

Classification	Classification Serum retinol levels (NFCS-FB, 2005)	Laboratory levels (Drs Du Buisson, Kramer Inc./Ing.)
Vitamin A deficiency	<10 µg/dl	< 100 µg /L
Marginal vitamin A status	10 – 19.9 µg/dl	100 – 199.9 µg/L
Adequate status	20 – 29.9 µg/dl	200 – 299.9 µg/L
Normal/ well-nourished status	> 30 µg/dl	> 300 µg/L

ix. Protein analysis

Visceral protein was measured using 125I-radioimmunoassay. Enzyme-linked immunosorbent assays were used to measure serum transferrin saturation (TSAT) and ferritin (Ramco Laboratories, Inc., Stafford, TX, USA) and interpreted (Table 5.9).

Table 5.9: Interpretation of serum protein status in children

Protein indicator	Classification	Half-life	Reference
Albumin	3.5 - 5.5 g/dl	18 - 20 days	Nutrition assessment
	35 - 55 g/l		
Transferrin	2.6 - 4.3 g/l	8 - 9 days	Wang, 1994
Pre-albumin	0.2 - 0.4 g/l	2 - 3 days	Wang, 1994

5.2.14. Dietary assessment

Dietary assessment involves measuring the quantity of foods consumed by an individual in one to several days or assessing the pattern of food use over several months (USDA, 2000). Conducting a dietary assessment was important in this study, as it assisted in determining the foods commonly consumed by the participants. A 24-hour recall was used to develop a food frequency questionnaire (FFQ). The FFQ was used to determine the frequency of food consumption by children and their portion size. The collected data were recorded in section C of the questionnaire.

Figure 5.2 shows the data collection procedure, which was conducted in phases. Phase 1 involved product preparation and analysis, whereby the safety and nutrient content of the product was assessed before consumption (Netshiheni *et al*., 2022). Biochemical and microbial activity of an instant maize porridge fortified with *MO* leaves and termite powder. Phase 2 involved baseline data collection, whereby an anthropometric, biochemical and dietary assessment of the children aged three to five years old was done before any intervention was introduced to assess their current growth, nutrient intake and nutritional status (Netshiheni *et al*., 2022). Micronutrient status and dietary patterns of preschool children in Thulamela Municipality). Phase 3 was an intervention, whereby participants were assigned to experimental and control groups. Children in the experimental group were fed with 170 g (1/2 cup) of instant-maize porridge fortified with *MO* leaves and termite powder and children of the control group was fed with 170 g (1/2 cup) of placebo; both groups were fed once a day in the morning, thrice a week, for a period of six months. In the last phase, the effect of consuming fortified instant-maize porridge was also determined. Baseline and post-intervention data was compared.

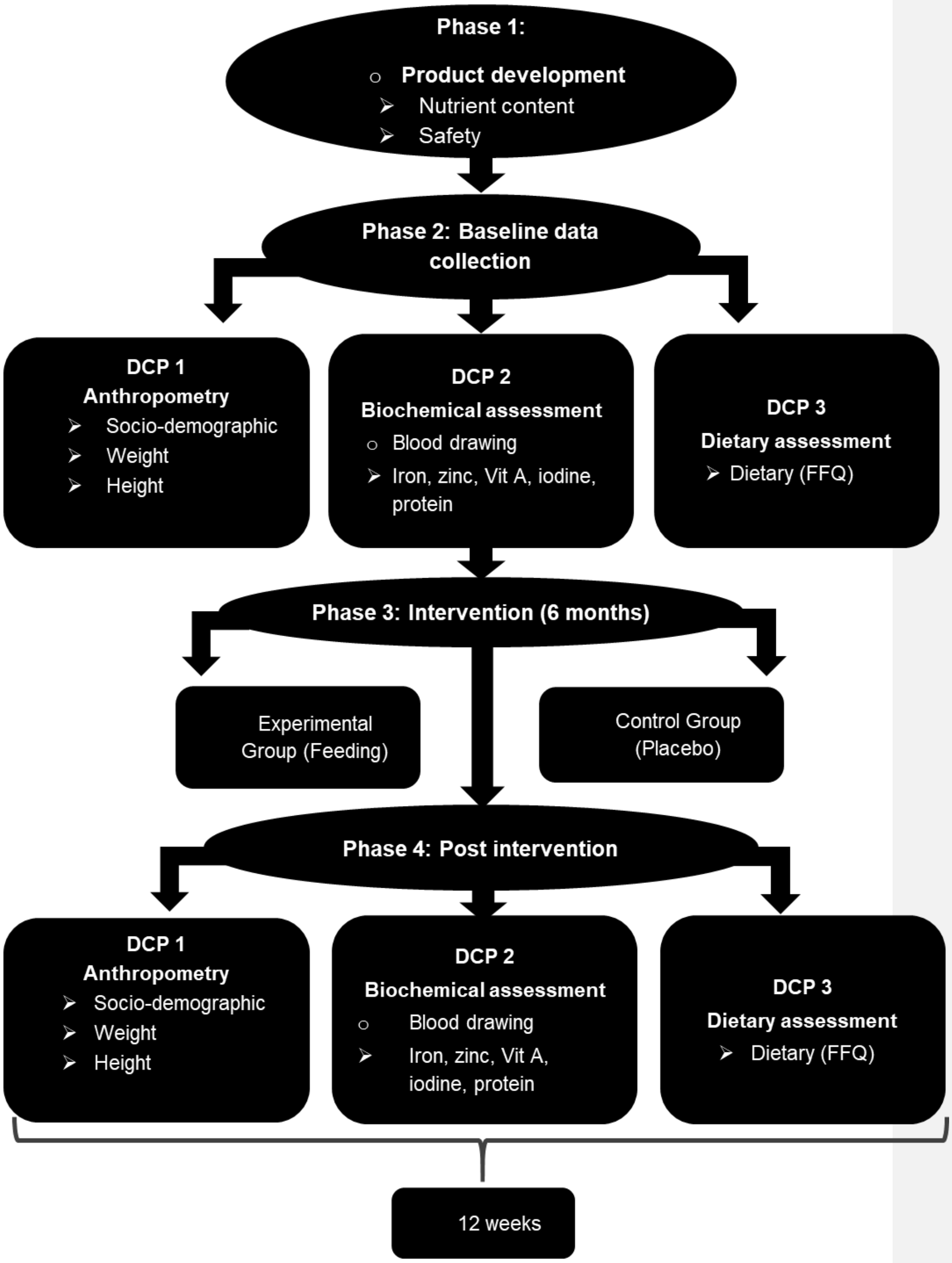

Figure 5.2. Data collection plan

Data collection venues

To ensure privacy of the participants during data collection, the principal of each participating preschool was requested to allocate a dedicated room for these purposes. Anthropometric measures were taken in a screened off area in the allocated venue.

5.2.15. Fieldworkers

Third year Nutrition students and graduates with a few years of work experience were recruited as fieldworkers, as they are already exposed to nutritional assessment and different techniques used to assess nutritional status. Professional nurses were provided by LANCET Laboratories for blood collection. The responsibilities of nutritionists included:

- Managing the data collection process
- Preparing equipment for fieldwork
- Arranging transport to the field
- Organising lunch packs for fieldworkers.

The responsibilities of nurses included:

- Drawing blood of preschool children and storing in vacutainer blood collection tubes.

The researcher was responsible for:

- Making appointments with preschool principals
- Training field workers on how to administer a questionnaire
- Anthropometric and dietary assessment
- Standardising method of data collection.

5.2.16. Validity

Validity is the extent to which the research findings accurately represent what is really happening in the situation (Welman *et al.,* 2005). Content validity was used in the study and ensured by:

- Developing a questionnaire following the objectives of the study and relevant literature
- The instrument was presented to promoters for scrutiny, and inputs and peer review provided yet another avenue for improvement
- The instrument was presented to the Faculty of Health Sciences Research Committee and University of Venda Higher Degrees Committee for approval
- The instrument was also pre-tested and the researcher interviewed participants using the local language.

5.2.17. Reliability

Reliability is a matter of whether a particular technique applied repeatedly to the same object would yield the same results, each time (Woods, 2006). The weighing scale was calibrated before use to ensure reliability and to provide accurate measurements. All analysis was carried out in triplicate and the average number was recorded down. A pre- test study was conducted to ensure clarity where the wording or phrasing may not be understood. Respondents were allowed to ask for clarification.

5.2.18. Statistical methods

Data was exported into Statistical Package for Social Sciences (IBM SPSS) and categorised as frequencies, mean, standard deviation, median and IQR. The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BMIZ).

5.2.19. Institutional approval

The proposal was submitted to the Faculty of Health Sciences, the University Higher Degrees Committee, and the Limpopo Department of Basic Education to seek permission to conduct the study. After permission was granted, the preschools were visited, and the researcher explained to the principals to obtain permission for including preschool children in the study.

5.2.20. Ethical considerations

Ethical clearance was obtained from the University of Venda Research Ethics Committee. The study was performed in accordance with the principles of the declaration of Helsinki (2013), Good Clinical Practices, and the laws of South Africa. The study was explained to preschool principals and the researcher sought their permission to conduct the study. Preschool principals were requested to call a meeting for the parents of preschool children. The researcher subsequently explained the purpose and procedure of the study to parents or legal guardians and gave them the consent forms to read, sign and return. Preschool children were also requested to give verbal assent. Parents or guardians who needed more clarity were requested to attend an information session before they completed the informed consent form. The researcher provided parents or legal guardians with an information sheet and explained the research and assessments, as well as the consent requirements if he/she allowed his/her child to participate in the study (Appendix A).

Although preschool children are vulnerable, this study involved no risk. The parents of the preschoolers and the preschool children had the right to withdraw from the study at any point, without being disadvantaged in any way. The children did not benefit directly from participating in the study but received a food and small bag of fruits at each one of the two data collection point as a token of appreciation for their time and commitment. Data obtained from the study was stored on a computer database in such a manner that it ensured the participants' confidentiality (use of codes instead of names).

5.3. RESULTS: POST-INTERVENTION RESULTS

5.3.1. Post-intervention socio-demographic characteristics of children under five years

About 120 preschool children from six primary schools participated in the present study. Their characteristics are summarised in Table 5.10. The majority (69.2%) of the respondents were mothers of the children, 30% were caregivers while only one child was cared for by the father (0.8%). Almost half of the parents were single (48.3%), 30.8% were married, 15.8% were living together and the remaining 5% were widowed. In terms of level of education of the parents, most had attended Grades 11–12 (45.8%), followed by tertiary education (26.7%). Most of the households did not have a child under five (43.3%), followed by one child (32.5%), two children (16.7%), and the remaining had three or more children under five (7.5%). In terms of the number of members of households older than five years, most had more than four members (71.7%), followed by four members (19.2%). Three quarters of the parents were not working. In most households, the mother is responsible for the household income (29.2%), followed by the grandparents (28.3%). Source of employment was mainly other (51.7%), followed by social grant (30%) and retail (12.5%). In terms of monthly income, the most earned was between R1 000–R2 000 (39.2%), followed by R2 001–R5 000 (24.2%). Almost 90% of the parents were receiving a child grant (88.3%) and more than half had any other form of grant (55%). Most had a traditional house (97.5%), with a tap indoors (98.3%) and pit latrine (85%). The majority had no special diet (95.8%). In terms of type of maize meal, most eat Magnifisan (40%) followed by Super Maize Meal (30.8%) and Tafelberg (20.8%). Over half had done deworming (54.2%). Also, more than half had taken vitamin A (56.7%). More than 30% had suffered illness in the past 15 days (31.7%). Almost all had RtHB (99.2%). All of them had blood and urine taken. (Table 5.10).

Table5.10: Socio-demographic characteristics at post-intervention (SAME AS ABOVE)

Characteristic	N = 120	%
Relationship with the child		
Mother	83	69.2
Father	1	0.8
Caregiver	36	30
Marital status of parent		
Single	58	48.3
Married	37	30.8
Widowed	6	5
Living together	19	15.8
Parent level of education		
Never attended school	2	1.7

Characteristic	N = 120	%
Grades 1–4	8	6.7
Grades 5–7	7	5.8
Grades 8–10	11	9.2
Grades 11–12	55	45.8
Tertiary	32	26.7
(Missing)	5	4.2
Children in the household under 5		
1 Child	39	32.5
2 Children	20	16.7
3 Children	3	2.5
4 Children	2	1.7
More than 4 children	4	3.3
None	52	43.3
Members older than 5 in the household		
2 Members	2	1.7
3 Members	9	7.5
4 Members	23	19.2
More than 4 members	86	71.7
Is the parent working		
Yes	30	25
No	90	75
Person responsible for house income		
Mother	35	29.2
Father	27	22.5
Grandparents	34	28.3
Mother and father	6	5
Other	18	15
Type of employment		
Health worker	2	1.7
Educator	4	3.3
Police	1	0.8
Retail	15	12.5
Social grant	36	30
Other	62	51.7
Monthly income		
Less than R500	3	2.5
R501–R1 000	18	15
R1 001–R2 000	47	39.2
R2 001–R5 000	29	24.2
R5 001–R10 000	8	6.7
R10 000 and above	7	5.8
Unknown	8	6.7

Characteristic	N = 120	%
Child grant		
Yes	106	88.3
No	14	11.7
Any other form of grant		
Yes	66	55
No	54	45
Type of house		
Traditional house	117	97.5
Brick and mortar (modern house)	1	0.8
Shack	2	1.7
Source of water		
Tap in house	118	98.3
Communal tap	2	1.7
Type of toilet		
Flush toilet in house	11	9.2
Flush toilet outside	6	5
Pit latrine	102	85
Bush	1	0.8
Special diet		
Allergic	4	3.3
Weight loss	1	0.8
None	115	95.8
Type of maize meal		
Ace	3	2.5
White Star	3	2.5
Super Maize Meal	37	30.8
Tafelberg	25	20.8
Magnifisan	48	40
Home-made	4	3.3
Deworming		
Yes	65	54.2
No	55	45.8
Vitamin A		
Yes	68	56.7
No	52	43.3
Illness suffered in the last 15 days		
Yes	38	31.7
No	82	68.3
RtHB		
Yes	119	99.2
No	1	0.8
Blood taken		
Yes	120	100

Characteristic	N = 120	%
Urine taken		
Yes	120	100

5.3.3. Cooking energy used at post-intervention period (SAME AS BASELINE)

The cooking energy used by the households of the 120 children is summarised in Table 5.11. Over 80% of the households use firewood coal (82.5%), more than two-thirds use electricity (67.5%), only 6.7% use gas, 1.7% use solar energy, while none of the households use paraffin, gel or cow dung.

Table5.11: Cooking energy used by the households of the 120 under-five children enrolled in the study

Cooking energy used	N=120	%
FIREWOOD COAL		
Yes	99	82.5
No	21	17.5
COAL		
Yes	2	1.7
No	118	98.3
ELECTRICITY		
Yes	81	67.5
No	39	32.5
GAS		
Yes	8	6.7
No	112	93.3
PARAFFIN		
No	120	100
GEL		
No	120	100
COW DUNG		
No	120	100
SOLAR		
Yes	2	1.7
No	118	98.3

5.3.4. Food preparation at post-intervention period (SAME AS BASELINE)

The food preparation used by the 120 households is summarised in Table 5.12. Almost 60% of the households use an open fire outside the house to prepare their food (57.5%), about 40% use open fire inside the house (38.3%), 10.8% use a gas stove, 3.3% use a paraffin stove, 57.5% use an electric stove, none of the households use coal or gel stoves, 14.2% use a microwave oven, and only one household uses Wonder Box or other method.

Table5.12: Food preparation

	N=120	%
OPEN FIRE OUTSIDE		
Yes	69	57.5
No	51	42.5
OPEN FIRE INSIDE		
Yes	46	38.3
No	74	61.7
GAS STOVE		
Yes	13	10.8
No	107	89.2
PARAFFIN STOVE		
Yes	4	3.3
No	116	96.7
ELECTRIC STOVE		
Yes	69	57.5
No	51	42.5
COAL STOVE		
No	120	100
GEL STOVE		
No	120	100
MICROWAVE		
Yes	17	14.2
No	103	85.8
WONDER BOX		
Yes	1	0.8
No	119	99.2
OTHER		
Yes	1	0.8
No	119	99.2

5.3.5. Household food production at post-intervention period (SAME AS BASELINE)

Analysis of household food production of the 120 households showed that 56.7% had a garden, almost a quarter had an orchard (23.3%), more than a fifth had a field in the household (22.5%), and 5% had a smallholder farm. (Table 5.13).

Table5.13: Household food production

Household food production	N	%
GARDEN		
Yes	68	56.7
No	52	43.3
ORCHARD		
Yes	28	23.3
No	92	76.7
FIELD IN HOUSEHOLD		
Yes	27	22.5
No	93	77.5
FIELD AWAY FROM HOUSEHOLD		
Yes	1	0.8
No	119	99.2
SMALLHOLDER FARM		
Yes	6	5
No	114	95
LARGE FARM		
No	120	100

5.3.6. Illness at post-intervention period

The illnesses experienced by the 120 children are summarised in

Table 5.14. The most common illnesses included throat problems (13.3%), headache (10%) and flu (9.2%).

Other less common problems included pneumonia (5%), chicken pox (5%), vomiting (4.2%), stomachache (3.3%), gastrointestinal problems (2.5%), worm infection (2.5%), earache (2.5%), mouth sores (1.7%), and hepatitis (1.7%). Only one child had experienced diarrhoea, ulcer or wound.

None of the children had a history of ear discharge, toothache, appendicitis, allergy, tumour, itching, epilepsy or malaria.

Table 5.14: Illnesses experienced by the 120 under-five children enrolled in the study

Illness	N=120	%
FLU		
Yes	11	9.2
No	109	90.8
THROAT PROBLEMS		
Yes	16	13.3
No	104	86.7

Illness	N=120	%
ASTHMA		
Yes	2	1.7
No	118	98.3
PNEUMONIA		
Yes	6	5
No	114	95
TB		
Yes	1	0.8
No	119	99.2
COVID-19		
Yes	2	1.7
No	118	98.3
STOMACH ACHE		
Yes	4	3.3
No	116	96.7
FEVER		
No	120	100
HEADACHE		
Yes	12	10
No	108	90
VOMITING		
Yes	5	4.2
No	115	95.8
DIARRHOEA		
Yes	1	0.8
No	119	99.2
CHICKEN POX		
Yes	6	5
No	114	95
WORM INFECTION		
Yes	3	2.5
No	117	97.5
HEPATITIS		
Yes	2	1.7
No	118	98.3
EARACHE		
Yes	3	2.5
No	117	97.5
EAR DISCHARGE		
No	120	100
EAR PROBLEM		
Yes	1	0.8
No	119	99.2

Illness	N=120	%
TOOTHACHE		
No	120	100
GASTROINTESTINAL PROBLEMS		
Yes	3	2.5
No	117	97.5
MOUTH SORES		
Yes	2	1.7
No	118	98.3
APPENDICITIS		
No	120	100
ULCER		
Yes	1	0.8
No	119	99.2
PILES		
Yes	2	1.7
No	118	98.3
WOUND		
Yes	1	0.8
No	119	99.2
ALLERGY		
No	120	100
TUMOUR		
No	120	100
ITCHING		
No	120	100
EPILEPSY		
No	120	100
MALARIA		
No	120	100

5.3.7. Child characteristics and anthropometrics at post-intervention

All 120 children (69 boys and 51 girls) had their anthropometrics also measured post-intervention. Their weight in kilograms (kg) post-intervention ranged from 10.6 kg to 30.5 kg, with mean (SD) of 18.0 (3.6) kg. Their height in centimeters (cm) ranged from 81 cm to 112.5 cm, with mean (SD) of 101.4 (5.4) cm.

The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BAZ). The weight-for-age z-scores (WAZ) ranged from -3.72 to 3.5, with mean (SD) of -0.12 (1.30). The height-for-age z-scores (HAZ) ranged from -4.61 to 2.73, with mean (SD) of -0.65 (1.00). The weight-for-height z-scores (WHZ) ranged from -4.94 to 4.50, with mean (SD) of

0.43 (1.61). The BMI-for-age z-scores (BAZ) ranged from -4.99 to 4.43, with mean (SD) of 0.41 (1.57).

5.3.8. Classification of children by anthropometrics at post-intervention

WAZ at post-intervention

Commented [A4]: Shouldn't it be numbered accordingly?

The majority (90%) were in the mild under-weight to normal to possible growth problem range; more than 60% of the children were having normal WAZ range. Seven children were underweight; three underweight and four severely underweight. Furthermore, five children were overweight or obese; four overweight and one obese (Figure 5.3).

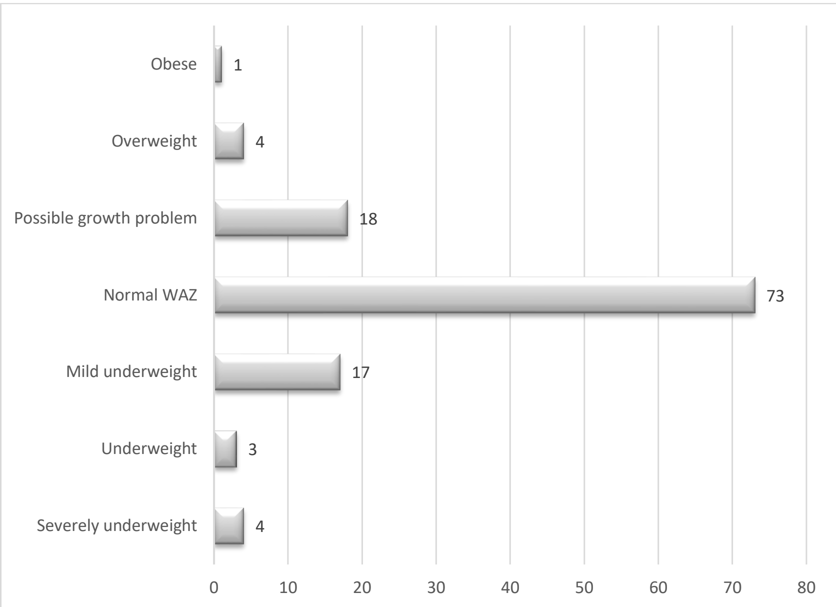

Figure 5.3. WAZ scores of study participants post-intervention

HAZ at post-intervention

The majority (112,93.3%) were in the mild stunted to normal height range; more than two thirds of the children had normal height range (81, 67.5%). Eight children were stunted, with one severely stunted (Figure 5.4).

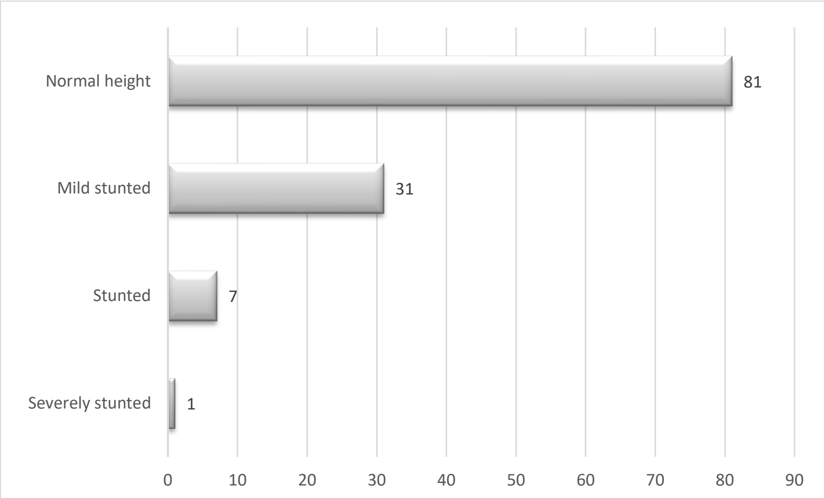

Figure 5.4. HAZ scores for study participants

WHZ at post-intervention

The majority (94,78.3%) were in the mild wasted to normal to possible risk of overweight range; almost half of the children had normal WHZ range (57, 47.5%). Three children were wasted and four had SAM. On the other hand, 16 (13.3%) were overweight and three were obese (Figure 5.5).

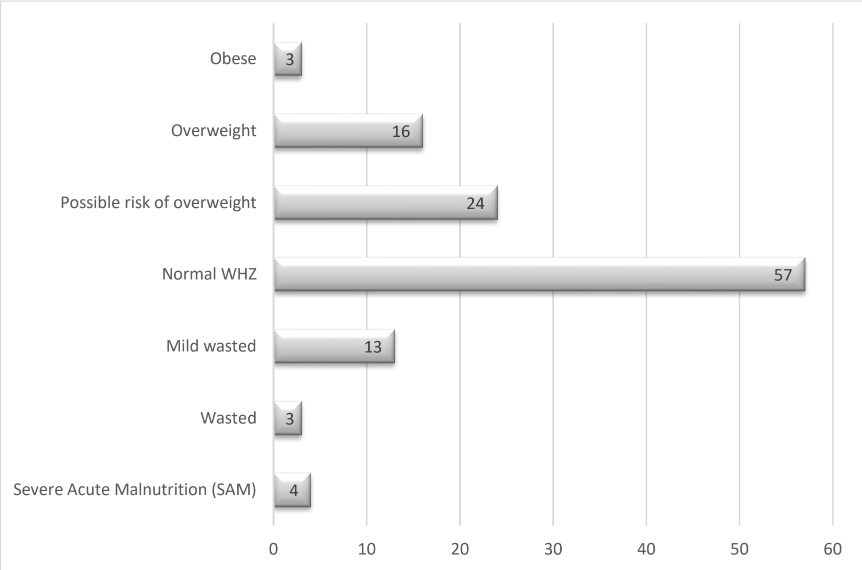

Figure 50.5: WHZ scores of study participants

5.3.9. Comparison of anthropometrics between intervention and control groups at post-intervention

Two independent samples t-test were done to compare the anthropometrics (WAZ, HAZ and WHZ) between the children who received intervention compared to those who received control at the point of post-intervention. There were no significant differences between intervention and control groups with respect to WAZ (p=0.762), HAZ (p=0.133), and WHZ (p=0.171) (Table 5.15).

Table 5.15: Comparison of anthropometrics between intervention and control groups at post-intervention

Anthropometric	Intervention			Control			P-value
	N	Mean	SD	N	Mean	SD	
WAZ	65	-0.16	1.21	55	-0.08	1.41	0.762
HAZ	65	-0.52	0.90	55	-0.80	1.10	0.133
WHZ	65	0.25	1.54	55	0.65	1.66	0.171

5.3.10. Comparison of anthropometrics from baseline to post-intervention for intervention group

Paired t-test was done to compare the anthropometrics (WAZ, HAZ and WHZ) between baseline and post-intervention in the intervention group. However, there were no significant differences between baseline and post-intervention in this group, with respect to WAZ (p=0.170), HAZ (p=0.596) and WHZ (p=0.173) (Table 5.16).

Table5.16: Comparison of anthropometrics from baseline to post-intervention for the intervention group

Anthropometric	Baseline			Post-intervention			P-value
	N	Mean	SD	N	Mean	SD	
WAZ	65	-0.20	1.19	65	-0.16	1.21	0.170
HAZ	65	-0.52	0.90	65	-0.52	0.90	0.596
WHZ	65	0.15	1.53	65	0.25	1.54	0.173

5.3.11. Comparison of anthropometrics from baseline to post-intervention for control group

Paired t-test was done to compare the anthropometrics (WAZ, HAZ and WHZ) between baseline and post-intervention in the control group. However, there were no significant differences between baseline and post-intervention in the control group, with respect to WAZ (p=0.227), HAZ (p=0.826) and WAZ (p=0.838). (Table 5.17).

Table 5.17: Comparison of anthropometrics from baseline to post-intervention for the control group

Anthropometric	Baseline			Post-intervention			P-value
	N	Mean	SD	N	Mean	SD	
WAZ	55	-0.03	1.39	55	-0.08	1.41	0.227
HAZ	55	-0.79	1.08	55	-0.80	1.10	0.826
WHZ	55	0.66	1.65	55	0.65	1.66	0.838

5.3.12. Comparison of baseline-to-post differences between intervention and control groups

Two independent samples t-test were done to compare the baseline-to-post differences in anthropometrics (WAZ, HAZ and WHZ) between the children who received intervention compared to those who received control at the point of post-intervention. There were no significant differences between intervention and control groups, with respect to WAZ (p=0.068), HAZ (p=0906) and WHZ (p=0.210) (Table 5.18).

Table 5.18: Comparison of baseline-to-post differences between intervention and control groups

Anthropometric	Intervention			Control			P-value
	N	Mean	SD	N	Mean	SD	
WAZ	65	0.04	0.25	55	-0.05	0.32	0.068
HAZ	65	-0.004	0.065	55	-0.009	0.292	0.906
WHZ	65	0.10	0.59	55	-0.007	0.249	0.210

5.3.13. Biochemical results at post-intervention

The biochemical results for the 120 children at post-intervention are shown in Table 5.19. Vitamin A had mean (SD) of 123.4 (62.2); however, its distribution is skewed with median of 117.5 and ranging from 40 to 425. Zinc had mean (SD) of 23.2 (17.4); also skewed with median of 19.1 and ranging from 5.7 to 169. Iron had mean (SD) of 18.1 (6.4) and seemed normally distributed and ranging from 6.7 to 31.0. Serum ferritin was also skewed with mean (SD) of 67.0 (40.9) and ranging from 11.6 to 211. Serum transferrin was normally distributed with mean (SD) of 38.6 (6.1) and ranging from 25.9 to 80.9. Also normally distributed were saturation with mean (SD) of 25.7 (10.0) and protein 71.1 (6.7). The rest of the biochemical elements were skewed, meaning they had outlying or extreme values—that is, median (Interquartile range [IQR]) were 38.1 (28.1, 53.6) for 24-iodine excretion, 1.4 (1.0, 2.0) for 24-creatinine, and 78.3 (61.8, 121.8) for iodine urine, respectively.

Table 519: Biochemical results at post-intervention

Biochemical element (N=120)	Mean(SD)	Median(IQR)	Range (Min, Max)
VITAMIN A (µg/L)	123.4 (62.2)	117.5 (72.2, 153.5)	(40,425)
ZINC (µg/dl)	23.2 (17.4)	19.1 (17.0, 21.75)	(5.7,169)
IRON (ng/mL)	18.1 (6.4)	18.5 (12.9, 23.0)	(6.7,31.0)
SERUM FERRITIN (ng/mL)	67.0 (40.9)	54.4 (40.8, 82.1)	(11.6,211)
SERUM TRANSFERRIN (g/L)	38.6 (6.1)	37.5 (35.4, 41.9)	(25.9,80.9)
SATURATION (%)	25.7 (10.0)	24.3 (18.1, 33.3)	(9.0,55.1)
PROTEIN (g/l)	71.1 (6.7)	72.0 (69.0, 74.0)	(29.1,84)
24-IODINE EXCRETION (µg/L)	47.2 (40.2)	38.1 (28.1, 53.6)	(9.7,331)
24-CREATININE (g/d)	2.5 (9.0)	1.4 (1.0, 2.0)	(0.2,95.9)
IODINE URINE (µg/L)	95.9 (61.9)	78.3 (61.8, 121.8)	(0.72,401)

SD: Standard Deviation, IQR: Interquartile Range

Using the Lancet Laboratory ranges, the researcher categorised the children as having deficiency, normal and excess levels of these biochemical elements (see Table 5.20).

Vitamin A (113–647) ug/dl

Only 14 children (11.7%) had normal vitamin A status at post-intervention.

Zinc (10.7–18.4) ug/dl

Four children (3.3%) had zinc deficiency, 55 (45.8%) had normal zinc levels, and 61 (50.8%) had excess zinc levels.

Iron levels (113–647) umol/l

According to Lancet Laboratories, normal iron is in the range (113–647) umol/l. Therefore, 10% of the children had iron deficiency.

Serum ferritin levels (13–400 ng/ml)

Three children had serum ferritin deficiency (2.5%), while the rest had normal serum ferritin levels (117, 97.5%).

Serum transferrin levels (26–47 umol/l)

One child had serum transferrin deficiency, the majority (117, 97.5%) had normal levels, and two children had excess levels.

Saturation (%) (20–55%)

Forty percent (n=48) of the children had low saturation while the rest (72, 60%) had normal saturation of 20 to 55%.

Protein (60–80 ug/dl)

Three children (2.5%) had low protein, the majority (n=116, 96.7%) had normal protein, and one child had high protein levels.

24-hour creatinine (0.98–6.04 mol/24h)

Fifty children (41.7%) had creatinine deficiency, the majority (n=68, 56.7%) had normal creatinine levels, and two children had excessive creatinine levels.

Iodine excretion (75–851 ug/day)

The majority of the children had low iodine excretion (108,90%) and only 10% (n=12) had normal iodine excretion.

Table5.20: Biochemical results in categories at baseline

Biochemical element	Categories	N=120	%
VITAMIN A (µg/L)	Deficiency	106	88.3
	Normal	14	11.7
ZINC (µg/dl)	Deficiency	4	3.3
	Normal	55	45.8
	Excess	61	50.8
IRON (ng/mL)	Deficiency	12	10.0
	Normal	108	90.0
SERUM FERRITIN (ng/mL)	Deficiency	3	2.5
	Normal	117	97.5
SERUM TRANSFERRIN (g/L)	Deficiency	1	0.8
	Normal	117	97.5
	Excess	2	1.7
SATURATION (%)	Deficiency	48	40.0
	Normal	72	60.0
PROTEIN (g/l)	Deficiency	3	2.5
	Normal	116	96.7
	Excess	1	0.8
24-IODINE EXCRETION (µg/L)	Normal	12	10.0
	Severe	108	90.0
24-CREATININE (g/d)	Deficiency	50	41.7
	Normal	68	56.7
	Excess	2	1.7

5.3.14. Comparisons of biochemical results between intervention and control at post-intervention

Since the data were skewed, it may not be appropriate to use the t-test. Therefore, using the nonparametric test (Mann-Whitney/Wilcoxon test), the following results were found (Table 5.21). There were significantly higher values in the intervention group compared to the control group at post-intervention for vitamin A ($p<0.001$), iron ($p<0.001$), serum ferritin ($p<0.001$), serum transferrin ($p=0.006$), saturation ($p<0.001$), and protein ($p=0.024$). However, for 24-iodine excretion ($p=0.001$) and 24-creatinine ($p=0.015$), the values were significantly higher in the control group than intervention at post-intervention. There was no significant difference in biochemical levels for zinc and iodine urine at post-intervention period.

Table5.21: Comparisons of biochemical results between intervention and control at post-intervention

Biochemical element N=120	Intervention (N=65) Median (IQR)	Control (N=55) Median (IQR)	P-value
VITAMIN A	128.0 (115.2, 189.0)	85.9 (60.0, 130.0)	<0.001
ZINC	19.0 (17.5, 22.1)	19.6 (16.1, 21.5)	0.38
IRON	20.1 (14.5, 25.0)	14.4 (11.0, 20.7)	<0.001
SERUM FERRITIN	74.2 (49.4, 102.1)	45.4 (33.8, 61.4)	<0.001
SERUM TRANSFERRIN	39.0 (36.0, 43.2)	36.5 (34.6, 39.9)	0.006
SATURATION	28.5 (22.0, 36.0)	21.0 (13.9, 30.7)	<0.001
PROTEIN	72.0 (70.0, 75.0)	71.0 (69.0, 73.0)	0.024
24-IODINE EXCRETION	34.0 (24.1, 41.2)	46.9 (30.7, 67.2)	0.001
24-CREATININE	1.0 (1.0, 1.9)	1.6 (0.9, 2.3)	0.015
IODINE URINE	88.0 (66.2, 122.3)	73.1 (61.1, 111.8)	0.092

5.3.15. Comparison of bio-chemicals from baseline to post-intervention for the intervention group

Paired t-test was done to compare the biochemical results between baseline and post-intervention in the intervention group. The levels of biochemical results in this group increased significantly from baseline to post-intervention for all the biochemical elements (p<0.001) except zinc (p=0.120) and iodine urine (0.901) (Table 5.22).

Table 5.22: Comparison of biochemical elements from baseline to post-intervention for the intervention group

Biochemical element	Baseline			Post-intervention			P-value
	N	Mean	SD	N	Mean	SD	
VITAMIN A	65	69.1	37.5	65	141.0	65.9	<0.001
ZINC	65	21.2	12.3	65	24.9	21.9	0.120
IRON	65	17.4	5.9	65	20.1	6.1	<0.001
SERUM FERRITIN	65	49.6	22.5	65	83.8	46.3	<0.001
SERUM TRANSFERRIN	65	35.3	4.2	65	39.3	5.2	<0.001
SATURATION	65	24.9	8.8	65	29.4	9.6	<0.001
PROTEIN	65	69.6	3.7	65	72.3	4.7	<0.001
24-IODINE EXCRETION	65	31.2	38.3	65	43.9	48.8	<0.001
24-CREATININE	65	1.1	0.5	65	1.3	0.6	<0.001
IODINE URINE	65	104.9	149.9	65	102.8	65.1	0.901

5.3.16. Comparison of bio-chemicals from baseline to post-intervention for the control group

Paired t-test was done to compare the levels of biochemical results between baseline and post-intervention in the control group. There was no significant difference from baseline to post-intervention for all the biochemical elements (p>0.05) except vitamin A, which increased from baseline to post-intervention (p<0.001) (Table 5.23).

Table5.23: Comparison of biochemical results from baseline to post-intervention for the control group

Biochemical element	Baseline			Post-intervention			P-value
	N	Mean	SD	N	Mean	SD	
VITAMIN A	55	76.2	35.3	55	102.6	50.6	<0.001
ZINC	55	21.7	9.97	55	21.2	9.3	0.292
IRON	55	15.9	6.1	55	15.8	6.0	0.087
SERUM FERRITIN	55	46.5	19.6	55	47.1	20.1	0.330
SERUM TRANSFERRIN	55	37.9	6.9	55	37.7	6.9	0.238
SATURATION	55	21.6	8.6	55	21.4	8.7	0.307
PROTEIN	55	69.6	8.3	55	69.6	8.3	0.518
24-IODINE EXCRETION	55	51.7	27.0	55	51.2	26.5	0.481
24-CREATININE	55	3.9	13.2	55	3.8	13.2	0.084
IODINE URINE	55	91.1	58.3	55	87.7	57.4	0.347

5.3.17. Comparison of baseline-to-post differences between intervention and control groups

Paired t-test was done to compare the baseline-to-post differences between intervention and control groups. The baseline-to-post increases in biochemical were significantly higher in Intervention compared to the control group for all biochemical results (p<0.001) except zinc (p=0.102) and iodine urine (p=0.941) (Table 5.24).

Table5.24: Comparison of baseline-to-post differences between intervention and control groups

Biochemical element	Intervention (N=65)			Control (N=55)			P-value
	N	Mean	SD	N	Mean	SD	
VITAMIN A	65	71.9	46.2	55	26.4	38.3	<0.001
ZINC	65	3.7	18.7	55	-0.6	4.1	0.102
IRON	65	2.7	4.0	55	-0.1	0.5	<0.001
SERUM FERRITIN	65	34.2	33.3	55	0.6	4.2	<0.001
SERUM TRANSFERRIN	65	4.0	3.3	55	-0.2	0.9	<0.001
SATURATION	65	4.5	4.0	55	-0.2	1.1	<0.001
PROTEIN	65	2.7	2.7	55	-0.1	0.6	<0.001
24-IODINE EXCRETION	65	12.7	14.7	55	-0.5	5.5	<0.001
24-CREATININE	65	0.3	0.3	55	-0.04	0.17	<0.001
IODINE URINE	65	-2.1	135.0	55	-3.5	27.0	0.941

5.3.18. Comparisons of dietary intake between intervention and control at post-intervention

Data on dietary patterns of preschool children at post-intervention are summarised in Table 5.25 as well as medians and IQR. About 20.5% of the children in the experimental group and 37.8% of the children in the control group had energy intakes below the RDA at post-intervention. Despite this, most children in both groups were given fat such as margarine and cooking oil at baseline as well as post-intervention. More than 70% of the children had poor vitamin A intake at baseline from both groups. However, at post-intervention, higher values were found in the intervention group compared to the control group for Vitamin A ($p<0.001$). The vitamin A intake increased in the experimental group at post-intervention. About 10% of the children also had poor iron intake at baseline from both experimental and control groups. Iron values in the present study were significantly higher ($p<0.001$) in the intervention group compared to the control group at post-intervention. The majority (96.7%) of the children had normal protein intakes.

Table 5.25: Dietary intakes of nutrients by preschool children as determined by the QFFQ (median, IQR) and percentages below 67% DRI at post-intervention

Nutrient	FFQ	N (%) FFQ deficiency
Energy (kJ)	6 889.40 (2 660.25)	54 (45)
Total protein (g)	63.14 (18.18)	2 (2.5)
Plant protein (g)	33.63 (16.35)	
Animal protein (g)	15.53 (9.43)	
Total fat (g)	38.06 (18.04)	
SFA (g)	8.49 (5.50)	
MUFA (g)	9.99 (7.11)	
PUFA (g)	11.18 (6.89)	
Carbohydrate (g)	182.93 (72.91)	1 (0.2)
Total sugars (g)	22.01 (19.50)	
Added sugar (g)	22.85 (16.23)	
Total dietary fibre (g)	22.`16 (8.11)	36 (18.1)
Calcium (mg)	257.47 (155.19)	96 (58.8)
Fe (mg)	9.07 (4.05)	13 (8.0)
Magnesium (mg)	289.44 (102.51)	1 (0.6)
Phosphorus (mg)	729.06 (335.79)	1 (0.6)
Zinc (mg)	6.06 (1.26)	1 (0.6)
Vitamin A (µg)	286.44 (289.14)	45 (45.4)
Thiamin (mg)	1.6 (0.32)	1 (0.6)
Riboflavin (mg)	0.91 (0.52)	8 (4.9)
Niacin (mg)	9.58 (5.18)	2 (1.2)
Vitamin B_6 (mg)	0.69 (0.31)	4 (2.5)
Folate (µg)	138.85 (76.87)	40 (24.7)
Vitamin B_{12} (µg)	2.39 (2.50)	13 (8.0)
Vitamin C (mg)	44.37 (31.34)	0 (0)
Vitamin D (µg)	3.66 (3.15)	73 (45.1)

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates; P/S ratio = polyunsaturated fat to saturated fat ratio.

Furthermore, means for vitamin A, iron and protein intakes increased at post-intervention in the experimental group compared to the control group. At baseline, vitamin A, iron and protein were below the RDA for both experimental and control groups. Mean nutrient intakes measured by the 24-HR is comparable to those measured by the Food Frequency Questionnaire at post-intervention, particularly for the experimental group rather than the control group (see 5.26).

Table 5.26: Mean percentage energy contribution of macronutrients of preschool children

Macronutrient	Reference ranges	FFQ
Protein	22 - 35%	18.18 ±1.97
Fat	21 - 31%	29.07 ± 5.88
SFA	< 10%	5.79 ± 1.99
MUFA	> 10%	8.59 ± 2.16
PUFA	6 - 10%	7.34 ± 2.20
CHO	55 - 65%	65.74 ± 5.98

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates.

Table 5.27 shows foods consumed by preschool children. The commonly consumed foods include fortified maize porridge, mangoes, maize meal, bananas, sweets, Simba chips, potatoes, bread, and milk.

Table 5.27: Commonly consumed foods by preschool children

FFQ	24-HRQ
Fortified maize porridge	Fat
Mangoes	Fortified maize porridge
Sugar	Maize meal
Maize meal	Mangoes
Sweets	Tea
Crisps	Bread
Potatoes	Chicken
Bread	Milk
Cabbage	*Amaranthus thunbergii*
Amaranthus thunbergia	Pumpkin leaves *(Cucurbita)*
Pumpkin leaves *(Cucurbita)*	Cabbage
Fish	Tomato and onion
Mafula	Mafula
Tea	Banana
Banana	Oranges
Oranges	Naartjies
Naartjies	Crisps
Rice	Eggs
Tomato and onion	Soups
Cookies	Beef
Apple	Potatoes
Eggs	Mabela
Spinach	Bread, white
Atchar	Rice
Chicken	Spinach
Cold drink	Margarine
Samp	Sweets

FFQ	24-HRQ
Beef	Fish
Milk	Apple
Vetkoek	Pumpkin
Margarine	Beetroot
Squash	Vetkoek
Macaroni	Squash
Soya	Beans
Beans	Avocado
Soup	Cookies

5.4. DISCUSSION OF RESULTS

5.4.1. Post-intervention socio-demographic characteristics of children under five years

About 120 preschool children, (69, 57.5%) boys and (51, 42.5%) girls, from six primary schools in Thulamela Municipality, Vhembe District, Limpopo Province, participated in the present study (Table 5.10). Limpopo is known to be one of the poorer regions in South Africa, with a population of almost six million (STATSSA, 2022). In the present study, the majority (69.2%) of the respondents were mothers of the children, 30% were caregivers while only one child was cared for by the father (0.8%). The following poverty indicators were observed in the present study: about 75% of mothers/guardians were unemployed and 88% were receiving either a child or pension grant. The unemployment results in this study are higher than what has been reported by other authors in previous years, and this could be one of the COVID-19 effects, as most people lost their jobs since 2020, because of the lockdown. Furthermore, 97.5% of residents in Limpopo still used traditional houses; this finding may imply a higher degree of poverty with poorer infrastructure in the rural areas of Limpopo. The poor infrastructure results observed in the present study are comparable to what Mamabolo *et al*. reported in 2006, and this shows that there has been no significant change in rural areas of Limpopo with regards to infrastructure.

In terms of number of members of household older than five years, most of the households had more than four members (71.7%), followed by four members (19.2%). In 2006, Mamabolo *et al*. reported that most households in Limpopo had large household size of more than four members. In addition, it has been evaluated and reported that household size may lead to competition for available food. Almost half of the parents were single (48.3%), 30.8% were married, 15.8% were living together and the remaining 5% were widowed. Most parents had attended Grades 11–12 (45.8%), followed by tertiary education (26.7%). A trend contradicting the results in the present study was observed in South Africa, where it was found that 25% of caregivers of children aged 1–9 years had no schooling (NFCS, 1999). In addition, Wamani et al. (2006) reported that 21% of mothers with children aged 0 to 23 months in rural Uganda had no schooling. On the other hand, Matthews *et al*. (2009) reported a percentage (39%) of caregivers who had no schooling in underdeveloped areas of North Western Nigeria.

Mushaphi *et al*. (2015) reported that the education level of mothers/caregivers contributes significantly to the quality of health and nutrition of a child. Mushaphi *et al*. (2015) further explained that educated mothers/caregivers process information on nutrition, acquire skills and display positive caring behaviours more than less educated mothers/caregivers; this may reflect on the child's nutritional status. In addition, the International Food Policy Research

Institute (IFPRI, 2012) also agrees that an improved educational level of mothers/caregivers can improve the child's nutritional status. This can be supported by a 43% total reduction in child malnutrition, which was observed from 1970 to 1995 after mothers/caregivers were educated on nutrition. Chen & Li (2008) and Semba *et al*. (2008) also observed the similar trend that mothers/caregivers without formal education or only primary education were less likely to contribute positively to the nutritional status of their children compared to mothers/caregivers who had secondary or college education. In the present study, three quarters of the parents were not working and 90% of those parents were receiving child grants. This could be because most mothers were students who were still studying either at high school or a tertiary institution, which resulted in lower family income.

According to the study conducted by Mushaphi *et al*. (2015), a higher number (74%) of the households who depended mainly on child support grants was recorded. In contrast Smuts et al. (2008) reported a much lower number of households that depended on child grants in rural districts of the Eastern Cape (37%) and KwaZulu-Natal (14%). In this study, most of the households were headed the mothers (29.2%). In terms of monthly income, the most earned was between R1 000–R2 000 (39.2%) followed by R2 001–R5 000 (24.2%). The income ranges of R1 001 to R2 000 in the present study is higher than what was reported by Mushaphi *et al*. (2015), who showed that 60% of the households' income was R1 000 or less per month in Limpopo. These findings are almost comparable to those of the NFCS (1999), which reported that 58% and 49% of households' income in South Africa, specifically in Limpopo, was below R1 000 per month.

5.4.2. Cooking energy used at post-intervention period (SAME AS BASELINE)

The cooking energy used by the households of the 120 children is summarised in Table 5.11. Over 80% of the households use firewood coal (82.5%), more than two-thirds use electricity (67.5%). The results in the present study are almost what Mushaphi *et al.* (2015) reported where more than 90% of the households relied on firewood for cooking in Limpopo. In 2008 Smuts *et al.* reported that 86% of the people in Eastern Cape and 71% in Kwazulu Natal were also using firewood for cooking. In contrast, the NFCS (1999) recorded that 49% of households in Limpopo with children aged one to nine years were using firewood/coal for cooking. Even though a higher percentage of households using firewood for cooking in Limpopo is reported than what NFCS reported in 1999, the same households also use electricity—but mainly for lights and other electrical appliances in the present study. Mamabolo *et al*. (2006) reported using firewood for cooking as one of the poverty indicators, meaning that even though there are some improvements in terms of lifestyle in Limpopo, poverty still exists.

5.4.3. Household food production at post-intervention period (SAME AS BASELINE)

As shown in Table 5.13, analysis of household food production of the 120 households showed that 56.7% had a garden, almost a quarter had an orchard (23.3%), more than a fifth had a field in the household (22.5%), and 5% had a smallholder farm. Even though most of the participants had either a home garden or orchard, producing food was still a challenge because of lack of water for irrigation and inadequate knowledge about modern farming. A lack of rural infrastructure, limited access to modern inputs and irrigating infrastructure, storage facilities, limited knowledge about modern farming techniques and access to credit all lead to low food production, limited participation in markets and a lack of investment (FAO, 2009). It is important that the government ensures that most rural people have access to land for food production, modern agricultural technologies, and credit, as that would help significantly in improving food security at household level, which in turn will result in adequate dietary intake and improved nutritional status of children in families (Mushaphi *et al*., 2015; Ajani, 2008).

Home gardens and small animal farming projects may also help in improving household food security and increase household income when a surplus is produced (vegetables and animals are sold, which can reduce poverty by generating income that will assist the family to buy other basic supplies). Promoting the production of horticultural crops with a high carotene content, such as carrots, pawpaw and leafy vegetables, increases access to and consumption of them, leading to improved vitamin A status of children (Babu, 2000; Faber *et al*., 2002b). However, home gardens and small animal farming should be integrated with nutrition education to ensure that the small farmers also consume the food they produce before they sell everything. Several studies have shown that the home garden, coupled with nutrition education, increased the intake of vitamin A-rich foods, leading to improved micronutrient status of children (Chakravarty, 2000; Faber *et al*., 2002a; Jones *et al*., 2005; Ruel & Levin, 2000).

5.4.4. Illness at post-intervention period

The illnesses experienced by the 120 children are summarised in

Table 5.14. The most common illnesses include flu (90.8%) and throat problems (87.5%). The high number of children who experienced flu in the present study could be due to the study being conducted in the second part of the year, which is cold compared to other seasons. Other less common illnesses included vomiting (4.2%) gastro-intestinal problems (2.5%) and about 0.8% of these children were affected with diarrhoea. The use of medications can lead to symptoms such as nausea, vomiting and diarrhoea, which result in loss of appetite and decreased food intake. Diarrhoea in most cases results in malabsorption of nutrients. Malabsorption may be a result of small-bowel bacterial overgrowth due to gastrointestinal

dysmotility or hypochlorhydria, and may also predispose the child to malabsorption (Miller, 2003:S134). None of the children had a history of TB, COVID-19, ear discharge, toothache, appendicitis, allergy, tumour, itching, epilepsy or malaria. Dannhauser & Veldman (2013) reported that illnesses can contribute to the development of malnutrition in children. Dannhauser & Veldman (2013) further explained that an illness can influence the food intake of the sick person, which in turn influences the nutritional and health status of the individual. Children are even more vulnerable to malnutrition due to their less developed immune system and their relatively high nutritional needs for proper growth and development. In addition, increased energy expenditure is regarded as one of the main causes of weight loss in individuals who are ill as their requirement for energy is approximately 10% more compared to those who are not ill. The food and nutrient intake of children who are ill or with advanced infection needs to provide for the increased needs over and above the needs for normal growth and development. Inability to meet these increased nutritional needs result in malnutrition (Semba & Tang, 1999:182). None of the children had a history of ear discharge, toothache, appendicitis, allergy, tumour, itching, epilepsy or malaria.

5.4.5. Child characteristics and anthropometrics at post-intervention

All the 120 children (69 boys and 51 girls) had their anthropometrics also measured post-intervention. Their weight in kilograms (kg) post-intervention ranged from 10.6 kg to 30.5 kg, with mean (SD) of 18.0 (3.6) kg. Their height in centimeters (cm) ranged from 81 cm to 112.5 cm, with mean (SD) of 101.4 (5.4) cm.

The WHO AnthroPlus software was used to calculate the z-scores (WAZ, HAZ, WHZ and BAZ). The weight-for-age z-scores (WAZ) ranged from -3.72 to 3.5, with mean (SD) of -0.12 (1.30). The height-for-age z-scores (HAZ) ranged from -4.61 to 2.73, with mean (SD) of -0.65 (1.00). The weight-for-height z-scores (WHZ) ranged from -4.94 to 4.50, with mean (SD) of 0.43 (1.61). The BMI-for-age z-scores (BAZ) ranged from -4.99 to 4.43, with mean (SD) of 0.41 (1.57).

5.4.6. Classification of children by anthropometrics at post-intervention

The majority (90%) were mild under-weight to normal to possible growth problem range; more than 60% of the children were having normal WAZ range (60.8%). Three underweight and four severely underweight. Furthermore, five children were overweight or obese; four overweight and one obese. (Figure 5.3). The majority (112, 93.3%) were in the mild stunted to normal height range; more than two thirds of the children were having normal height range (67.5%). Eight children were stunted with one severely stunted. (Figure 5.4). The majority (78.3%) were in the mild wasted to normal to possible risk of overweight range; almost half of the children were having normal WHZ range (47.5%). Three children were wasted and four had SAM. On the other hand, 16 (13.3%) were overweight and three were obese. (Figure 5.5).

5.4.7. Comparison of anthropometrics between intervention and control groups at post-intervention

Two independent samples t-test were done to compare the anthropometrics (WAZ, HAZ and WHZ) between the children who received intervention compared and the control group at the point of post-intervention. There were no significant differences between intervention and control groups with respect to WAZ (p=0.762), HAZ (p=0.133), and WHZ (p=0.171). The results in the present study are similar to what Faber *et al*. (2012) reported, where anthropometric values did not significantly change according to the 95% CI for the median difference (E = [0; 0]; C = [0; 0]) at post-intervention. Furthermore, when the two groups (experimental and control) were compared at post-intervention, there was no statistical difference between the experimental group and control group regarding HAZ (95% CI for the median difference [0; 0]), WAZ (95% CI for 96 the median difference [0; 0]), WHZ (95% CI for the median difference [0; 0]) and BMI/A z-scores (95% CI for the median difference [0; 0]). Mushaphi *et al*. (2015) also reported similar results to that of the present study and that of Faber *et al.* (2002), with a nutrition education programme not making a significant difference to the nutritional status of children. Mushaphi *et al*. (2015) further reported that the prevalence of stunting, underweight, wasting and overweight did not change (95% CI for the median difference [0; 0]) in both groups at post-intervention. In this study, the intervention was conducted for six months, which is regarded a shorter period to be able to see anthropometric significant changes. Moreover, even though the experimental group presented some changes in some children, it was difficult to facilitate catch-up growth in stunted children after six months of intervention. In contrast, Walsh *et al*. (2002) reported results that differ from the present study where the nutritional status of children was determined after an intervention study was conducted in the Free State and Northern Cape for a period of over two years; as a result, the intervention significantly improved the weight for age of boys and girls in urban areas, and of boys in one rural area, the two year period of the study conducted by Walsh *et al*. (2002) was long enough to present significant changes in anthropometry. In addition, Ghoneim *et al*. (2004) indicated that the number of children aged two to five years from three day care centres in Egypt who were stunted and wasted, significantly improved their status after one year of intervention.

Paired t-test was done to compare the anthropometrics (WAZ, HAZ and WHZ) between baseline and post-intervention in the intervention and control groups. However, there were no significant differences between baseline and post-intervention in the intervention group with respect to WAZ (p=0.170), HAZ (p=0.596) and WHZ (p=0.173). There were also no significant differences between baseline and post-intervention in the control group with respect to WAZ (p=0.227), HAZ (p=0.826) and WAZ (p=0.838) (Table 5.2 and Table 5.3). Furthermore, when two groups (E and C) were compared at post-intervention, there was no statistical difference between the experimental group and control group regarding HAZ (95% CI for the median difference [0; 0]), WAZ (95% CI for 96 the median difference [0; 0]), WHZ (95% CI for the median difference [0; 0]) and BMI/A z-scores (95% CI for the median difference [0; 0]). The results in the present study are similar to what Faber *et al*. (2012) reported where, anthropometric values did not significantly change according to the 95% CI for the median difference (E = [0; 0]; C = [0; 0]) at post-intervention.

Contrary to the current study, research done by Walsh *et al*. (2002) and Ghoneim *et al*. (2004) reported an improvement in the anthropometric status of the children and that could be because nutrition education was combined with a feeding programme, which may have contributed to the significant changes. Furthermore, the majority (77.6% of control group to 82.5% of experimental group) of children had a normal HAZ at baseline when using the WHO (2009) classification system. The less or no difference observed at baseline and post-intervention in the control group could be due to that the children in the control group did not change their diet and that no intervention was introduced to them.

5.4.8. Biochemical results at post-intervention

The biochemical results for the 120 children at post-intervention are shown in Table 5.15. Vitamin A had mean (SD) of 123.4 (62.2); however, its distribution is skewed with median of 117.5 and ranging from 40 to 425. Zinc had mean (SD) of 23.2 (17.4); also skewed with median of 19.1 and ranging from 5.7 to 169. Iron had mean (SD) of 18.1 (6.4) and seemed normally distributed and ranging from 6.7 to 31.0. Serum ferritin was also skewed with mean (SD) of 67.0 (40.9) and ranging from 11.6 to 211. Serum transferrin was normally distributed with mean (SD) of 38.6 (6.1) and ranging from 25.9 to 80.9. Also normally distributed were saturation with mean (SD) of 25.7 (10.0) and protein 71.1 (6.7). The rest of the biochemical elements were skewed, meaning they had outlying or extreme values, that is, median (Interquartile range [IQR]) was 38.1 (28.1, 53.6) for 24-Iodine excretion, 1.4 (1.0, 2.0) for 24-creatinine, and 78.3 (61.8, 121.8) for iodine urine, respectively.

SD: Standard Deviation, IQR: Interquartile Range

Using the Lancet Laboratory ranges, the researcher categorised the children as having deficiency, normal and excess levels of these biochemical elements. Only 14 children (11.7%) had normal Vitamin A status at post-intervention. Four children (3.3%) had zinc deficiency, 55 (45.8%) had normal zinc levels, and 61 (50.8%) had excess zinc levels. According to the Lancet Laboratories, normal iron is in the range (113 to 647) umol/l. Therefore, 10% of the children had iron deficiency. Three children had serum ferritin deficiency (2.5%), while the rest had normal serum ferritin levels (117, 97.5%). One child had serum transferrin deficiency, the majority (117, 97.5%) had normal levels, and two children had excess levels. Forty percent (n=48) of the children had low saturation while the rest (72, 60%) had normal saturation of 20 to 55%. Three children (2.5%) had low protein, the majority (n=116, 96.7%) had normal protein, and one child had high protein levels. Fifty children (41.7%) had creatinine deficiency, the majority (n=68, 56.7%) had normal creatinine, and two children had excessive creatinine levels. Most of the children had low iodine excretion (108,90%) and only 10% (n=12) had normal iodine excretion.

5.4.9. Comparisons of biochemical results between intervention and control at post-intervention

Since the data was skewed, it was not appropriate to use the t-test. Therefore, the nonparametric test (Mann-Whitney/Wilcoxon test) was used in the present study. Significantly higher values were found in the intervention group compared to the control group at post-intervention for vitamin A ($p<0.001$) (Table 5.15). The median vitamin A intake showed a tendency towards increase in the experimental group at post-intervention. The higher vitamin A values reported in the present study may be due to the consumption of a maize porridge fortified with *MO* leaves as they are known to be rich in vitamin A (Netshiheni *et al*., 2019). Netshiheni *et al*. (2019) further reported that fresh *MO* leaves contain four times the vitamin A (carotene) than that found in carrots. Therefore, this means that the fortified maize porridge used in the experimental group of the present study could be essential for the growth and metabolism of all body cells in children and useful in producing rhodopsin (visual purple), a complex compound containing retinol and protein. Additionally, the results in the present study are also in agreement with what was done by Gibson *et al*. (2003) amongst preschool children in rural Malawi where vitamin A intake was higher in the intervention group after one year of implementing a community-based nutrition education programme. Faber *et al*. (2002a) also reported that one year after implementing nutrition education together with home-based food production in rural KwaZulu-Natal, increased dietary vitamin A intake rose significantly in children aged two to five years. In addition, the improvement observed in the present study in the experimental group on the intake of vitamin A, is within the EAR and RDA. Furthermore, Mushaphi *et al*. (2015) presented results which confirm that when children are given vegetables three to five times per week, their micronutrient status is improved. The studies done by Dannhauser *et al.* (2000) in the Free State and by Mamabolo *et al*. (2006) in the central region of Limpopo revealed that the diet of most children lacked variety, resulting in a low intake of most micronutrients, including vitamin A.

Iron values in this study were significantly higher (p<0.001) in the intervention group compared to the control group at post-intervention. The higher iron values could be a result of the consumption of maize porridge fortified with *MO* and termites, and this could be because of substitution effect, since fresh Moringa leaves are reported to have over three times the amount of iron than spinach (Edward *et al*., 2005). The increase in iron intake in the present study is almost similar to what was reported by other authors, where the iron (E = 95% CI for the median difference [0.04; 1.89]; C = 95% CI for the median difference [0.5; 2.5]) intake increased significantly (E = 95% 136 CI for the median difference [1.41; 70.1]; C = 95% CI for the median difference [55.7; 117.0]) in the experimental group Mamabolo *et al*. (2006). On the contrary, Gibson *et al*. (2003) indicated that total iron of the intervention study conducted in rural Malawi did not change in children of the experimental group after implementing community-based dietary intervention. A similar observation was made by Mamabolo *et al.* (2006), who found the intake of iron to be inadequate. Furthermore, Mamabolo *et al*. (2006) indicated that most children aged one to three years in the central region of Limpopo consumed inadequate amounts of most micronutrients due to a diet that lacked variety. The preschool feeding programme may also have played a role in increasing the intake of iron, since the majority of children attending preschool were sometimes given vegetables such as spinach and fruit at least twice a week, as also reflected in 24-hour recall. Furthermore, the intake of fortified maize meal and bread by the South African government may have contributed to the overall improvement in the intake of iron, since these products are enhanced with this nutrient.

Significantly higher protein (p=0.024) values were observed in the intervention group compared to the control group at post-intervention. This could be because placebo was introduced to this group. In addition, their diet never changed at both baseline and post-intervention, while the increase in protein intake especially in the experimental group in this study could be attributed to what Netshiheni *et al*. (2019) reported that although cereals are known to contain more carbohydrate and low amount of protein and essential minerals, among cereals, maize contains higher amounts of protein than other cereals (Iken & Amusa, 2010). In addition to the previous statement, *MO* leaves and termites contain substantial amounts of protein, and therefore the fortified maize porridge with Moringa and termites could help in improving the crude protein in weaning food products for infant and children (Netshiheni *et al*., 2019) in African countries (Anigo *et al*., 2009). The protein content of the present study is significant, as it may meet infant protein requirements and boost the immune system against diseases (Moyo *et al*., 2013); therefore, introducing proper nutrition and health to infants and children under the age of five is essential in the first 1 000 days of life, and should be prioritised.

The levels of biochemical results in the intervention group increased significantly from baseline to post-intervention for all the biochemical elements ($p<0.001$) except zinc ($p=0.120$) and iodine urine (0.901). 24-iodine excretion values were significantly higher in the control than intervention at post-intervention. There was no significant difference in biochemical levels for iodine urine at post-intervention period (Table 5.15). The iodine values in the present study are contrary to what the researcher expected, especially because when maize porridge was fortified with *MO* and termites, the fortified porridge's iodine content increased from 10 g/l in the control to 47 g/l in this study. In contrast, when a *MO* leaf and spinach-fed diet was composited, there was a significant increase in urinary excretion of thiocyanate and iodine of rats. Comparing iodine results in the present study with that of other authors was very challenging, and there is less or no literature available on iodine intake of an intervention study similar to this one.

There was no significant difference in biochemical levels for zinc at post-intervention period (Table 5.15). Even though children who were three years old improved in terms of zinc intake those who were four years and above did not improve. Moreover, it was reported that children aged four years and above have a higher zinc requirement as compared to those aged three years and below. The previous statement can be supported by observations made by Faber *et al*. (2002) where the zinc intake was reported to be adequate among children aged one to three years, while for children aged four to eight years, it was inadequate. The zinc results in the present study and in the study conducted by Faber *et al*. (2019) are contrary to other authors, as it was reported that the consumption of the dual food fortified fermented sorghum porridge using *MO* leaf powder and baobab fruit pulp for porridge can be promoted to fight against zinc deficiencies among children aged 6–12 months and children aged 12–36 months. In addition, in the study done by Gibson *et al*. (2003), the median zinc intake was greater in the experimental group after implementation of a community-based nutrition education programme. In the present study, the 24-hour recall showed that many children were given chicken feet, hearts or liver in one or two meals per week, and these are good sources of zinc. Since the observations made in the present study were not significant when the control and experimental groups were compared, it could have been possible that the 24-hour recall and dietary assessment questionnaire was not sensitive enough to detect the effects of the fortified maize porridge with *MO* and termites.

5.4.10. Comparisons of dietary intake between intervention and control at post-intervention

Data on dietary patterns of preschool children at post-intervention are summarised in Table 5.28 as well as medians and IQR. About 20.5% of the children in the experimental group and 37.8% of the children in the control group had energy intakes below the RDA at post-intervention. Looking at the baseline assessment, a significant observation was made between the energy intake of the experimental and control groups. The significant change in the number of children whose energy intake is below the RDA from baseline to post-intervention could be attributed to that even though *MO* is low in dietary fat, the dietary fat content in termites along with the one from margarine consumed by the preschool children in the present study might have contributed to increasing energy intakes. Furthermore, dietary fat also provides energy, which is essential for children's growth and development (Gallagher, 2008). The improvement observed in the experimental group may reflect the possible effect of consuming a fortified maize porridge, as there were no energy improvements in the control group at baseline. Despite this, most children in both groups were given fat such as margarine and cooking oil at baseline as well as post-intervention. More than 70% of the children had poor vitamin A intake at baseline from both groups. However, at post-intervention, higher values were found in the intervention group compared to the control group at post-intervention for vitamin A ($p<0.001$). The vitamin A intake increased in the experimental group at post-intervention. The higher vitamin A values reported in the present study may be due to the consumption of a maize porridge fortified with *MO* leaves as they are known to be rich in vitamin A (Netshiheni *et al*., 2019). The increase in vitamin A values in this study is compared to what Mushaphi *et al*. (2015) reported after a nutrition education was given in an intervention study. About 10% of the children also had poor iron intake at baseline from both experimental and control groups. Iron values in the present study were significantly higher ($p<0.001$) in the intervention group compared to the control group at post-intervention. The higher iron values in the present study could be a result of the consumption of maize porridge fortified with *MO* and termites—and this could be because of substitution effect, since fresh Moringa leaves are reported to have over three times more iron than spinach (Edward *et al*., 2005). The preschool feeding programme may also have played a role in increasing the intake of iron, since the majority of children attending preschool were sometimes given vegetables such as spinach and fruit at least twice per week as also reflected in 24-hour recall. The majority (96.7%) of the children had normal protein intake. This could be because placebo was introduced to this group. In addition, their diet never changed at both baseline and post-intervention while the increase in protein intake especially in the experimental group in the present study could be attributed to what Netshiheni *et al*. (2019) reported, that although cereals are known to contain more carbohydrate and low amounts of protein and essential minerals, however, among cereals, maize contains higher amount of protein than other cereals (Iken and Amusa, 2010). In addition to the previous statement, *MO* leaves and termites contain substantial amounts of protein and, therefore, the fortified maize porridge with *MO* and termites could help in improving the crude protein in weaning food products for infant and children (Netshiheni *et al*., 2019) in African countries (Anigo *et al*., 2009). The protein content of the present study is significant as it may meet infant protein requirement and boost immune system against diseases (Moyo *et al*., 2013); therefore, introducing proper nutrition and health to infants and children under the age of five is essential, and should be prioritised.

Table 5.28: Dietary intakes of nutrients by preschool children as determined by the QFFQ (median, IQR) and percentages below 67% DRI at post-intervention

Nutrient	FFQ	N (%) FFQ deficiency
Energy (kJ)	6 889.40 (2 660.25)	54 (45)
Total protein (g)	43.04 (16.18)	3 (2.5)
Plant protein (g)	26.44 (12.35)	
Animal protein (g)	13.51 (8.53)	
Total fat (g)	34.06 (17.44)	
SFA (g)	8.45 (5.49)	
MUFA (g)	9.95 (6.19)	
PUFA (g)	11.18 (6.89)	
Carbohydrate (g)	206.93 (84.91)	1 (0.6)
Total sugars (g)	24.01 (19.51)	
Added sugar (g)	27.85 (16.37)	
Total dietary fibre (g)	16.56 (7.11)	31 (19.1)
Calcium (mg)	257.47 (155.19)	118 (72.8)
Fe (mg)	7.67 (3.85)	13 (8.0)
Magnesium (mg)	244.47 (104.52)	1 (0.6)
Phosphorus (mg)	726.06 (331.79)	1 (0.6)
Zinc (mg)	5.08 (2.26)	1 (0.6)
Vitamin A (µg)	206.74 (237.14)	80 (49.4)
Thiamin (mg)	0.79 (0.32)	1 (0.6)
Riboflavin (mg)	0.91 (0.52)	8 (4.9)
Niacin (mg)	9.58 (5.18)	2 (1.2)
Vitamin B_6 (mg)	0.69 (0.31)	4 (2.5)
Folate (µg)	130.85 (76.87)	40 (24.7)
Vitamin B_{12} (µg)	2.39 (2.50)	13 (8.0)
Vitamin C (mg)	44.37 (31.34)	0 (0)
Vitamin D (µg)	3.66 (3.15)	73 (45.1)

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates; P/S ratio = polyunsaturated fat to saturated fat ratio.

Furthermore, means for vitamin A, iron and protein intake increased at post-intervention in the experimental group compared to the control group. At baseline, vitamin A, iron and protein were below the RDA for both experimental and control groups. Mean nutrient intake measured by the 24-HR is comparable to those measured by the Food Frequency Questionnaire at post-intervention, particularly for the experimental group rather than the control group. The macronutrients' mean contributions to energy intake were observed to be within the ranges made by the WHO (year), except for the mono-unsaturated fatty acids (Table 5.29) at baseline rather than at post-intervention.

Table 5.29: Mean percentage energy contribution of macronutrients of preschool children

Macronutrient	Reference ranges	FFQ
Protein	10 - 15%	12.18 ±1.67
Fat	15 - 30%	22.07 ± 5.68
SFA	< 10%	5.77 ± 1.89
MUFA	> 10%	6.59 ± 2.16
PUFA	6 - 10%	7.34 ± 2.20
CHO	65 - 75%	65.74 ± 5.98

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; CHO = carbohydrates.

Table 5.30 shows foods consumed by preschool children. The commonly consumed foods include fortified maize porridge, mangoes, maize meal, bananas, sweets, Simba chips, potatoes, bread, and milk. The data stating that fortified maize porridge is at the top of the list is supported by the high vitamin intake found in the experimental group at post-intervention compared to the control group. The higher fat intake can explain the fortified maize porridge and fat being at the top of the list.

Table 5.30: Commonly consumed foods by preschool children

FFQ	24-HR
Fortified maize porridge	Fat
Mangoes	Fortified maize porridge
Sugar	Maize meal
Maize meal	Mangoes
Sweets	Tea
Crisps	Bread
Potatoes	Chicken
Bread	Milk
Cabbage	*Amaranthus thunbergii*
Amaranthus thunbergia	Pumpkin leaves *(Cucurbita)*
Pumpkin leaves *(Cucurbita)*	Cabbage
Fish	Tomato and onion
Mafula	Mafula
Tea	Banana
Banana	Oranges
Oranges	Naartjies
Naartjies	Crisps
Rice	Eggs
Tomato and onion	Soup
Cookies	Beef
Apple	Potatoes

FFQ	24-HR
Eggs	Mabela
Spinach	Bread, white
Atchar	Rice
Chicken	Spinach
Cold drink	Margarine
Samp	Sweets
Beef	Fish
Milk	Apple
Vetkoek	Pumpkin
Margarine	Beetroot
Squash	Vetkoek
Macaroni	Squash
Soya	Beans
Beans	Avocado
Soup	Cookies

Dietary fat helps in the digestion, absorption and transportation of fat-soluble vitamins in the body (Gallagher, 2008). Furthermore, dietary fat also provides the body with energy, which is essential for children's growth and development (Gallagher, 2008).

Limitation of the study

The prevalence of HIV/AIDS status was not determined in the present study. HIV/AIDS infection is associated with weight loss, wasting and an increased risk of opportunistic infection AIDS (FANTA, 2004); these could have had an influence on the impact of the nutrition intervention on the nutritional status of the children. However, the anthropometrics of the children in both groups were not significantly different after intervention, and thus it is unlikely that there was any association with HIV. In addition, when using the 24-hour recall and FFQ methods, there is a possibility that mothers/caregivers under or over-report the consumption of food items (Gibson, 2005; Lee & Nieman, 2007). Furthermore, the participants are more likely to withhold or alter information about what their children ate because of poor memory or embarrassment, or to please or impress the researcher. However, under- or over-reporting was minimised by using the average of three 24-hour recalls and two FFQs on different days, probing and using food models and household utensils to ensure that food portion size was determined as accurately as possible.

5.5. Conclusion

The biochemical results confirmed that there was a significant improvement in nutritional status of the experimental group as compared to that of the control group after six months' intervention. In addition, maize porridge fortified with *MO* leaves and termite powder can potentially have a significant effect in reducing anaemia and protein energy malnutrition. Thus, improving iron, protein and vitamin A status of children under the age of five. Therefore, an awareness of the nutritional benefits of instant-maize porridge fortified with termite and *MO* leaf powders should be promoted to increase its adoption at household level and in schools with feeding programs and health facilities to introduce termite and *MO* leaf blends as part of their daily meals. This could be one of the sustainable strategies to alleviate malnutrition in developing countries.

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5.7. Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

5.8. Authors' contributions

K. R. Netshiheni was the project leader responsible for project design, data collection, analysis, and manuscript drafting. C. N. Nesamvuni and L. F. Mushaphi supervised the project and proofread the manuscript.

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5.10. Data availability

The data supporting this study's findings are available from the corresponding author, K. R. N., upon reasonable request.

5.11. Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

CHAPTER SIX:

6.1. GENERAL CONCLUSION AND RECOMMENDATION

The addition of *MO* leaves and termite powders to maize porridge has resulted in a significant increase in the nutritional value of maize porridge. The fortified sample contained higher amounts of potassium, iodine, folic acid and vitamin A content. The implication, therefore, is that fortified maize porridge could be one of the sustainable strategies to eradicate malnutrition in children under five years. Maize is a staple food for most South Africans and is consumed almost every day for breakfast as thin or thick porridge with meat or vegetables by both children and adults.

Regarding microbial activity, there was no significant difference between the fortified and unfortified samples at $p<0.05$ in coliform and total viable bacteria counts. Listeria monocytogenes and Salmonella were also found to be absent in both fortified and unfortified samples. Moreover, the fortified sample did not contain Salmonella or Staphylococcus aureus, compared to the unfortified sample, which had 20 staphylococcus counts. The absence of Listeria monocytogenes and Salmonella may mean that fortified maize porridge in the present study could be safe for consumption. It was also observed that there were no significant differences between the study intervention and control groups for wasting, stunting and underweight. The intervention was conducted for six months, a shorter period to see significant anthropometric changes. In addition, even though the experimental group presented some changes in some children, it was difficult to facilitate catch-up growth in stunted children after six months of intervention.

The biochemical results of the preschool children in the present study confirmed a significant improvement in nutritional status of the experimental group compared to that of the control group after six months of intervention. Moreover, about 80% of the children had inadequate vitamin A intake, and 10% had iron deficiency, which could be attributed to the high consumption of cereal-based foods eaten daily in Limpopo province. Therefore, the maize porridge fortified with *MO* leaves and termite powder can significantly reduce anaemia and protein energy malnutrition, thus improving the iron, protein and vitamin A status of children under the age of five years. Hence, an awareness of the nutritional benefits of instant-maize porridge fortified with termite and *MO* leaf powders should be promoted as part of daily meals to increase its adoption at the household level, in schools with feeding programs and health facilities. It is recommended that the effectiveness of consuming termite and *MO* leaf-fortified porridge should be assessed on preschool children with SAM for up to a year.

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RESEARCH ETHICS COMMITTEE

UNIVEN INFORMED CONSENT

APPENDIX A: Letter of information

Title of the Research Study :

The effect of consuming instant-maize porridge fortified with termite (*Kalotermitidae*) and *Moringa oleifera* leaf powders on the nutritional status of children aged three to five years old in the Vhembe District, Limpopo Province, South Africa (RCT)

Principal Investigator/s/researcher : Netshiheni, Khavhatondwi Rinah, Masters

Co-Investigator/s/supervisor/s : Nesamvuni Cebisa Noxolo, PhD

: Mushaphi Lindelani Fhumudzani, PhD

Brief introduction and purpose of the study:

Malnutrition is responsible for the deaths of eight million children under the age of five years worldwide (Rajaratnam, 2010). Over one-third of child deaths are due to undernutrition, mostly from increased severity of diseases such as pneumonia and diarrhoea (Corka *et al*., 2017). Undernutrition is a serious health problem caused by a deficiency of essential micronutrients (vitamins and minerals) in a diet, which affect the growth and development of children and also increase the risk of death from common childhood illness (Black *et al*., 2010). Mason *et al*. (2001) estimated that globally, 140 million children under five years had vitamin A deficiency. It has also been estimated that, worldwide, 127 million preschool children were sub-clinically vitamin A deficient (West *et al*., 2002).

Aim of the study

The aim of the study is to investigate the effect of consuming instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders on the nutritional status of children aged three to five years old in the Vhembe district.

Objectives of the study

Objectives for Phase 1

- To determine the nutrient content of instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders
- To determine the microbial activities of instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders.

Objectives for Phase 2

- To assess the anthropometric status (height and weight) of children aged three to five years in the Thulamela Municipality.
- To assess the micronutrient status of children aged three to five years old in the Thulamela Municipality.
- To determine the dietary intake of children aged three to five years in the Thulamela Municipality.

Objectives for Phase 3

- To standardise the preparation method and portion sizes of the instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders
- To feed children aged three to five years old for six months in the Thulamela Municipality.

Objectives for Phase 4

- To determine the effect size of consuming instant-maize porridge fortified with termite and *Moringa oleifera* leaf powders using Cohen's d
- To compare baseline (Phase 2) to post-intervention data (Phase 4).

Outline of the Procedures:

Phase 1 :

This PhD study is a continuation of the MSc work done by Netshiheni *et al*. (2017), where the effect of *Moringa oleifera* (*MO*) leaves and termite powders on the nutritional and sensory properties of instant-maize porridge was studied. In the previous study, proximate (protein, moisture, ash, fibre, fat and carbohydrates), thermal (gelatinisation, viscosity and texture) and physical (colour) properties were studied. Sensory evaluation of the product was carried out by using 60 untrained panellists on the following sensory attributes: appearance, texture, taste and aroma—and overall acceptability of fortified instant-maze porridge. A nine-point hedonic scale, varying from dislike extremely (score 1) to like extremely (score 9) was used according to Mbata *et al*. (2009). This phase involves product preparation and analysis, whereby the safety and nutrient content of the product is assessed. The product will be prepared according to Netshiheni *et al*., 2017 (*see the procedure below*).

Study design

The factor levels will be: T_0-control (100% maize, 0% *MO* leaves and 0% termites); T_1 - Experimental (80% maize, 5% cooked *MO* leaves and 15% termite powders). The research type will be quantitative. Each experiment will be done in triplicate and the statistical model will be: $Yij = M + Ti + Eij$

where: Yij = Observation

M = Overall mean

Ti = Effect of i^{th}treatment on instant maize porridge

Eij = Random error

Experimental site

The samples will be prepared at the Department of Food Science and Technology, School of Agriculture, University of Venda, Thohoyandou, South Africa.

Product preparation

Maize meal, salt, sugar, stainless steel pots, stainless steel and wooden spoons will be purchased from the local supermarket. *MO* leaves will be purchased from local farmers and termites will be purchased from street vendors in Thohoyandou. The samples will be transported to the Department of Food Science and Technology and stored at room temperature (25 °C) until they are prepared and analysed.

Microbial and nutrient analyses

Microbial analysis will be done to check the safety of the product and also to verify if the quality of the raw materials (maize meal, *MO* leaves and termites) used are within specification before consumption or placed on the market. Additionally, laboratory testing is important for the development of new products, including, for example, the choice of ingredients or components, the design of food processing and shelf-life studies. It is important to analyse this fortified instant-maize porridge to assess the amount of essential micronutrients contained in the product and which are also required by children for proper growth and development—and if it will address micronutrient deficiency in children. The microbial activities, the following micronutrients (iron, zinc, iodine, folic acid and vitamin A) and phytic acids will be analysed using standard procedures shown in Table 0.31.

Table 3.10. Microbial and nutrient analysis

Analysis/component	Equipment	Method	Reference
Microbial	Incubators and Agar sterilizers	Dilution machine and dispensers	Aspirata Food and Beverages Laboratory
Iron	Thermo ICap 6200 inductively coupled plasma optical emission spectrometry (ICP-AES).	*Microwave accelerated reaction system* (MARS microwave digester)	De Bruyn Spectroscopic Solutions (Bryanston, South Africa)
Zinc	Thermo ICap 6200 inductively coupled plasma optical emission spectrometry (ICP-AES).	*Microwave accelerated reaction system* (MARS microwave digester)	De Bruyn Spectroscopic Solutions (Bryanston, South Africa)
Iodine	Thermo ICap 6200 inductively coupled plasma optical emission spectrometry (ICP-AES).	*Microwave accelerated reaction system* (MARS microwave digester)	De Bruyn Spectroscopic Solutions (Bryanston, South Africa)
Folic acid	Hewlett Packard HPLC	*In house method 003, (HPLC) SANAS*	The South African Grain Laboratory NPC (SAGL)
Phytic acid	Hewlett Packard HPLC	*In house method 003, (HPLC) SANAS*	The South African Grain Laboratory NPC (SAGL)
Vitamin A	Hewlett Packard HPLC	Reverse-phase High Performance Liquid Chromatography (HPLC) technique	Aspirata Food and Beverages Laboratory & Amin & Cheah, 2003
Vitamin C	Hewlett Packard HPLC	Reverse-phase High Performance Liquid Chromatography (HPLC) technique	Aspirata Food and Beverages Laboratory & Amin & Cheah, 2003

Phase 2

In this phase, baseline analysis will be conducted, whereby anthropometric, biochemical and dietary assessments of the children aged three to five years old will be done before any intervention is introduced to assess their current growth, nutrient intake and nutritional status.

Study design

A randomised controlled trial (RCT) will be used to determine the effect of consuming instant-maize maize porridge fortified with *MO* leaves and termite powders on the nutritional status of children aged three to five years old. Participants will be assigned to experimental and control groups. The research type will be quantitative. The study will also be registered with *trials.gov* after the protocol is approved.

Study population

The study population will be 387 children aged three to five years from Thulamela Municipality and their mothers. The municipality has 12 296 preschool children from 224 preschools. Preschool children are more vulnerable to micronutrient deficiency, which can have long term, irreversible, negative effects on neurological and cognitive outcomes (Prado & Dewey, 2014). However, if these deficiencies are detected and treated in the early stages, the outcomes thereof could be reversed, decreasing child mortality and mobility by 15% (WHO, 2015); it can also help in increasing the IQ score of children under the age of five years.

Study area

The Thulamela Local Municipality is in the Limpopo Province of South Africa. Its municipal boundaries were greatly altered after the South African municipal elections, 2016, when much of the area that formerly belonged to the municipality, including the town of Malamulele, was incorporated into the newly formed Collins Chabane Local Municipality. It is named after the Thulamela ruins located near the Pafuri Gate of the Kruger National Park.

Eligibility criteria

Preschool children will be screened before intervention to check if ever they had severe obvious congenital abnormalities and immune suppression diseases. HIV/AIDS, TB etc. will also be screened as these could affect the nutritional status of children, as malnutrition and some of immune suppression diseases are closely related and can affect each other. For example: according to Food and Nutrition Technical Assistance (FANTA, 2004), HIV infection affects nutrition through increasing resting energy expenditure, reductions in food intake, nutrient malabsorption and loss, and complex metabolic alterations that culminate in the weight loss and wasting common in AIDS. Furthermore, weight loss and wasting are associated with increased risk of opportunistic infections. The immune system can be impaired as a result of these diseases, thus contributing to malnutrition. Preschool children will also be screened to see if they were dewormed, have any known allergy or are allergic to some of the ingredients used to produce the product, and if they had received any supplement—such as vitamin A six months before the intervention—as these could affect the results.

Inclusion

All children aged three to five years old within the selected preschools and whose parents consented to all three assessment occasions will be included in the study.

Exclusion criteria

Children with known immune suppression diseases, those visiting Thulamela Municipality during data collection and those with known allergies to maize, *MO* or termites will not be eligible for participation in this research.

Sampling

A simple random sampling will be used for the selection of 20 preschools (10 for control and 10 for experimental) in the Thulamela Municipality. A systematic sampling will be used for the selection of 10 children aged three to five years old within the 20 selected preschools. A list of children aged three to five years will be obtained from the preschools and the k^{th} value will be calculated. The study population size is 387 children aged three to five years and their mothers. However, 60% (232) of the actual population size will be used for budgetary reasons—and is also statistically significant.

Subject recruitment

The researcher will visit the preschools three times in the same week for recruitment purposes.

Visit one—The researcher will distribute letters to preschool principals outlining the purpose and procedure of the study and agree on a date to meet parents.

Visit two—The researcher will meet parents of the preschoolers and explain the purpose and procedure of the study. The researcher will also give consent forms to parents to read and return after being signed.

Visit three —During this visit, a session will be conducted with parents who need more clarity and would like to ask questions about the research before providing consent. Signed consent forms will also be collected from parents during this visit.

Pilot study

A pilot study will be conducted to ensure the validity of the questionnaires whereby 10 mothers will be interviewed. During the pilot study, mothers or guardians will be asked to respond to questions. A pilot study will be conducted to check if the participants understand the wording and phrasing of the questions. Participants in the pilot study will not be included in the final study.

Instrument development

The questionnaire will be developed following the objectives of the study and relevant literature. The instrument will be presented to promoters for scrutiny and inputs. Peer review will provide yet another avenue for improvement. It will also be shown to the School of Health Sciences Research Committee and University of Venda Higher Degree Committee for approval. The instrument will also be pre-tested and piloted. Furthermore, the researcher will interview participants using local language to ensure validity. The instrument will consist of three sections: section A (socio-demographic information); section B (anthropometry) and section C (dietary). The questionnaire will be developed by the researcher in English and translated to Tshivenda by the Department of Linguistics.

Measurements/Assessments

Anthropometric measurements

Anthropometric assessments will be performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry (Stewart et al., 2011). The measurements will be taken in duplicate using calibrated equipment and will be recorded on section B of the questionnaire.

i. Weight

The children will be weighed without shoes and wearing light clothing. The subjects will stand still in the middle of the scale's platform without touching anything and with body weight equally distributed on both feet (Norton & Olds, 1996). The weights will be taken using a Tanita bathroom solar scale. The scale is accurate within 0.1 kg. The zero weight on the scale's horizontal beam will be checked periodically. The scale will also be checked continuously against an electronic scale. A solar scale will be used to avoid error in places where there is no electricity. Weight will be taken twice and the average will be calculated. This will be done to ensure accuracy.

ii. Height

Height will be taken without shoes and socks (bare feet) with feet flat on the floor, heels close together and against the wall. Heels, shoulder blades, and buttocks will also be against the wall. The shoulders will be in a relaxed position, arms at the sides and the head in the Frankfort horizontal plane (looking straight ahead) (Norton & Olds, 1996). Heights will be taken using a portable height meter fitted with a metal tape measure that determines to the nearest millimeter. The measure will be taken twice and the average will be calculated to ensure accuracy.

Biochemical assessment

Procedure for blood specimen collection

A professional nurse will be responsible for blood collection. They will gather the tubes and supplies needed for drawing the blood, position the participant in a chair and wash their hands. The nurse will select a suitable site for venepuncture, by placing the tourniquet three to four inches above the selected puncture site on the participant and put on non-latex gloves and palpate for a vein. When a vein is selected, they will cleanse the area in a circular motion and also allow the area to air dry. They will then ask the participant to make a fist—the participant should avoid "pumping the fist." The nurse will grasp the participant's arm firmly using their thumb to draw the skin taut and anchor the vein, then swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15–30 degree angle with the arm surface. When the last tube is filling, the nurse should remove the tourniquet. They will remove the needle from the participant's arm using a swift backward motion and place gauze immediately on the puncture site. The nurse will ask the participant to apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1–2 minutes, they will tape a fresh piece of gauze or plaster to the puncture site and dispose of contaminated materials/supplies in designated containers (WHO, 2017). The equipment needed for this fieldwork includes the following: vacutainer blood collection tubes for blood sample collections and a cooler box with ice packs inside for blood storage during sample collection.

Iron, zinc, iodine, folic acid and vitamins A and C status of the children will be analysed using standard procedures.

Data collection venues

To ensure privacy of the participants during data collection, the teacher of each participating preschool will be requested to allocate a dedicated room for these purposes. Anthropometric measures will be taken in a screened off area in the allocated venue. Blood of preschool children will be drawn inside the appointed room by the teachers.

Fieldworkers

BSc in Nutrition graduates with a few years work experience and a professional nurse will be recruited and used as fieldworkers, as they are already exposed to nutritional assessment and different techniques used to assess nutritional status.

The responsibilities of these fieldworkers will include:

- Managing the data collection process
- Preparing equipment for fieldwork
- Arranging transport to the field
- Organising lunch packs for fieldworkers

- A professional nurse will be responsible for drawing blood of preschool children and store in vacutainer blood collection tubes.

The researcher will be responsible for:

- Making appointments with preschool principals
- Training field workers on how to administer a questionnaire
- Assisting fieldworkers with anthropometric and dietary assessment
- Standardised method for data collection.

Data collection point 1: Anthropometric measurements (weight and height) of preschool children will be taken at this point by the BSc in Nutrition graduates in the presence of parents/caregivers.

Data collection point 2: BSc in Nutrition graduates will also be responsible for administering the questionnaire (socio-demographic information and dietary assessment) to mothers of the preschool children.

Data collection point 3: A professional nurse will be responsible for drawing the blood of preschool children and storing it in vacutainer blood collection tubes. The collection of urine samples by the nutrition graduates will also be done at this point.

Phase 3: Intervention

Participants will be assigned into experimental and control groups by a statistician using a software package. Two factors will be considered—which will be the consumption of 100% instant-maize porridge by the control group and fortified (80% maize, 15% termites and 5% *MO*) maize porridge by the experimental group—for a period of six months. The experimental group will be fed with 170 g (1/2 cup) of instant-maize porridge fortified with *MO* leaves and termite powders and the control group will be fed with 170 g (1/2 cup) of placebo both once a day in the morning, twice a week for a period of six months, whereby they will be fed in the first three months within preschools; in the next three months the process of feeding will be repeated again. According to the Nutrition Information Centre of the University of Stellenbosch, it is recommended that children under the age of five years should consume 170 g of cooked cereal once a day (NICUS, 2005). The experimental and control porridges will be packaged in plastic bags with two different colours, mixed in bowls of which the colours match the packaging colours, and dished up in cups or bowls of the same colours as the packaging and mixing bowls.

Phase 4: Post-intervention

In this phase, the effect size of consuming fortified instant-maize porridge will be determined using Cohen's diet to see if the effect is strong, medium or weak. Anthropometric, biochemical and dietary assessment of children aged three to five years old in the Vhembe District will be conducted to determine the effect of consuming instant-maize porridge fortified with *MO* leaves and termite powders (See Phase 2 above for data collection procedure). Baseline and post-intervention data will also be compared.

Institutional approval

The proposal will be submitted to the School and University Higher Degree Committee, Department of Health and the Limpopo Department of Basic Education to seek permission to conduct the study. The study will also be registered with *trials.gov* after the protocol is approved. Once permission has been granted, the researcher will visit the Department of Basic Education in Vhembe District for permission to approach the randomly selected preschools. The preschools will be visited and the research will be explained to the principals to obtain permission for including preschool children in the study.

Ethical considerations

Ethical clearance will be obtained from the University of Venda Research Ethics Committee. The study will be performed in accordance with the principles of the declaration of Helsinki (2013), Good Clinical Practices, and the laws of South Africa. The study will be explained to preschool principals and it will also be requested that they call a meeting for the parents of the preschool children. The researcher will subsequently explain the purpose and procedure of the study to parents or legal guardians and give the consent forms to read, sign and return. Preschool children will also be requested to give verbal assent. Parents or guardians who will need more clarity will be requested to attend an information session before they can complete the informed consent form. The researcher will provide the parents or legal guardians with an information sheet and explain the research and assessments, as well as the consent requirements if he/she allows his/her child to participate in the study.

Risks or discomforts to the participant:

Although preschool children are vulnerable, this study involves no risks. If any physical or mental health related problems are identified during the course of the assessments, the participants will be referred to the appropriate school authority for follow up with parents/legal guardians.

Benefits:

The preschool children will not benefit directly from participating in the study, but will receive a small bag of fruits and a flyer on ***the importance of consuming fortified maize porridge*** at each one of the two data collection points as a token of appreciation for their time and commitment.

Reason/s why the participant may be withdrawn from the study:

The parents of the pre-scholars and the preschool children will have the right to withdraw from the study at any point without being disadvantaged in any way.

Remuneration:

The preschool children will not benefit directly from participating in the study, but will receive a small bag of fruits and a flyer on ***the importance of consuming fortified maize porridge*** at every one of the two data collection point as a token of appreciation for their time and commitment.

Costs of the Study: None

Confidentiality:

Data obtained from the study will be stored on a computer database in such a manner that it maintains the participants' confidentiality (use of codes instead of names).

Research-related injury: No research related injury will be incurred

Persons to contact in the event of any problems or queries:

(Please contact the researcher Miss KR Netshiheni, (015 962 8334.), my supervisor (Dr CN Nesamvuni and Dr LF Mushaphi, 015 962 8653 and 015 962 8334) or the University Research Ethics Committee Secretariat on 015 962 9058. Complaints can be reported to the Director: Research and Innovation, Prof GE Ekosse on 015 962 8313 or Georges Ivo.Ekosse@univen.ac.za

General:

Potential participants must be assured that participation is voluntary and the approximate number of participants to be included should be disclosed. A copy of the information letter should be issued to participants. The information letter and consent form must be translated and provided in the primary spoken language of the research population

APPENDIX B: consent form

CONSENT

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, (*Netshiheni KR*), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _,
- I have also received, read and understood the above written information (*Participant Letter of Information*) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

Full Name of Participant	Date	Time	Signature
I, …………………….	**……………**	**…………**	**…………………..**

(Name of researcher) herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

Full Name of Researcher

……………………….. Date………………………….. Signature…………………………..

Full Name of Witness (if applicable)

………………………… Date………………………….. Signature…………………………..

Full Name of Legal Guardian (If applicable)

………………………… Date………………………….. Signature…………………………..

Please note the following:

Research details must be provided in a clear, simple and culturally appropriate manner and prospective participants should be helped to arrive at an informed decision by use of appropriate language (Grade 10 level–use Flesch Reading Ease Scores in Microsoft Word), selecting of a non-threatening environment for interaction and the availability of peer counselling (Department of Health, 2004)

If the potential participant is unable to read/illiterate, then a right thumb print is required and an impartial witness, who is literate and knows the participant e.g. parent, sibling, friend, pastor, etc. should verify in writing, duly signed that informed verbal consent was obtained (Department of Health, 2004).

If anyone makes a mistake completing this document e.g. a wrong date or spelling mistake, a new document has to be completed. The incomplete original document has to be kept in the participant's file and not thrown away, and copies thereof must be issued to the participant.

References:

Department of Health: 2004. *Ethics in Health Research: Principles, Structures and Processes*

http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

Department of Health. 2006. *South African Good Clinical Practice Guidelines*. 2nd Ed. Available at:

http://www.nhrec.org.za/?page_id=14

APPENDIX C: Letter to request permission to conduct the research study

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
EDUCATION

Ref: 2/2/2 Enq: Mabogo MG Tel No: 015 290 9365 E-mail:MabogoMG@edu.limpopo.gov.za

Netshiheni KR
University of Venda
Private bag x5050
Thohoyandou
0950

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH

1. The above bears reference.

2. The Department wishes to inform you that your request to conduct research has been approved. Topic of the research proposal: **"THE EFFECT OF CONSUMING INSTANT-MAIZE PORRIDGE FORTIFIED WITH TERMITE (KALOTERMITIDAE) AND MORINGA OLEIFERA LEAF POWDERS ON THE NUTRITIONAL STATUS OF CHILDREN AGED 3 TO 5 YEARS OLD IN THE VHEMBE DISTRICT, LIMPOPO PROVINCE ,SOUTH AFRICA(RCT)".**
3. The following conditions should be considered:

3.1 The research should not have any financial implications for Limpopo Department of Education.

3.2 Arrangements should be made with the Circuit Office and the schools concerned.

3.3 The conduct of research should not in anyhow disrupt the academic programs at the schools.

3.4 The research should not be conducted during the time of Examinations especially the fourth term.

3.5 During the study, applicable research ethics should be adhered to; in particular the principle of voluntary participation (the people involved should be respected).

REQUEST FOR PERMISSION TO CONDUCT RESEARCH: NETSHIHENI KR

CONFIDENTIAL

Cnr. 113 Biccard & 24 Excelsior Street, POLOKWANE, 0700, Private Bag X9489, POLOKWANE, 0700
Tel: 015 290 7600, Fax: 015 297 6920/4220/4494

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APPENDIX D: Questionnaire

Date of data collection……………………..Subject Code:

Name of the interviewer: …………………………………

TITLE OF THE RESEARCH PROJECT:
The effect of consuming instant-maize porridge fortified with termite (*Kalotermitidae*) and *Moringa oleifera* leaf powders on the nutritional status of children aged 3 to 5 years old in the Vhembe district, Limpopo Province, South Africa (RCT).

REFERENCE NUMBER:
PRINCIPAL INVESTIGATOR:
Khavhatondwi Rinah Netshiheni

ADDRESS:
Department of Human Nutrition and Dietetics, University of Venda, P/Bag X5050, Thohoyandou
CONTACT NUMBERS:
0678034348.

Office hours: 015 962 8334.

DEMOGRAPHIC DATA

1.Relationship with the child

	Code	Mark with an X
Mother	1	
Father	2	
Caregiver	3	

2. Marital status of parent/caregiver

	Code	Mark with an X
Single	1	
Married	2	
Widowed	3	
Living together	4	
Divorced	5	

3.Highest education of parent/caregiver

	Code	Mark with an X
Never attended school	1	
Grade 1 – 4	2	
Grade 5 – 7	3	
Grade 8 – 10	4	
Grade 11 – 12	5	
Tertiary	6	
ABET	7	

4. How many children (younger than 5 years) live in the household?

	Code	Mark with an X
1 Child	1	
2 Children	2	
3 Children	3	
4 Children	4	
More than 4	5	
None	6	

5. How many family members live in the household?

	Code	Mark with an X
1 Member	1	
2 Members	2	
3 Members	3	
4 Members	4	
More than 4	5	

6. Is the parent/caregiver working?

	Code	Mark with an X
Yes	1	
No	2	

7. Who is responsible for household income?

	Code	Mark with an X
Mother	1	
Father	2	
Grandparents	3	
Mother and Father	4	
Other, Specify …………………………	5	

8. Type of income/employment

	Code	Mark with an X
Health worker	1	
Educator	2	
Police	3	
Retail	4	
Social grant, Specify…………	5	
Other, Specify ……………….	6	

9. Monthly income of the family/household

	Code	Mark with an X
Less than R500	1	
R501 – R1000	2	
R1001 – R2000	3	
R2001 – R5000	4	

R5001 – R10000	5	
R10000 above	6	
Unknown	7	

10. Does the child receive Child Grant.

1.	2.
Yes	No

11. Any other form of grant in the household

1.	2.
Yes	No

Comment:……………………………………………………………………………………

BIOPHYSICAL ENVIRONMENT

12. Type of house

Type (a)	**(yes 1; no 2)**	**Number of rooms (b)**
Traditional house		
Brick and mortar (Modern house)		
Shack		
Other		

13. Source of Water

	Source	**(yes 1; no 2)**
13.1	Tap in house	
13.2	Tap outside	
13.3	Communal tap	
13.4	River	
13.5	Tank (rainwater harvest/bore hole/communal tap/municipal water)	
13.6	Bore hole	
13.7	Spring/ well	
13.8	Other	

14. Type of Toilet

Code	**Type**	**Tick appropriate boxes**
14.1	Flush Toilet in house	
14.2	Flush Toilet outside	
14.3	Pit latrine	
14.4	Bush	
14.5	Other	

15. Does the child follow any of the below special diets?

Type	**Code**	**Tick appropriate boxes**
Diabetic	1	
Allergic	2	
Weight loss	3	
None	4	

16. What type of maize-meal porridge does your child eat?

Type	**Code**	**Tick appropriate boxes**
Ace	1	
Iwisa	2	
White star	3	
Super maize meal	4	
Tafelberg	5	
Magnifisan	6	
Home-made	7	
Madoda	8	
Cup final	9	

17. Type of energy used for cooking in the household.

Code	**Type of energy**	**(yes 1; no 2)**
17.1	Firewood	
17.2	Coal/ charcoal	
17.3	Electricity	
17.4	Gas	
17.5	Paraffin	
17.6	Gel	
17.7	Cow dung/Matoko	
17.11	Solar energy	
17.10	**Other**	

FOOD SYSTEMS INVENTORY

18. Place of food purchase

Code	**Place**	**(yes 1; no 2)**
18.1	Local formal shops	
18.2	Spaza shops	
18.3	Shoprite	

18.4	Pick n Pay	
18.5	Spar	
18.6	Boxer	
18.7	USave	
18.8	Checkers	
18.9	Choppies	
18.10	Game	
18.11	Street vendors	
18.12	SaveMor	
18.13	OK grocery	
18.14	Pick up wild foods	
18.15	Shops unknown	
18.16	Other	

19. Household food production (plants)

Code	**Type**	**(yes 1; no 2)**
19.1	Garden	
19.2	Orchard/ Fruit tree	
19.3	Field in the Household	
19.4	Fields away from household	
19.5	Small holder farm	
19.6	Large farm	
19.7	**Other**	

20. Places for food storage

Code	**Type**	**(yes 1; no 2)**
20.1	Refrigerator	
20.2	Deep Freezer	
20.3	Dry room storage	
20.4	Cup board	
20.5	Not specific	
20.6	**Other**	

21. Resources for food preparation

Code	**Type**	**(yes 1; no 2)**
21.1	Open fire outside	
21.2	Open fire inside/Tshitangani	
21.3	Gas stove	
21.4	Paraffin stove (primus)	
21.5	Electric stove	
21.6	Coal stove	
21.7	Gel stove	
21.8	Microwave	
21.9	Wonder box	
21.10	Other	

Code

SECTION B: Clinical Assessment Record Form

22. Name of the preschool……………………………………………
23. Child's name ………………………………………………………
24. Child's ID……………………………………………
25. (ii.) Date of Birth……………… day………. Month………………year
26. (iii.) Age…………………………
27. Gender:

1	2
Female	Male

28. Did the child receive Deworming in the past 3 months?

1	2
Yes	No

29. Did the child receive Vitamin A in the past 3 months?

1	2
Yes	No

30. (a) Body weight……………………………… kg
(b) Body weight…………………………… kg
© Average weight…………………………….. kg
31. (a) Height……………………………… cm
(b) Height……………………………… cm
(c) Average height…………………………… cm
32. (a) MUAC……………………………… cm
(b) MUAC……………………………… cm
© Average MUAC……………………………. cm

33. Has your child suffered from any health conditions in the last 15 days?

1.	2.
Yes	No

34. If Yes, type of disease…………………………

35. Does the child have a Road to Health Booklet (RtHB)?

1.	2.

Yes	No

36. Blood sample taken:

1.	2.
Yes	No

37. If no, what is the reason:
…………………………………………………………………………………………
………………………………………………………………………………………

38. (a)Time of blood collection………………………………

(b)Time of the previous meal……………………………

Code

N.B: This should be done once per month.

39. Health risk checklist

Numbers	Diseases	Yes 1; No 2
	Diseases of the respiratory system	
1.	Flu/mukhushwane	
2.	Throat problems/zwilonda zwa mukuloni	
3.	Asthma/bronchitis	
4.	Pneumonia	
5.	TB (Tuberculosis)	
6.	COVID- 19 (Signs)	
	Signs and symptoms, not elsewhere classified	
7.	Stomachache/ulunwa lunwa thumbuni	
8.	Fever/mutetemelo	
9.	Headache/ urema ha thoho	
10.	Vomiting/utanza	
	Infections and parasitic diseases	
11.	Diarrhoea /utshuluwa	
12.	Chickenpox/maruda	
13.	Worm infections /nowana	
14.	Hepatitis /uzwimba tshivhindi	
	Diseases of the ear	
15.	Earache/ uvhavha ha ndevhe	
16.	Ear discharge/ubva malwa ndevheni	
17.	Ear problem/inflammation/uzwimba	
	Diseases of the digestive system	
18.	Toothache / urema ha mano	
19.	Gastrointestinal problem/stomach-ache and nausea/ulunwa lunwa thumbuni na utanza	
20.	Mouth sores or disease/zwilonda mulomoni	
21.	Appendicitis	

22.	Ulcer/zwilonda zwa thumbuni	
23.	Piles	
	Diseases of the skin	
24.	Wound/tshilonda	
25.	Allergy	
26.	Tumor or abscess/	
27.	Itching/uthothona	
28.	Epilepsy/vhuladze ha uwa	
29.	Malaria	

24-hour recall

Code

Section: Dietary Patterns and Intakes of children

Please describe the foods (meals and snacks) that the child ate yesterday during the day and night, whether at home or outside the home. Start with the first food eaten in the morning.

Write down all food and drinks mentioned by the respondent. When the respondent has finished probe for meals and snacks not mentioned

				Household	**Preschool**	**Outside Of home**
Meal	**Yes 1; No 2**	**Time of meal**	**Food item description**			
Breakfast						
Snack						
Lunch						
Snack						
Dinner						

Snack						

[Household level: consider foods eaten by the child, and exclude foods purchased and eaten outside of the home or at the preschool]

When the respondent recall is complete, fill in the food groups based on the information recorded above. For any food groups not mentioned, ask the respondent if a food item from this group was consumed.

Question number	Food group	Examples	Yes = 1 No = 2
40.	CEREALS	corn/maize, rice, wheat, sorghum, millet or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products) + *insert local foods e.g. ugali, nshima, porridge or pastes or other locally available grains*	
41.	VITAMIN A RICH VEGETABLES AND TUBERS	pumpkin, carrots, squash, or sweet potatoes that are orange inside + *other locally available vitamin-A rich vegetables (e.g. red sweet pepper)*	
42.	WHITE TUBERS AND ROOTS	white potatoes, white yams, white cassava, or other foods made from roots	
43.	DARK GREEN LEAFY VEGETABLES	dark green/leafy vegetables, including wild ones + *locally available vitamin-A rich leaves such as amaranth, cassava leaves, kale, spinach etc.*	
44.	OTHER VEGETABLES	other vegetables (e.g. tomato, onion, eggplant), including wild vegetables	
45.	VITAMIN A RICH FRUITS	ripe mangoes, cantaloupe, apricots (fresh or dried), ripe papaya, dried peaches + *other locally available vitamin A-rich fruits*	
46.	OTHER FRUITS	other fruits, including wild fruits	
47.	ORGAN MEAT (IRON RICH)	liver, kidney, heart or other organ meats or blood-based foods	
48.	FLESH MEATS	beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds	
49.	EGGS	chicken, duck, guinea hen or any other egg	
50.	FISH	fresh or dried fish or shellfish	
51.	LEGUMES, NUTS AND SEEDS	beans, peas, lentils, nuts, seeds or foods made from these	
52.	MILK AND MILK PRODUCTS	milk, cheese, yogurt or other milk products	
53.	OILS AND FATS	oil, fats or butter added to food or used for cooking	
54.	RED PALM PRODUCTS	Red palm oil, palm nut or palm nut pulp sauce	
55.	SWEETS	sugar, honey, sweetened soda or sugary foods such as chocolates, candies, cookies and cakes	
56.	SPICES, CONDIMENTS, BEVERAGES	spices (black pepper, salt), condiments (soy sauce, hot sauce), coffee, tea, alcoholic beverages OR *local examples*	

57. Total yes:	Yes = 1 No = 2

APPENDIX E: Ethical clearance certificate

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Ms KR Netshiheni

Student No:
11602027

PROJECT TITLE: **The effect of consuming instant-maize porridge fortified with termite and Moringa oleifera leaf powders on the nutritional status of children aged 3 to 5 years old in Thulamela Municipality, South Africa (RCT).**

PROJECT NO: **SHS/19/NUT/02/2803**

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Dr CN Nesamvuni	University of Venda	Promoter
Dr LF Mushaphi	University of Venda	Co - Promoter
Ms KR Netshiheni	University of Venda	Investigator – Student

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: April 2019
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: ....
Name of the Chairperson of the Committee: Senior Prof. G.E. Ekosse

UNIVERSITY OF VENDA
DIRECTOR
RESEARCH AND INNOVATION
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APPENDIX F: Editorial letter

27 February 2023

This is to confirm that I have done the language editing of Ms KR Netshiheni's thesis, (Student #11602027), with the title: *THE EFFECT OF CONSUMING INSTANT-MAIZE PORRIDGE FORTIFIED WITH TERMITES AND MORINGA OLEIFERA LEAF POWDERS ON THE NUTRITIONAL STATUS OF CHILDREN AGED THREE TO FIVE YEARS OLD IN THULAMELA MUNICIPALITY, SOUTH AFRICA (RCT).*

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APPENDIX G: Turnitin report

Final Thesis Submission
by Khavhatondi Netshiheni

Submission date: 07-Mar-2023 08:12PM (UTC+0200)
Submission ID: 2031354326
File name: Netshiheni_KR_PhD_Thesis_submission_05_March_2023.docx (1.41M)
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Final Thesis Submission

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