EFFICACY OF MORINGA OLEIFERA AND MORINGA STENOPETALA LEAVES ON GROWTH PERFORMANCE, HAEMATO-BIOCHEMICAL PROFILES AND GUT MICROBIOTA IN BROILER CHICKEN

Ebenezer Udofia Nathaniel

A Thesis Submitted to the Department of Agriculture and Natural Resources in Partial Fulfilment of the Requirements for the Conferment of Doctor of Philosophy Degree in Agricultural and Rural Development of Kenya Methodist University

June, 2021

DECLARATION AND RECOMMENDATIONS

DECLARATION

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

Signed……………………… Date…8-9- 2021

Nathaniel Ebenezer Udofia (AGR-4-0422-1/2017)

RECOMMENDATION

This thesis has been submitted for examination with our approval as University supervisors

Signature.... ....Date…10/09/2021

Dr. Mworia Mugambi, Ph.D

School of Science and Technology, Kenya Methodist University

Signature:……………………………… Date…12/09/2021

Dr. Jared M. Onyancha, PhD

School of Pharmacy, Mount Kenya University

Signature: .... Date…12/09/2021

Dr. William Ncene, PhD

School of Science and Technology, Kenya Methodist University

COPYRIGHT

All rights reserved. No part of this thesis may be reproduced, stored in any retrieval system or transmitted in any form or by any means, electronically, mechanically, by photocopying or otherwise, without prior written permission of the author or Kenya Methodist University

DEDICATION

This thesis is dedicated to Regina, Mfon and Anietie. Also, to Chris and Anne Gingles for their supports in the entire period of the study.

ACKNOWLEDGEMENTS

I thank the Almighty God for giving me all that was needed to complete my Doctor of Philosophy Study. I appreciate all members of my family for standing by me throughout the period of my study. The completion of this thesis has been achieved through the tireless efforts of my University Supervisors: Dr. Mworia Mugambi and Dr. William Ncene of Kenya Methodist University as well as Dr. Jared M. Onyancha of Mount Kenya University, Thika. I am grateful to Dr. John Muchiri (Ph.D), Dr. Laititi Mutunga (Ph.D) late Prof. Peter Kamau (Ph.D) and late Prof. Ellis Njoka (Ph.D) for their guidance.

I am thankful to the staff members of the Kenya Methodist University Library who helped me throughout the period as well as the staff of Mount Kenya University demonstration farm. I am grateful to Mount Kenya University Research Centre for provision of laboratory equipment, apparatus, reagents and services for phytochemical screening. I acknowledged Unga East Africa Limited for the use of their facility for formulation of the experimental feeds and sample analysis. Also acknowledged is the Kenchic Kenya Limited for provision of experimental chicks and sample analysis. My appreciation goes to all those who contributed either directly or indirectly to the successful completion of this thesis especially Moriasi Gervason Apiri, Mandela Nelson Elias, Geredine Kandie, Kenneth Mburugu and Isah Abdul Wahab for their varied contributions. I am indebted to Healing Hands International Inc. for unwavering support throughout the period of this study. God bless you!

ABSTRACT

Antibiotic growth promoters (AGP) have been used for decades to enhance production of meat, poultry growth acceleration, as well as improved disease prevention. The extensive use of antibiotics has led to development of resistant pathogens causing deleterious environmental and public health effects thus the need for antibiotic growth promoters for the poultry industry. The objective of this study was to assess the effectiveness of *Moringa oleifera* and *Moringa stenopetala* leaves on growth, blood and gut microbiota in broiler chicken. The study was carried out at the Mount Kenya University demonstration farm, Thika. Three hundred one-day-old mixed sex Cobb 500 broiler chicks from Kenchic Ltd, Kenya were used in a completely randomized block study design and twenty treatments used in the experiment. Maceration using aqueous and ethanol was used as the extraction method. The results were analysed using Minitab Version 19.1. First, a one-way analysis of variance (ANOVA) was used; thereafter, Fisher's Least Significant Difference was used at 0.05 Level of significance. Results proved that powders from *M. Stenopetala* and M. oleifera are potent growth promoters. This was evidenced in the body weight gained by the chicks served with the feeds supplemented with graded levels of the studied powders. Similar result was obtained for the ethanolic and aqueous leaf extracts of the studied plants as growth promoters in terms of weight gains. The study showed that both plant leaves did not negatively affect the studied haematological parameters. Upon determination of the effects of the studied plant powders on the selected biochemical parameters, it was concluded that no adverse effects were exhibited. It was also concluded that the studied plant powders and the studied extracts influenced gut microbiota. It was further concluded that the treatment groups of chicken which received the leaf powders of *M. oleifera* and *M. Stenopetala* had significantly low total coliform counts ($p<0.05$).The study showed that the leaf powders of both *M. oleifera* and *M. Stenopetala* are endowed with a variety of important nutrients known to promote health in animals and humans. The results showed that the leaf powders of *M. oleifera* has significantly higher concentration of the assayed vitamins than those in the leaf powder of *M. Stenopetala* ($p<0.05$). The two plant powders possess pharmacologically important phytochemicals which are associated with proper growth, melioration of oxidative stress, antimicrobial activity, and immunomodulation as well as health promotion. *M. oleifera* leaf powder has significantly higher phenolic content than that of *M. Stenopetala* ($p<0.05$). From this study, the use of *M. oleifera* and *M. Stenopetala* leaf powders as alternative and safer broiler chicken feed supplements is encouraged. Studies geared towards further validation, authentication of the use of *M. oleifera* and *M. Stenopetala* in other poultry species are encouraged.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATIONS .... **ii**
DECLARATION .... **ii**
RECOMMENDATION .... **ii**
COPYRIGHT .... **iii**
DEDICATION .... **iv**
ACKNOWLEDGEMENTS .... **v**
ABSTRACT .... **vi**
LIST OF TABLES .... **xi**
LIST OF FIGURES .... **xii**
LIST OF APPENDICES .... **x**
ABBREVIATIONS AND ACRONYMNS .... **xiii**
OPERATIONAL DEFINATION OF TERMS AND DEFINITIONS .... **xv**
CHAPTER ONE **1**
INTRODUCTION **1**
1.1 Background of the study 1
1.2 Statement of the problem 6
1.3 General Objective 8
1.4 Specific Objectives 8
1.5 Research Hypotheses 8
1.6 Justification of the Study 9
CHAPTER TWO **11**
LITERATURE REVIEW **11**
2.1 Introduction 11
2.2 Importance of poultry industry 11
2.3 Trends on broiler meat production 12
2.4 Broiler chicken production systems 13
2.5 Broiler productivity and performance 16
2.6 Broiler chicken nutrition 18
2.7 Broiler haematological and biochemical parameters 48

2.8 Broiler gut microbiota ... 52
2.9 *Moringa oleifera* and *M. stenopetala* as feed additive ... 56
2.10 *Moringa oleifera* and *stenopetala* effect on growth performance of broilers ... 74
2.11 Effects of *M. oleifera and stenopetala* on haemato- biochemical parameters ... 76
2.12 Effects of *M. oleifera* and *stenopetala* on the gut microbiota ... 78
2.13 Phytochemicals and nutrient in *M. oleifera* and *stenopetala* extracts ... 79
CHAPTER THREE ... 90
RESEARCH METHODOLOGY ... 90
3.1 Introduction ... 90
3.2 Site description ... 90
3.3 Experimental chicken and management ... 90
3.4 Plant materials and extracts ... 91
3.5 Dosage and extract administrations ... 93
3.6 Feed preparation ... 94
3.7 Experimental design ... 95
3.8 Data collection procedure ... 98
3.9 Determination of haematological and biochemical parameters ... 99
3.10 Effects of *M. stenopetala* and *M. oleifera* leaf extract gut microbiota ... 100
3.12 Proximate analysis ... 103
3.13 Data analysis ... 109
3.14 Ethical considerations ... 109
CHAPTER FOUR ... 111
RESULTS AND DISCUSSION ... 111
4.1 Effects of *M. oleifera* and *M. stenopetala* extracts on growth performance ... 111
4.2 *M. Oleifera* and *Stenopetala* Leaf Powder and Biochemical Parameters ... 123
4.3 Effects of *M. stenopetala* and *oleifera* extracts on the gut microbiota ... 141
4.4 Phytochemical and nutrient composition of *M. oleifera* and *stenopetala* leaf ... 146
CHAPTER FIVE ... 162
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS ... 162
5.1 Introduction ... 162
5.2 Summary of the study **... 162**
5.3 Conclusions Drawn from this Study ... 164

5.3 Recommendations 166
REFERENCES 168
APPENDICES 205

LIST OF APPENDICES

Appendix 1: Day-old Experimental chicks 205
Appendix 2: Experimental Chicks after Acclimatization 206
Appendix 3: Feeding of Experimental Chicks 207
Appendix 4: Extract Being Administered to Experimental Chick 207
Appendix 5: Locating and Sterilizing the Brachial Vein for Blood Extraction........ 208
Appendix 6: Methods for the Determination of Biochemical Parameters........ 209
Appendix 8: Methods for quantitative phytochemical analysis of *Moringa powder* ... 217
Appendix 9: Analysed Results 220
Appendix 10: Gut microbiota analysis results 249
Appendix 11: Certificates of analysis of *M. oleifera* and *M. stenopatala* feeds........ 250
Appendix 12: Letter of approval to use MKU Research centre facilities........ 251
Appendix 13: Setup for the determination of crude lipid content. 252
Appendix 14: Determination of Crude Protein the Studied Plants 253
Appendix 15: Map of *Moringa oleifera* Lam distributions in the world........ 254
Appendix 16: Research Permit 255

LIST OF TABLES

Table 2.1: Nutrient requirements for broilers (expressed as percentage of diets) 23

Table 2.2 Composition of dried leaf powder of *Moringa oleifera* per 100 g of edible portion of leaves 81

Table 2.3 Proximate composition, mineral and vitamin contents of raw leaves of *Moringa stenopetala* 84

Table 3.1 Composition of Broiler experimental diet (%) 95

Table 3.2 Experimental layout 96

Table 4.1 Effects of the leaf powders of *M. oliefera* and *M. stenopetala* 125

Table 4.2 Effects pf the aqueous and ethanolic leaf extracts of *M. oliefera* & *stenopetala* on heamotologic parameters of experimental broiler chicks 127

Table 4.3 Effects of the leaf powder of *M. oleifera* and *M. stenopetala* on serum biochemical parameters of experimental broiler chicks 135

Table 4.4 Effects of the leaf aquoeus and ethanolic Extacts of *M.oleifera* and *M.stenopetala* on serum bio 138

Table 4.5 Qualitative phytochemical composition of leaf powders of *Moringa*......... 147

Table 4.6 Qualitative phytochemical composition of the aqueous and ethanolic leaf extracts of *M. oleifera* and *M. stenopetala* 148

Table 4.7 Relative abundancies of selected phytochemicals in *Moringa oleifera and Moringa stenopetala* leaf powder 149

Table 4.8 Proximate composition of selected parameters in leaf powders of *Moringa* 152

Table 4.9 Proximate concentration of essential amino acids in the leaf powders 154

Table 4.10 Concentration of selected vitamins in leaf powders of *M. oleifera* and *M. stenopetala* 156

Table 4.11 Mineral element concentration in leaf powders of *M. oleifera* and *M. stenopetala* 160

LIST OF FIGURES

Figure 2.1: Showing aerial parts and stem of Moringa tree 62

Figure 2.2: Showing Moringa leaves 63

Figure 2.3: Showing the pods, flowers and seeds of Moringa oleifera 65

Figure 2.4: Showing Moringa oleifera tree (Captured by Ebenezer Udofia) 74

Figure 4.1: Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's feed intake 112

Figure 4.2: Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's Body weight 114

Figure 4.3: Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's Feed conversion ratio (FCR) 115

Figure 4.4: Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's feed intake 116

Figure 4.5: Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's Body weight 117

Figure 4.6: Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's Feed conversion ratio (FCR) 119

Figure 4.7: Effects of the leaf powder supplements on the total coliform count of gut microbiota in broilers 142

Figure 4.8: Effects of the aqueous and ethanolic leaf extracts of M. oleifera and M. stenopetala on the total coliform count of gut microbiota 144

ABBREVIATIONS AND ACRONYMNS

Abbreviation	Meaning
AGP	Antibiotic growth promoters
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BSLF	Black soldier fly larvae fat
CRD	Completely randomized design
DMRT	Duncan multiple range test
EDTA	Ethylene diamine tetra-acetic acid
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
FE	Feed efficiency
g	Grams
Hb	Haemoglobin
HPAL	Hepatic pathogenic Arian influenza
KEMRI	Kenya Medical Research Institute
MCV	Mean corpuscular volume
MOEE	Moringa oleifera ethanol extract
MOEW	Moringa oleifera water extract
MOP	Moringa oleifera powder
MSEE	Moringa stenopetala ethanol extract
MSEW	Moringa stenopetala water extract

MSP	Moringa stenopetala powder
M. oleifera	Moringa oleifera
M. stenopetala	Moringa stenopetala
Mg	Milligrams
NACOSTI	National Commission for Science, Technology, and Innovation
NAGP	Non–Antibiotic growth promoters
OECD	Organization of Economic Corporation and Development
P^H	Power of hydrogen or potential for hydrogen
PVC	Packed cell volume
RBC	Red blood cells
USDA	United States Department of Agriculture
WBC	White blood cells

OPERATIONAL DEFINATION OF TERMS AND DEFINITIONS

Terms	Operational definition
Actinobacteri	These are a bacteria phylum that are gram - positive
Ad libitum feeding	The feeding management through which animals are given as much feed as they want.
Antibiotics growth promoters	Are antibiotics added to the feed of food animals to enhance their growth rate and production performance
Bacteroidetes	These are a phylum of gram negative bacteria
Biosecurity	A set of fundamental practices and strategies taken to prevent the entry and transmission of pathogens in the farms
Efficacy	The performance of an intervention under ideal and controlled conditions
Endocrinological functions	Endocrine system is responsible for regulating range of bodily functions through the release of hormones
Firmicutes	These are bacteria phylum, mostly gram positive
Feed additives	products used in animal nutrition for improving the quality of feed and thereby the quality of food of animal origin
Feed supplements	are the compounds that are added at low rate to animal feeds without changing considerably
Gut health	Refers to the balance of microorganisms that live in the digestive tract
Infections	An invasion and multiplications of pathogens
Intestinal microbiota	It was formally called gut flora, these are microbes

	population in the intestinal track
Immunological functions	The immune system defends the body against pathogens. White blood cells play a major role.
Metabolic functions	Liver is the main metabolic organ in the body and has a major role in carbohydrate, fat and protein metabolism.
Metabolizable Energy	The total digestible energy after feacal and urinary energy loss.
Muscle catabolism	This means breaking down of complex muscles into tissues
Pathogen	Disease causing organism
Phytochemicals	These are compound in plants or chemical of plant origin
Prebiotics	These are non-digestive food (fibre) that serve as food for friendly bacteria in the gut
Probiotics	Live microorganisms with beneficial health effects when consumed
Proteobacteria	These are considered a major phylum of bacteria with wide variety of pathogens
Thermos-neutral environment	This refers to an environment with the range of temperature where animals and poultry are most comfortable and productive
Traditional medicine	This refers to the skills, knowledge and practices based on experiences, theories and beliefs indigenous to different cultures

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Poultry farming is a great contributor of protein diet supply in many countries (Samboko et al., 2018). Farming of chicken, turkeys, ducks, geese, ostrich, guinea fowl, doves and pigeons constitutes poultry farming of which chicken accounts for over 90% of the entire global poultry population which have been successfully domesticated by human, for ages, to supply meat and eggs (Boschloo*,* 2019). Poultry production is rated as the fastest growing section of the global supply chain of meat and the developing economies have taken a leading role (Advisory & Down, 2019).

The United States is leading in poultry meat production followed by China then India despite several disease outbreaks like the avian influenza, which threaten productivity. African countries like Ghana imports chicken meat mainly from Europe and Brazil due to the imbalance between demand and supply. The low production is linked to increased feed costs and inadequacies in farm management practices affects production progress in Ghana (FAO, 2018). In Kenya, the annual poultry meat production is about 605,000 million tonnes (Ministry of Agriculture, 2019). The demand of poultry meat is projected to increase to 164.6 metric tonnes in 2030 (Carron et al., 2017).

The advantage of poultry industry is the short turnover period. In addition to providing opportunities to increased poultry exports, increasing poultry production stimulates growth in global import demand for feeds and other inputs that generates investment opportunities (Boschloo, 2019). However, this is challenged by the high cost of feed

especially energy and protein sources (Galvan et al., 2016). As the production of poultry makes continuously essential contributions in the world meat supply, poultry feeds have become an increasingly vital component of the integrated food chain. The demand for livestock products including poultry is increasing due to global population growth, while at the same time, there are increasing safety concerns (Bettencourt et al., 2015).

It has been reported that products of livestock origin account for close to 30 percent of global agricultural value. In addition, they provide 19 % of rated food production value, as well as offer 16% of energy and 34% of protein in current human diets (D'Odorico et al., 2018). The consumers' demand for meat, eggs, milk and other livestock products is dependent on the availability of well balanced and safe animal feeds (Ministry of Agriculture, 2019). Broiler chicken are fed on variety of feeds which have varying energy and protein levels (Neves et al., 2014). Chicken nutrition should contain at the minimum of thirty-eight (38) nutritive composition with the right concentration and balance (Réhault-Godbert et al., 2019).

Dietary requirement is based on growth, feed efficiency, and quality of poultry meat (Klasing, 2015). An appropriate composition and balance of by-products of cereals, proteins of plant origin, mineralised and vitamin supplements, grains, feed additives, and fats is required for effective performance and good health. Broilers require sufficient minerals, water, proteins, vitamins and water (FAO, 2014). Furthermore, nutritional supplements in feeds, non-nutritive additives are also being used to promote chicken performance and health (Velmurugu, 2012).

Feed supplements or additives are compounds that are added at low concentrations to animal feeds without changing considerably their composition, speed up the growth and thereby improve animals' performance in terms of body size and weights (Cardoso & Almeida, 2013). Some feed additives are probiotics, prebiotics, exogenous enzymes, and organic acids (Anjum & Chaudhry, 2010). These additives are used to modulate gut microbiota so as to enhance production efficiency and constituent health of poultry (Alayande et al., 2020). Inclusion of antibiotic growth promoters (GP) in feed is aimed at increasing effective feed conversion ratio with the goal of improving poultry performance (Mehdi et al., 2018).

Despite the importance of antibiotics in the control and management of infections in broiler production, research has shown that extensive use of antibiotics not only causes antibiotic-resistance, but also responsible for presence of drug residues in the environment and meat, which, is detrimental to both human and animal health (Manyi-Loh et al., 2018).With increase in consumers consciousness about safety of feed additives, there is a growing need to search for safer feed supplement alternatives (Lillehoj et al., 2018). Additionally, due to facts on antibiotic resistance, pressure is mounting to decrease reliance of antibiotics when producing poultry (Mahfuz & Piao, 2019a).

To address the antibiotic resistance issue in broiler production, inclusion of herbs, herb extracts and products of medicinal plants in poultry feed has been proposed since they promote growth while acting as antibiotic substitutes (Aroche et al., 2018). Many tropical plants have beneficial values such as antimicrobial and antioxidant properties. It

is evident that approximately two-third of global plant species contain medicinal properties. Presence of differentiated phytochemical components is perceived to be the basis of medicinal value in aforementioned plants. Many of the plant species have been studied for their possible medicinal applications (Napagoda et al., 2018).

As reported by the World Health Organization (WHO), approximately eighty percent of global population is reliant on traditional medicinal products to cater for their healthcare needs, and most of this involves the use of plant extracts and their bioactive components (Ekor, 2014). The function of plants of medicinal properties in disease prevention or control is accredited to their antioxidant capabilities of their components, which are sometimes related to polyphenol compounds (Batool et al., 2019).

The role of medicinal plants in preventing or controlling disease has also been attributed to the antioxidant properties of their constituents, sometime associated with polyphenolic compounds (Batool et al., 2019). Humans have for centuries used plant products as their source of traditional medicine and food (Jamshidi-Kia et al., 2018). Similarly, natural spices and herbs have been used extensively as food additives in farm animal production (Kumar et al., 2014).

The effects of phytochemicals in poultry may arise from the fact that they stimulate exudation of digestive enzymes; improve appetite; stimulate immune functions; have anti-inflammation, antibacterial, and anthelmintic properties; and possess antioxidant abilities (Achilonu et al., 2018). Prebiotics are the non-digestible foods (fibres from fruits, vegetables, and starches) that beneficially affect the host through selective growth

stimulation in addition to influencing growth of beneficial colonic bacteria. Report has shown that prebiotics alters gut microbiota, reduce invasion by pathogens like Salmonella enteritis and Escherichia coli as well as reduce cholesterol (Davani-Davari et al., 2019).

There is an increasing attention in development and evaluation of natural antioxidants for animal husbandry from tropical plant materials. Among those tropical plants, *Moringa* species is showing great potentials (Boukandoul et al., 2018). It grows very fast and withstands drought conditions. *Moringa oleifera* Lam originated in Himalayas in north-western India and is found mostly in sub-tropical and tropical regions (Khor et al., 2018). According to Saini et al. (2016), the extract of *Moringa oleifera* Lam leaves have antioxidant potential, calcium, proteins, vitamin C, β-carotene and natural antioxidants including flavonoids, phenolics, ascorbic acid, and carotenoids. *Moringa stenopetala* Bac is also thought to have a similar medicinal and nutritional properties as *Moringa oleifera* Lam (Singh et al., 2019) .

Utilisation of *Moringa oleifera* and *Moringa stenopetala* as plants with medicinal value by Africans and Asians dates back to centuries ago, the leaves and immature fruits are also used as food in human sustenance (Tamilselvi & Arumugam, 2019). The *Moringa* plant has a panacea of medicinal and preventive properties. Evidence has proven that alcohol, hydro alcohol, and aqueous extracts of *Moringa oleifera* leaves contain multiple biological properties such as antimicrobial, antioxidant, anti-inflammatory, vital organs protective, pain-relieving, anti-peptic ulcer, antihypertensive, anticancer, anaphylactic, and other immune boosting actions (Jacques et al., 2020; Stohs & Hartman, 2015).

The leaf extracts of Moringa plant has antimicrobial activities over some disease such as Staphylococcus aureus, E. coli, Salmonella typhi, Shigella species in humans. The leaf extracts of *Moringa stenopetala* has shown considerable antimicrobial and antifungal action. It is suggested that *Moringa oleifera* and *Moringa stenopetala* could possibly possess a potential for formulation of antibiotic preparations (Abrar et al., 2017; Elangovan et al., 2014).

Studies like that of Hafsa et al. (2019) showed that *Moringa oleifera* leaf additives fed to broiler poultry led to reduced hindgut count of Salmonella together with E. coli; however, Lactobacillus spp incremented. The study further suggested that inclusion of *Moringa oleifera* extract in feed could promote growth performance and antioxidant activities. Additionally, it can also modulate gut microbiome and protect against pathogenic microbe, with no adverse effects on the broiler chicken(Kumar et al., 2018)**.**

Considering medicinal and nutritional significance of *Moringa* plant, it is possible that it could be used as natural component of feeds and as a safe antibiotics replacement that can increase the effectiveness of broiler production. However, *M. oleifera* and *M. Stenopetala* is inadequately used in Kenya. *Research* studies evaluating the appropriate safe and efficacious levels to be used in poultry feeds are limited. Therefore, the study sought to evaluate efficacy of *Moringa oleifera* and *Moringa stenopetala* leaves on gut microbiota, growth performance, and blood in broiler chicken.

1.2 Statement of the problem

Broiler chicken rearing relies on antibiotics as a means of improving meat production because they accelerate growth, increase conversion of feed, and provide a better

method of controlling diseases. Even though in-feed antibiotics improve poultry performance, the use of synthetic antibiotic growth promoters has been found to cause environmental and public health risks including allergic reactions, carcinogenicity, drug sensitization, drug toxicity and immunopathological diseases (Manyi-Loh et al., 2018). The development of antibiotic resistant pathogens, presence of antibiotics residues in meat and concerns over extensive use of antibiotics in poultry and livestock has raised global interest in limiting their use in livestock farming. As such, growing concerns from farmers, consumers, and health practitioners across the world resulted into a ban on the use of antibiotics growth promoters in European union in 2006 (Salaheen et al., 2017).

This developing issue on poultry production necessitates alternative approaches using a natural agent with similar beneficial effects of growth promoters to improve feed efficiency, growth performance and subsequently production in the absence of antibiotic growth promoters (AGP). An alternative strategy to AGP is the use of Moringa, a tree known to be laden with medicinal and nutritional properties in addition to its economic value. The Moringa plants are found throughout most of the tropical and sub-tropical climates, including Kenya. *Moringa oleifera* and *Moringa stenopetala* extract possesses important medicinal properties. However, their use in poultry as a gut microbiota enhancer in feeds of broiler chicken has not been evaluated. This study report provides scientific data for the efficacy levels of the two plants to be used as poultry feed additives.

1.3 General Objective

To evaluate the efficacy of *Moringa oleifera* and *Moringa stenopetala* leaves on growth, haemato-biochemical profiles, and gut microbiota in broiler chicken.

1.4 Specific Objectives

i. To determine the efficacy of *Moringa oleifera* and *Moringa stenopetala* leaf powders and extracts on growth performance in broiler chicken.

ii. To determine efficacy of graded levels of *Moringa oleifera* and *Moringa stenopetala* leaf powders and extracts on blood composition in broiler chicken.

iii. To analyse the efficacy of graded levels of *Moringa oleifera* and *Moringa stenopetala* leaf powders and extracts on gut microbiota in broiler chicken.

iv. To compare phytochemical, nutrient and proximate compositions in *Moringa oleifera* and *Moringa stenopetala* leaf powder and extracts.

1.5 Research Hypotheses

i. *Moringa* specie leaf powders and extracts have significant effects on the growth of broiler chicken

ii. Different levels of *Moringa* species leaf powders and leaf extracts have significant effects on broiler Chicken hematologic and biochemical parameters

iii. Different levels of *Moringa* specie leaves powders and extracts have significant effects on gut microbiota of broiler chicken

iv. There is a significant difference between the nutrient, phytochemicals and proximate composition of Moringa specie leaf extracts

1.6 Justification of the Study

The mounting concerns over antimicrobial resistance from the consumers, regulatory bodies, medical and veterinary field has prompted the need to replace antibiotic growth promoters (AGP) with other alternative approaches that will enhance poultry performance and health (Cervantes, 2015). However, withdrawing antimicrobials will restrict growth rates of poultry, thereby affecting the efficiency of production and possibly threaten food security (Salaheen et al., 2017).

Elimination of antibiotic- based growth promoters has caused a significant increase in poultry infections, which has further degraded performance of farm animals generally. Therefore, the trend and need of finding available alternatives has increased significantly. This provides a strong justification to explore and develop alternative but safer growth promoting sources for broiler poultry production. The use of *Moringa* plant as feed additives is seen as a viable alternative to AGP. Both *Moringa oleifera* Lam and *Moringa stenopetala* are locally available in Kenya and can be used in the production of poultry feed in a cost- effective way. The leaves of the both species are reported to have antioxidant potential and so it is imperative to investigate its efficacy as a potential alternative growth promoter for broiler chicken.

Notwithstanding, the use of *Moringa* as nutritional supplement in broiler production, several studies have depicted varying results on the implications of powdered Moringa leaves and extract on performance of chicken growth, blood parameter and microbiota at different graded levels, thus the need for the study. Evidence is required to develop feeding strategies so as to improve broiler production practices.

Although, limited studies have been conducted on *Moringa* leaves as protein supplement in broiler chicken diets with varying results, there are very few studies related to its efficacy as gut microbiota enhancer in broilers. This study sought to investigate the effectiveness of *Moringa oleifera* together with *Moringa stenopetala* leave powders and extracts on blood parameters, growth performance as well as gut microbiota in broiler chicken.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter provides an in-depth review of literature in relation to the poultry industry, poultry production system, productivity performance; nutrition; non-nutritive feed additives; antibiotic growth promoters in broiler production; Botanical supplements in broiler nutrition;poultry gut microbiota**;** *Moringa* tree; *Moringa stenopetala* Bac *Moringa oleifera* Lam. *Moringa oleifera* Lam leaves; its origin, distributions; uses ; cultivation and soil conditions. The review was drawn from both primary and secondary literature using different search engines such as google, google scholar and data bases including PubMed, Scopus, AGRICOLA (EBSCO).

2.2 Importance of poultry industry

The most commonly consumed food is meat and eggs, providing energy, protein, and other nutrients to humans. It remains the most desired food for dietary value and taste. Additionally, poultry farming in developing countries is the fastest growing sector (FAO, 2013). Due to rapid population growth in developing countries, the need for chicken products is rising and it is projected that production of poultry meat is becoming the most eaten animal meat globally (Carrasco et al., 2019). The development of sustainable agriculture and food security continues to pose a major challenge especially in the developing nations threatening achievement of the targets set by the sustainable development goal 2 (United Nations, 2015).

Being a platform necessary for comprehending sustainable agricultural practices, poultry farming is an important component for accelerating creation of food systems; and, development of sustainable economic and agricultural systems (HLPE, 2016) . There is diversity in poultry production because of different factors that include feed base; poultry breed, housing and the production systems. Poultry also has the potential to cover the current protein intake gap given the ability of exotic poultry to easily adapt to environmental conditions without compromising returns on investment or demanding the use of expensive production technologies (Yusuf et al., 2016) .

2.3 Trends on broiler meat production

According to FAO (2018), the global trends on broiler meat production showed that United States led production with estimated 121.6 million tonnes. China reportedly is still the second largest world producer despite the decline in 2017 due to the highly pathogenic avian influenza (HPAI) outbreaks. The third producer, India, showed continued growth due to increased local demand, resulting from rapid urbanization, change in diet practices and higher incomes. Due to the changes, farming in commercial farms rose subjecting small household farming to drop. Many African countries like Ghana imports chicken meat mainly from Europe and Brazil due to the shortage experienced because local production of poultry cannot meet the demand. The low production linked to increased feed costs and inadequacies in farm management practices affects production progress in Ghana (FAO,2018)

Chicken constitutes 98% of the poultry population in Kenya, while the rest are ostrich, quail, ducks, geese, doves, turkey and guinea fowl. Seventy percent (70%) of chicken

are free-ranging traditional breeds. The annual poultry meat production is about 605,000 million tonnes (Ministry of Agriculture, 2019). Due to the improvement of people's socioeconomic status and the continuous recovery of the poultry system, it is estimated that the demand for poultry meat will increase from 54.8 tons up to 164.6 tons in 2000 and 2030 respectively (Carron et al., 2017). Despite the increasing production of meat poultry due to the cost of feeds, population growth doubling in Kenya as shown in the census release (2019) continues to cause unmet demand of chicken product (KNBS, 2019).Additionally, attaining sustainable production of poultry products that meet both environmental health and standards has become a multifaceted endeavour (Alders et al., 2018). The country can therefore satisfy an elevated demand for poultry meat under the supposition that trade systems continue to be the same and the costs incurred as outputs and ones invested as inputs remain constant (Carron et al., 2017).

2.4 Broiler chicken production systems

Production systems in low-income countries is very dynamic, resulting from the rapidly growing population combined with income and has led to increased demand of poultry products (Liverpool-Tasie et al., 2019). Broiler production done under different agro-ecological zones and production vary worldwide. There is also a variation in poultry production needs between high- and low- income nations. These variances even exist in nations where some areas favour a particular approach to livestock production (Rust, 2019). The practice of poultry production regardless of the scale is essential in improving the livelihood of people worldwide. Studies have shown that poultry keeping in developing countries enables socioeconomic improvements since even the poorest

households practise it. Research projects undertaken in Bangladesh demonstrated the positive influence of chicken rearing on the livelihood of women (FAO, 2014). Additionally, chicken possesses religious and cultural values especially in the African society. Compared to other livestock, chicken reproduce so fast making its contribution to the economy very significant. It is also ecologically friendly and requires very small space (Fitsum, 2014).

Poultry productivity depends on a number of parameters including herd size, breed, feed, lodging structure, wellbeing, available innovations, bio-security among other factors (Njuguna, 2018). Poultry production systems range from very simple shelters to those with fully automated systems (Terie, 2016). Poultry production systems can be classified based predominantly on scale as follows: (i) semi-concentrated, little to medium scale, market-situated, business poultry creation, (ii) customary, broad, backyard poultry production, and large scale intensified poultry production at industrial level (Cox, 2014). In addition, production systems may be classified depending on the feeding systems, flock size and ecological zones as: (i) Semi-subsistence systems and (ii) Semi-commercial system (Gizaw & Tegegne, 2015).

In Kenya, poultry production systems include free-range/scavenging, semi-intensive and intensive systems (Chaiban et al., 2018). Enhancing the yield of poultry through breeding and better poultry production systems escalates the chances of fair distribution of food and poverty alleviation in households (Liverpool-Tasie et al., 2019).

Traditional poultry systems utilises low feeds input, partially relying on scavenging (Melesse, 2014). It also requires minimum veterinary services, exposed to low level of

biosecurity, high off-take rates but suffers high mortality rates. The low investment after initial purchase of stock of chicken, little local grain given and smaller housing structure makes it easy to sustain. Apart from the indigenous poultry which are reared in the homesteads, some crossbreed and exotic breeds like the Kuroiler chicken in Kenya may be kept under this system (Fitsum, 2014).

Inadequate disease management of indigenous chicken may not only cause death but also inhibits production in terms of growth rate and egg yield. This ultimately results to low returns on investments and consumption impacting negatively on the livelihoods of the people who live in the rural areas especially (Zamxaka, 2016). Trading of chicken in these systems of production is characterised by lack of proper hygiene, lack of proper cold storage, and inappropriate market practices. However, the growing need for indigenously reared meat provides a window of opportunity for traditional system in future (FAO, 2018).

Small-scale or semi-commercial poultry production is a vital tool in alleviating poverty and starvation in low-income countries (FAO, 2013). It is a substitute to meeting the demand for chicken meat in the rural areas. It is categorised as small to medium scale intensive production system because of the medium input of feed, water and veterinary service and least risk bio-security as compared to large-scale production. Most poultry farmers prefer large scale commercial farms as their source of starting. This approach is assumed to be superior to the backyard ones (El-Menawey et al., 2019).

The industrial production system is an extremely rigorous production system where a farmer keeps an average of 10,000 poultry under enclosed environments with a moderate

to high level of bio-security level. The exotic breeds kept under this system demand high inputs as compared to other types of production systems. The market sale system is reliant on sale of meat products to large scale wholesalers and hyper-stores (Tewodros, 2019). Broiler farming in Kenya practised under large-scale production system is done in urban and peri-urban of Mombasa and Nakuru. Also it occurs in Nairobi, Nyeri, and Kisumu because market is assured in comparison to the villages where local chicken rearing continues to lead (Carron et al., 2017). The advantage is that little space is required and so scarcity of land resource is not a problem. However, high prices of feed and antibiotic resistance is a major constraint to this type of production system (FAO, 2018).

2.5 Broiler productivity and performance

Compared to other livestock, poultry converts food into meat and eggs more efficiently (Mbuza et al., 2017). The health of poultry is assessed using performance as an outcome-based measure. Feeding of broilers should always be a diet appropriate to age and genetics, also containing adequate nutrients that meets requirement (OIE, 2015). Various indicators such as residual feed intake (RFI), apparent metabolizable energy (AME), feed conversion ratio (FCR), time to achieve market weight, and body weight gain (BWG) are used to measure poultry productivity. Variability in performance in flocks resulting from changes in productivity can be affected by microbiota composition (Carrasco et al., 2019).

Feed conversion ratio is computed by dividing the sum of feed consumed by the number of eggs produced or weight gained by the entire flock. That is, feed conversion ratio

equals input divided by output. A smaller value is indicative of a higher efficiency of feed conversion by an animal (Carré, 2015). For broiler producers, a feed conversion ratio of 1.0 means that their chickens gain 1 kilogram of weight for every 1.0 kilograms of feed consumed. Effective feed conversion ratio to improve poultry performance can be attained by genetic improvements and the inclusion of growth promoters (GP) or growth promoter alternatives.

According to Mehdi et al. (2018), growth promoters are used as agents for improving poultry growth and feed conversion ratios. These promoters are classified into Antibiotic growth promoters (AGP) which are commonly used in current poultry production systems. Another classification is Non–Antibiotic growth promoters (NAGP): an alternative to AGP is NAGP. They do not have risks resulting to microbial resistance or undesired deposits in meat (Hao et al., 2014). The use of NAGP not only affects the rate of development of healthy intestinal flora, but also stability of digestion, as well as better feeding efficiency (Shroha et al., 2019)

Antibiotics naturally produced from lower types of fungi and certain bacteria or synthetically produced are used to prevent propagation and kill bacteria (Carvalho & Santos, 2016). They are utilised to routinely control and cure infections in poultry. However, data shows that the massive use of antibiotics has caused a greater problem of antibiotic resistance coupled with notable presence of antibiotic residues in meat and environment, which has proven impactful on health of humans and animals (Manyi-Loh et al., 2018). Thus, there is an increasing need to source for alternatives to the prevention of bacterial diseases and minimize the spread of resistant bacteria (Mehdi et al., 2018).

Due to the resistance of bacteria to existing antibiotics and their residual effects on humans and animals, research is currently interested in identifying effective alternatives (such as phytochemicals, probiotics, and prebiotics) that can enhance the host's response Immunity to pathogen infection (Selaledi et al., 2020). The pressure is due to the global concern on the limitations of the synthetic drugs and chemicals due to the anticipated toxicity and adverse effects (Salaheen et al., 2017).

2.6 Broiler chicken nutrition

The paradigm shift from indigenous poultry production into intensive commercial ventures has caused systemic challenges whose solution is in innovative production methods in accordance with changing world developments. In most countries, the size of units and scale of poultry system has shifted to industrial level production (Carron et al., 2017). Feed preparation aims at developing well-balanced diet that includes suitable amounts of organically accessible nutrients necessary for poultry. In addition to the nutritional supplements in feeds, contemporary formulations constitute non-nutritive elements due to their efficacious properties that promotes performance and health (Velmurugu, 2012).

Nutrition and environmental factors play key roles in poultry production (De Bruyn et al., 2015). Provision of sufficient amount of nutritionally adequate feed ingredients to the animal is important for efficient production of animal products (Yadav & Jha, 2019). The purpose of supplying adequate amount of nutritionally balanced feed ingredients requires careful evaluation and good understanding of the nutrients in each feed ingredient formulated into a mixed diet (FAO, 2012). Feed ingredients that contain

protein are formulated into diets to supply amino acids for meeting physiological needs of the chicken. The efficient production of animal products requires correct amount of nutritionally adequate feedstuffs in formulated diets. Attainment of this goal requires an understanding of the digestion characteristics and utilization of amino acids in the feed ingredients (Kong & Adeola, 2014). Poultry have a simplistic digestive system constituting a non-functional caeca with the exception of ostriches and geese whose caeca is highly developed (Ravindran, 2013). Digestive tract in poultry includes a storage organ which is the crop, the gizzard, which is a grinding organ. In broiler chicken, the feed passes from the mouth to the cloaca within three hours and is digested and absorbed in this period (Rodrigues & Choct, 2018).

Broiler chicks perform better if allowed immediate access to feed. In meeting the increasing demands for broiler meat, broilers are raised to attain table weight in a shorter period (Beski et al., 2015). It is therefore important to choose ingredients to provide maximize nutrient availability (Beski et al., 2015). The most critical determinant of chicken intestinal growth is the attention of chicks in the early post hatch stages, enteric infections, the type of feed elements and the supplements that enhance protection of intestinal wall mucosa (Jha et al., 2019). Healthy gut is essential for poultry to perform essential physiological functions and achieve optimal productivity (Kogut, 2019). Nutritional modification is a favourable approach for positively manipulating the gut microbiota of poultry. Routine usage of alternative supplements that possess growth effects on intestinal mucosa promotes improved growth rates, manner in which digestion takes place, and gut health of broilers (Souza et al., 2020).

Commercial poultry feeding has an economic impact as it contributes largely to high cost of production. Broilers' energy requirements for instance contribute up to 70% of the cost of feeds, additionally the grain variety, and the manufacturing technique contributes to the financial sustainability and poultry performance in a different way. Normally, broiler chicken are fed on variety of feeds which have varying energy and protein levels .The benefits of using processed feedstuff is evident but contributes to escalated cost for manufacturing (Neves et al., 2014).

Environmental effects on chicken are low as compared to other animals. Poultry converts feed faster and efficiently with a resultant increased nutrition demand (Tallentire et al., 2018). Chicken nutrition should contain at the minimum, thirty-eight (38) nutritive composition with the right concentration and balance (Réhault-Godbert et al., 2019). Dietary requirement is based on factors such as, growth, feed efficiency, and quality of poultry meat among others depending on the type of poultry. The feed ratios take into the account the proportion of nutrients that are digestible, absorbed and metabolized through the normal pathway (Klasing, 2015).

The gastrointestinal system in poultry is very simple but efficient as compared to other livestock like goats. The digestive tract of poultry includes the gut along which food passes down to where the wastes are excreted. Other organs include the liver and the pancreas- enzyme producing organs, the crop (storage), and the gizzard, which is a grinding organ. In broiler chicken, the feed passes from the mouth to the cloaca within three hours and is digested and absorbed in this period. To make up for the relatively fast and shortened digestive tract, chicken needs easily digestible, nutrient-balanced feeds.

Therefore, the feed has to be good quality and contain more easily digestible ingredients if the good chicken production is desired (Holland, 2013).

Chicken regulates their feeding patterns based on various factors such as temperature, level of activity, and physiological states that demand different energy levels. Therefore, their daily feed requirements must have vitamins, amino acids, minerals, and water (Klasing, 2015). These components can be found in diets formulated using aggregated ingredients such as grains, animal by-products, fats, mineral supplements, plant protein, and feed additives (Bain et al., 2016). This approach is reported to give optimal health, performance, and productivity in poultry (FAO, 2014).

Feed ingredients in relation to broiler rearing are categorised as fat and oils, vitamins, grains, proteins, and supplemental additives. In addition to water, these feed classes provide sufficient nutrients and energy requisite for health, proper growth, and reproduction. The calories necessary for metabolism and production of good quality meat are provided primarily by carbohydrates and fats and to some extent, proteins in the feed (Poultry Hub, 2021)

2.6.1 Broiler dietary requirement

Both water soluble and fat- soluble vitamins are essential elements for proper functioning of poultry immune system and maintenance of proper health and wellbeing of birds. When poultry feed on a high-energy diet, they reduce consumption on feed therefore diet should comprise of relatively more minerals, vitamins, and amino acids (Klasing, 2015). Large amounts of minerals like sodium, calcium from limestone or crushed sea shells, and phosphorus are needed since they are responsible for skeletal

growth and normal development. Other minerals such as cobalt, manganese, zinc, and iodine are required in minute quantities because their lack thereof leads to adverse health consequences in poultry (Poultry Hub, 2021)

Crude proteins are not necessary for broiler chickens but they should be supplied to ensure there are enough components for synthesis of amino acids that are non-essential (Ullrich et al., 2019). They can be sourced from corn and soy beans although their amount can be reduced in case synthetic amino acids are utilized. Current studies about lysine assert that high amount of lysine is required for maximum growth of broilers (Cerrate & Corzo, 2019). Other suggested methods of improving gut health, feed consumption and productivity is through modification of the physical aspects of the feed including the inclusion of coarse grain particles and manipulation of nutritional fiber composition. This method works, as it has shown to stimulate the foregut of poultry. The insoluble non-starch polysaccharides have been described as a good example. It enhances gut health, waste quality and dietary use by increasing the gizzard and crop activity, increasing enzyme production in the digestive system and enhancing microbial fermentation in the hind gut (Kheravii et al., 2018).

The nutrient values constructed on normalized food intake levels among birds reared in thermos neutral surroundings while consuming diets with specified energy content are as indicated below.

Table 2.1

Nutrient requirements for broilers (expressed as percentage of diets)

AGE	**0–3 WK**	**3–6 WK**	**6–8 WK**
KCAL AME_N/KG DIET	3,200	3,200	3,200
CRUDE PROTEIN	23.00	20.00	18.00
ARGININE	1.25	1.10	1.00
GLYCINE + SERINE	1.25	1.14	0.97
HISTIDINE	0.35	0.32	0.27
ISOLEUCINE	0.80	0.73	0.62
LEUCINE	1.20	1.09	0.93
LYSINE	1.10	1.00	0.85
METHIONINE	0.50	0.38	0.32
METHIONINE + CYSTINE	0.90	0.72	0.60
PHENYLALANINE	0.72	0.65	0.56
PHENYLALANINE + TYROSINE	1.34	1.22	1.04
PROLINE	0.60	0.55	0.46
THREONINE	0.80	0.74	0.68
TRYPTOPHAN	0.20	0.18	0.16
VALINE	0.90	0.82	0.70

Source (Poultry Hub, 2021)

Nutritional fats are a major source of calories, vital macronutrients and fat-soluble micronutrient. Growth performance can be influenced by the kind of fat utilized in poultry feed formulation. The type of fat can also affect the fatty acid structure of meat and can be deleterious for poultry health and dietary quality for the consumers.

Additionally, the quality and composition of feed are vital for determining carcass quality in poultry (Kanakri et al., 2018). Other sources of fats researched on like the black soldier fly larvae fat (BSLF) have been used to replace soy bean oil with promising results in terms of better productivity, carcass characteristics and meat quality in broilers, thereby the proposition that this component be made part of modern chicken feed (Schiavone et al., 2017).

Dietary approaches such as reduction of an amount of feed can increase the efficacy of feed efficiency (FE) of poultry at a given nutrient concentration. Decreasing feed intake causes improved nutrient digestibility and or post-absorptive metabolism and ultimately compensatory growth. Additionally, feeding on coarser feed influences causing enlargement of the gizzard improves digestibility and impacts on diet use (Aftab et al., 2018).

2.6.2 Broiler feed supplementation

Supplements in poultry feeds are exogenous enzymes, probiotics, organic acids and prebiotics (Anjum & Chaudhry, 2010). Their use to modulate microbiota of the gut has successfully resulted in improved health and production efficiency of poultry (Alayande et al., 2020). Proper understanding of gut ecology in conjunction with benefits and harms of feed supplements on modulating gut microbiota is a requisite for optimal chicken production (Yadav & Jha, 2019).

Antibiotics are among the feed supplements commonly used in animal feed formulation. There are other feed supplements originating from plant products and are referred as phytochemical feed supplements or phytobiotic (Lillehoj et al., 2018). Phytochemical

compounds refers to the parts such as seeds, fruits, roots, and leaves of various tropical plants and spices including coriander, oregano, thyme, garlic, rosemary and cinnamon as well as respective plant extracts in the form of essential oils (Bor et al., 2016). The pathway in which phytochemicals act on poultry is not fully understood, but it can be explained by their antibacterial properties (Lee et al., 2013), antiviral (Lillehoj et al., 2018), oxidative-resistant activity (Mohammadi and Kim, 2018), anti-inflammatory (Lee et al., 2013), enhancement of the immune system (Kothari et al., 2019) and as a result, improvement in poultry performance. Besides efficacy, application of phytochemical feed supplements to livestock also has to be safe to animals, humans and the environment.

Antibiotics as growth promoters work by inhibiting pathogens and reducing competition between the host and bacteria to provide nutrients to the host, which results into more nutrients to the host with less bacterial propagation (Vieco-Saiz et al., 2019)**.** Poultry feed preparations also contain some ingredients known as “feed additives”. It constitutes less than 0.05 percent of feed composition, which helps in maintaining the health status, consistency and production efficiency in large-scale poultry production systems. Feed additives are an important components of chicken nutrition (Wina, 2018).

2.6.3 Antibiotic growth promoters in broiler production

Utilization of antimicrobials together with stringent biological hazard security and hygienic practices has helped to avert serious negative effects from numerous poultry infections. This resulted in growth of poultry production industries over the past decades (Mehdi et al., 2018). Various antibiotics are used in poultry farming and the most

commonly used is tetracycline, bacitracin, tyrosine, salinomycin, virginiamycin and bambermycin for commercial production of broilers in North America (Glasgow et al., 2019). Tetracycline use is more in America, as it represents more than two thirds of the therapeutic agents used in poultry farming in the United states (Jamal et al., 2017).

Usage of antimicrobials across Europe is at 37% only (Carvalho & Santos, 2016). Utilization of antibiotics growth promoters is prohibited in European Surveillance of Veterinary Antimicrobial Consumption (EVAC) member nations (Schar et al., 2018). Antibiotic growth promoters when given at sub therapeutic levels effectively promotes growth and provides protection against infections through modulation of immunity and gut biota (Mehdi et al., 2018). Modification of microbiota of the gut of chicken affects the immunity and health of chicken. Furthermore factors including nutritional composition, exposure to disease causing microbes, housing, and use of antibiotics affect gut microbiota (Carrasco et al., 2019).

Although the mechanism of action supporting antimicrobial facilitated growth promoters is unknown (Gaddet et al., 2017), it is thought that antibiotics facilitates remodeling of microbial composition (structure and diversity) and is produced in large quantities in the gut to provide enough microbiota for growth (Mehdi et al., 2018). A theory proposed by Francois (1961) and Visex (1978) tried connecting the efficacy of antibiotic growth promoters relative to antibacterial action as it was assumed that their growth was by reduction in the composition of the gut microbiota. This was thought to decrease nutrient competition with corresponding reduction in detrimental metabolites from microbial (Gaddet et al., 2017). However, Niewold (2007) disputed the theory and suggested that

the beneficial properties of antimicrobial resulted from effective interactions among immune cells instead of having growth restriction on microbiota. Niewold (2007) forwarded a theory that antimicrobials stimulated production of pro-inflammation cytokines associated with decreased appetite coupled with extensive muscle catabolism.

Anti-inflammatory effects from AGP inhibits energy wastage and focuses the same on productivity (Niewold, 2007). Even though it seems clearer that inclusion of antibiotic growth promoter in feed causes modification of microbiota biodiversity (Gaddet et al., 2017), the bottlenecks to actually connect specific microbial species to improved growth and identification of pathways to modify microbiota to desired ones remains (Lin, 2014). Antibiotics as growth promoters include inhibiting pathogens and reducing the competition between the host and bacteria, of which, ensures availability of sufficient nutrients for the host without bacterial growth (Vieco-Saiz et al., 2019).

Non-therapeutic alternatives such as feed additives can substitute AGP use in poultry production (Mehdi et al., 2018). Supplements are mainly added as a way of enhancing poultry growth efficiency, increase capacity of laying, enhance feed usage, and avert incidences of disease (Hassan et al., 2018). However, any additives utilized in feed has to be permitted for consumption and utilized as instructed with regards to the concentrations level and period of feeding. Additives available are specific for the type and age of poultry being fed (Poultry Hub, 2021). Use of safe additives and other naturally available alternatives as a replacement of AGP might possibly be important in poultry production (Hassan et al., 2018).

Tropical plants have wide ranging functional attributes like antimicrobial, antioxidant, antifungal, immune stimulating properties and feed intake enhancement. Many of these effects are associated with different phytochemicals such as alkaloids, flavonoids and saponins among other (Achilonu et al., 2018). In formulating poultry feed, energy, protein, and feed additives to supply vitamins, minerals and amino acids are required (Ravindran, 2014). Over the years, plant products have been used as valuable sources of natural products for maintaining animal and human health. In fact, plants have been determined to contain substances with chemical properties of therapeutic and preventive importance (Hamuel, 2012).

2.6.4 Exogenous enzymes additives

Since the 1980s, exogenous enzymes use in rations is recognized in the poultry industry and has been suggested as a probable alternative to AGPs. It is the most commonly used additives for growth improvement and enhancement of feed conversion (Alagawany et al., 2018). Enzymes are proteins that facilitate particular biochemical responses and also target specific substrates (Pirgozliev et al., 2019). Generally, livestock are reported to be unable to assimilate about 15- 25% of the rations they consume (Imran et al., 2016). Even though poultry and gut microflora do release a lot of enzymes, it is not specific, which is required for whole assimilation of normal rations or to moderate anti-nutritional factors in feed that slows digestion (Poultry Hub, 2021).

There are 2500 known classes of enzymes, the most commonly used enzymes are the non-starch polysaccharidases (NSPases) that attach to the non-starch polysaccharides (NSP) contained in gelatinous cereals (such as barley, wheat, triticale) as well as

phytases which are known to work on phytate-complexes which are comprised within plant related ingredients (Imran et al., 2016). Phytase enzymes (50%) are widely used in the world as compared to the rest of the enzymes used in livestock production (Imran et al., 2016). Reaction towards enzymes during digestion in poultry is dependent on factors such as; nutrients concentration in the diet, food type, quantity of enzymes, genetic strain and poultry age (Alagawany et al., 2018).

According to Wallis (1996), apart from Aspergillus used commonly in the food industry, exogenous enzymes are currently derived from genes encoding. Many enzymes cloned include; phytases, xylanases and β-glucanases, they are produced in several commercial systems. Other inexpensive enzymes produced from microorganisms are appropriately selected and multiplied. Microbes used include; bacteria (Bacillus lentus, B. subtilis, B. stearothermophils and B. amyloliquifaciens), Yeasts (Sacharomyces cerevisiae) and Fungi (Asperigillus niger, A. oryzae and Triochoderma longibrachiatum) (Alagawany et al., 2018).

Exogenous enzymes act by ensuring complete digestibility of feed taken by poultry and this decreases the negative environmental effects by decreasing litter production. These additives are proteins in nature that are eventually assimilated or excreted, leaving no deposits in meat or eggs (Imran et al., 2016). None of the enzymes that animals produce is able to hydrolise non-starch polysaccharides present in food. Dietary non-starch polysaccharidases can catabolize chemical bonds holding NSP sugar units and remarkably decrease the thickness of gut content. Hind gut viscidness facilitates whole digestion and assimilation of nutrients, decreased bacterial growth and enhanced gut

health (Abdulla et al., 2017) . The NSPases can also generate oligosaccharides which may act as prebiotics at the same time increase the liver antiradical ability of broilers chicken (Pirgozliev et al., 2019)

Phytase is mainly utilized to intensify the degradation of phytate and use of phosphorus, and possibly facilitate increased prececal amino acid digestibility (Borda-Molina et al., 2019). According to Selle and Ravindran (2007), plant phosphates in the range of 600 g/kg to 700 g/kg exist as phytate. Though birds have the capacity to produce their own phytase, they are normally augmented by bacterial ones since endogenous phytase is not enough for optimum hydrolysis. Phytases work by hydrolyzing bonds between inositol ring and phosphate groups in phytate resulting into more food available for absorption. Phytases are beneficial in enhancing performance, increasing the liver antioxidant ability, mineral retention, energy, and amino acid availability for poultry growth (Pirgozliev et al., 2019).

2.6.5 Probiotics additives

According to FAO and WHO, probiotics or direct fed microorganisms are “live strains” from well selected lineages of microorganisms such that their adequate administration benefits the host (FAO, 2002). However, probiotic additives should be of prescribed standards by The International Scientific Association for Probiotics and Prebiotics [ISAPP]. It is a requirement that probiotics be microorganisms that are alive and meet minimum quantity, useful to the animal and be favourable to the physiology of the gut. Due to their efficacy and effectiveness in promoting animal growth, probiotics are generally utilized in chicken rations (Śliżewska et al., 2019).

Microorganisms that have been selected and tested to be safe and efficacious and considered as probiotic include; lactobacilli acidophilus, Streptococci faecum, Bifidobactrium bifidum, Enterococcus faecum and Bacillus species, L. salivarius, L. caset, L. cellobious, S.thermophilus, Torulopis spp, Aspergillus oryzae, Bacillus licheniformis etc. (Gaddet et al., 2017; Jin et al., 1997). These feed additives significantly prevent pathogenic bacteria which are listed as Listeria monocytogens, Campylobacter jejuni, Staphylococcus aureus, Salmonella enteritidis, Clostridium pefrigens, Escherichia coli, Yersinia enterocolitica, Eimeria sp, S. typhimurium, and candida albicans (Gaddet et al., 2017; Hassan et al., 2018; Jin et al., 1997)

Studies have suggested that directly fed microbials act by transforming pathogenic gut microbiota to important commensals. As reported by Ferdous et al., (2019), these probiotics assist in the production of bactericin, lactic acid and hydrogen peroxide, which creates a very unfavourable environment for many harmful microbes. It also decreases the oxidation-reduction capacity in the intestines which hinder aerobic harmful microorganisms, inhibits the poisonous amines and ammonia build up, and produces essential digestive enzymes and B-vitamins, lastly acting as stimulating the animals' appetite.

Production of acetic and lactic acid by Lactobacillus in the gut is said to prevent proliferation of harmful Gram-negative organisms, the action of this bacteria is reliant on acids depended on the p^H. Apparently, the lower the p^H the higher the concentration of acids in an undissociated form , which is bactericidal and germicidal action (Jin et al., 1997). Many more benefits of probiotics in poultry productivity outlined by (Jin et al.,

1997) include; preservation of animal gut after antibiotic treatment, reinforcement of immunity due to increased levels antibody titres, immune cells, and immunoglobulin. It also moderates lipid metabolism and reduces cholesterol content, balances gut physiology and enhances mineral assimilation. Probiotics also acts by detoxifying fungi especially mycotoxins and improves faecal composition by decreasing ammonia and faecal water contents (Jadhav et al., 2015).

Other recent reports such as Hassan et al., (2018), on the beneficial effects of probiotics have also demonstrated favorable effects on the chicken weight, feed conversion ratio, with reduction in death rates. Use of probiotics infeed in broiler nutrition comprising Bacillus sp, in broiler diets has shown to considerably enhance growth performance and carcass quality as compared to antibiotic growth promoters. Similarly, it has been shown that probiotic additives singularly, could potentially increase weight and better dietary conversion ratio as compared to all other alternative additives. Moreover, probiotic additive in feed, might be the best choice in place of AGPs (Ferdous et al., 2019).

According to Blajman et al., (2014), literature reviews done from the 1980s' of so many randomized research trials confirmed that probiotics are highly efficacious in poultry productivity. Probiotics use in feed demonstrated their extent of efficacy in aspects of feed conversion and growth rates in broiler chicken. The studies reviewed established that probiotics led to added chicken weight and lowered feed conversion ratio. It also, concluded that administration of probiotics was better through drinking water than infeed (Gaddet et al., 2017; Jadhav et al., 2015).

In conclusion, probiotics in intensive broiler production is best in feed additives alternative. However, use of probiotic in combination with other alternatives may probably be more advantageous than utilizing it singly to attain the same action as that of AGP. Leveraging on probiotics and proper management and poultry practices optimum growth productivity will be achieved (Gaddet et al., 2017; Jadhav et al., 2015; Jin et al., 1997).

2.6.6 Prebiotics additives

In addition to probiotics, prebiotics have proven themselves as a promising alternative in poultry production because they can pass through the digestive tract and promote and maintain beneficial symbiotic relationship between the host microbiota and the gastrointestinal (GI) tract (Gaddet et al., 2017). According to FAO (2007), prebiotics are "inanimate food ingredients that provide host health benefits related to the regulation of the microbiota". All the other definitions are anchored on the premise that, prebiotics given in feed to poultry are indigestible in the foregut, non-absorbable, but are beneficial as it selectively stimulates the proliferation, digestion, and composition of commensal normal flora in the gut thereby eliminating harmful ones (Solis-cruz et al., 2018).

As authored by Micciche et al. (2018), prebiotics function by changing the host gut microbiota through a mechanism that produces favorable benefits to the involved animal. It not only has a positive effect on the intestinal environment, but also has a positive effect on systemicity, thereby improving production parameters such as laying capacity, ration of feed conversion, mortality, and body weight.

Prebiotics have the capability of eliminating colonization of pathogens of community healthiness importance. Examples are Clostridium perfringens Esherischia coli, Campylobacter, and Salmonella. In young chicken for example, the digestive tract biota keeps being altered so fast as the chicken grows. Normally, Clostridiacea and Enterobacteriaceae pathogen are found to colonize the hindgut of a seven-day-old chickens (Jha et al., 2019). However, in a 35-day-old bird, the hindgut is colonized by normal flora suchas Lactobacillaceae and Clostridiacea. Infeed prebiotics when administered, cause growth and balance of favorable gut bacteria colonies of young broilers birds, by increasing the proliferation of Lactobacilli and Bifidobacteria and reducing the number of pathogenic Coliform (Teng & Kim, 2018)

In one of the reviews on the role of prebiotics in intestinal biota of broilers, the mode of action discussed included; change of the caeca microorganism balance, development of the enterocytes, and enhancement of the host immune response. When prebiotics are ingested, they are fermented by intestinal normal flora, releasing bacteriocine, lactic acid, and short-chain fatty acids (SCFA): this by products are effective against harmful bacteria. The gut epithelial cell are well developed leading to improved assimilation of nutrient hence improved animal performance and productivity (Teng & Kim, 2018).

The main types of prebiotics are either non-starch polysaccharides (NSP) or oligosaccharides. This include galactooligosaccharide, mannan oligosaccharide (MOS), fructooligosaccharide, oligofructose, maltooligosaccharide, lactulose, isomaltooligosaccharide, lactitol, galactooligosaccharide, inulin, xylooligosaccharide, soya-oligosaccharide and pyrodextrins (Solis-cruz et al., 2018; Teng & Kim, 2018).

When formulating prebiotic additives, it is of paramount importance that the correct dosage is prescribed; otherwise, chicken may experience adverse effects like flatulence and diarrhea as side effects. However, prebiotics may be used as prophylaxis and also for a prolonged duration without side effects (Śliżewska et al., 2019)

Use of alternative to AGPs could improve poultry production and the economy generally. This is achieved through successful modulation of pathogens and commensal microorganism in the chicken. Infeed prebiotic use is beneficial in enhancing intestinal health, immune system, prevention of harmful microbiome, and growth performance. Additionally, the variety of additive, dosage, formulation of the main diet, the type of bird and the host gut ecosystem determines the efficacy of prebiotics (Solis-cruz et al., 2018).

2.6.7 Symbiotic feed additives

The aggregation of both prebiotics and probiotics is also referred as symbiotic because of their synergistic action. These preparations containing this mixture are utilized in livestock diet. The mixture is thought to favorably affect the host by enhancing its endurance, continual growth of probiotics and selective boosting of growth or breakdown of useful microorganisms in the digestive tract (Gaddet et al., 2017). Gibson and Roberfroid (1995) described symbiotic as "a mixture of probiotics and prebiotics that improves the survival and implantation of live microbial supplements in the gastrointestinal tract and selectively stimulating the growth and/or metabolism of one or a limited number of beneficial bacteria, thereby benefiting the host and improving its health" For instance, probiotics influence intestinal stability favorably, and can form a

protective barrier along the intestinal wall. Conversely, prebiotics are a source of energy and nutrients for probiotic bacteria (Śliżewska et al., 2019)

Even though data regarding influence of symbiotic in livestock is scarce, use of symbiotic to influence gut microbiota in livestock is promising. Reports on the synergistic mechanism of probiotics and prebiotics against bacterial gut colonization are documented. Furthermore, there is a consensus that symbiotic additives are more effectives as compared using the mixture separately (Śliżewska et al., 2019). In the study by Yitbarek et al. (2015) combination of probiotics and yeast-derived carbohydrates demonstrated weight gain relative to the control group.

Further, to demonstrate the effectiveness of symbiotic, the effects of injectable prebiotics and symbiotic in 35- day-old broiler poultry was evident, when results showed a percentage increase in glycolytic fibres of pectoral muscle from chicken treated with a symbiotic additive with inulin included as compared to prebiotic treatment singularly (Dankowiakowska et al., 2018). In hotter climatic conditions, studies have demonstrated that symbiotic seems to be more effective. According to Lara and Rostagno (2013), high temperatures is a significant ecological challenge faced in chicken production, it hinders productivity and endurance capability leading to poor carcass quality and performance. One of the studies done to evaluate the effect of symbiotic additive fed to broiler chicks in hot environments confirmed that symbiotic positively influenced growth performance and productivity. There was increased weight gain, reduced presence of gut E. coli, Salmonella, and Shigella, and reduced excreta ammonia. The study concluded that

symbiotic is an effective feed supplement especially in hot climatic regions (Abdel-Wareth et al., 2019)

The major probiotics strains commonly utilised in formulation of symbiotics preparations include Lactobacilli, S. boulardii, Bifidobacteria spp, B. coagulans etc., whereas prebiotics are oligosaccharides (galacto-oligosaccharides, fructooligosaccharide, and xyloseoligosaccharide), natural sources (yacon roots, etc.) inulin. Although the formulation of symbiotics seems to be extremely difficult and requires further research, formulation of symbiotics should meet specific criteria of having useful and health benefits to the host. The preparation is similar to that of prebiotic and probiotic used singly (Śliżewska et al., 2019).Additionally, the symbiotics mixture is a significant health determinant. As mentioned above, the symbiotic design not only promotes the growth of symbiotic bacteria, but also promotes the growth of certain native strains (autochthonous specific) in the intestine. Therefore, symbiosis seems to show promise to change the composition of the gut microbiota (Hamasalim, 2016).

2.6.8 Organic acids, antimicrobial proteins, and bacteriophages feed additives

Other additive commercially available in poultry production include organic acids, antimicrobials proteins, bacteriophages, and pigments, these are used to enhance poultry performance and productivity. Just as the other additives discussed above, infeed organic acids also enhance growth, improve rate of feed conversion and consumption. Organic acids are preservation compounds that are utilised commonly for defence as it hinders multiplication of bacteria and fungi. The structure of this carboxylic type of acid

contains an hydroxyl chemically functional group at the α-carbon with examples like lactic acid, malic acid, and simple monocarboxylic acids such as formic, proprionic, and acetic acid. According to Maroccolo (2013), this group of acids act on bacteria as its non-dissociated acids have the ability to pass through the lipophilic bacteria cell wall then go in to interfere with enzymatic responses, and the transport system, hindering proliferation and protecting against certain pathogenic bacteria such as Campylobacter and Escherichia coli(Mehdi et al., 2018).

Infeed citric acid (2%) for example, has exhibited to enhance chicken gut cell multiplication, regeneration of epithelial wall and elongation of villi height (Mohammadagheri et al., 2016). Butyric acid which is a carboxylic acid that is saturated is usually released in hindgut due to carbohydrate metabolism. Addition of butyric acid to broiler feed has also revealed effects on growth performance. Availability of this butyric acid generates energy to the intestinal epithelial cells and promotes the proliferation and division of cells. This effect enhances feed conversion and utilization. One of the experiments confirmed that whether butyric acid (BA) and fermentable carbohydrates (FC) added to coarse feed enhances performance of chicken fed with poor digestible proteins sources. The results showed that compared with chicken fed a light diet, the predicted ileal protein digestibility was higher, the stomach pH was lower, the villus height was increased, the crypt depth was shallower, and the appendix branched chain fatty acids (BCFA) were reduced (Qaisrani et al., 2015a).

Apart from decrease in pathogenic bacteria, organic acid neutralizes ammonia release and reduces the predisposition to re-infection. Just like other additives, the efficacy of

organic acids in chicken is reliant on the assortment of diet and the buffering capacity (Dhama et al., 2014) Alteration in gut microbiota and proliferation in commensal bacteria such as Lactobacillus demonstrate that, organic acid can be utilized as infeed additive in place of AGPs (Mehdi et al., 2018).

Studies have confirmed the antimicrobial effect in poultry rearing. A review by Wang et al. (2016) observed that, over and above the antimicrobial properties AMPS possess, it is capable of overcoming resistance unlike the commonly used antibiotics. Moreover, it has influence on broiler growth performance, feed digestibility, intestinal stability and alimentary canal microbiome. It concluded by affirming that, AMPs are potential substitute applications to AGPs used in poultry farming (Wang et al., 2016).

Bacteriophages are bacteria infecting viruses and are used as alternative to antibiotic growth promoters; they are also used in feeds to modulate poultry gut bacteria. Further, phages that are developed through genetically modification are utilized as "gene carriers" for the biosynthesis and degradation of food and genetic modulation of the celiac bacteria (Gagliardi et al., 2018). Various studies document the antibacterial capabilities of bacteriophages (phages); use of in feed phages has shown action against harmful bacteria: Escherichia coli, S. enteritidis and Campylobacter. Jejuni (Suresh et al., 2018).

Discovered by Twort in 1915 and further characterised by D'Herelle, bacteriophages also known as bacterial parasites are freely available viruses whose life cycle, is linked with bacterial cell. The viruses do not have any enzyme composition or cell structure

necessary for normal food absorption, synthesis of proteins or development of fresh virion elements, this characteristically incomplete nature of phages makes it dependent on live cells to propagate. As compared to antibiotics, phages act by specifically selecting and infecting only one strain, group or serotype of bacteria. This mechanism has made it possible for it to be applied to targeted treatment for slow healing morbidities. Interestingly, this mode of action does not result to any damage to gut normal flora (Wernicki et al., 2017)

Although bacterial resistance is reported, Bragg et al. (2014) explains that, phages are assumed to have changed alongside the specific host; so, if the bacteria develop resistance, the bacteriophages could also mutate to outdo the resistance mechanism. Moreover, the bacteriophages count specific for a bacterial host are sufficient, therefore resistance to phages is not taken as a big threat. This makes it vital for development of bacteriophage treatments which contain several combinations specific for many different receptors. This means that, the likelihood of resistance developing will be reduced. Stains such as Staphylococcus aureus, which are genetically homogenous, may be more prone to bacteriophages targets as compared to Escherichia coli, which is genetically heterogeneous (Gigante & Atterbury, 2019).

2.6.9 Phytobiotics feed additives

Phytobiotic supplements are non-nutritive components present in a plant-based diet that exert protective or disease-preventing effects. By including them in the diet, they provide many opportunities to improve livestock production. It represent a diverse group of natural products some of which may be nutritionally valuable and many others have

no nutritional value (Willett et al., 2019). According to Zhang et al. (2015), Phytobiotics, also known as phytogenic or phytochemical feed additives (PFA) are natural derivatives from plants added to feeds to improve poultry productivity (Mehdi et al., 2018). It is also described as non-nutritive, secondary plant metabolites, produced by plants and protect plants from diseases, pests, stress, and physical damage (Kamboh et al., 2018) . Plant-derived bioactive agents have attracted more and more attention in improving the growth performance and immune response of broilers (Hassan et al., 2018). The main bioactive agents are polyphenols, the composition and concentration is different in plants, vary in parts of the plant e.g. roots, leaves or bark, geographical location, harvesting time, ecological factors, method of preparation and how they are stored (Lillehoj et al., 2018).

It is recommended that potential antimicrobial substitutes should have the same useful effects of AGPs, and should cause maximum livestock growth performance and good feed conversion ratios (Lillehoj et al., 2018).These bioactive compounds are generally in fruits (e.g. grapes, cherries, strawberries, and raspberries), vegetables (broccoli, cabbage, carrots, onions, tomatoes,), legumes (e.g. beans, soy foods, and nuts), whole grains (e.g. wheat bread, seeds), fungi, herbs and spices. Many types of spicy and herbal products (e.g., garlic, rosemary, marjoram, yarrow, oregano, ginger, coriander, greenish tea, cinnamon, and black cumin) all have undergone testing in poultry production for viability as AGP substitutes (Lillehoj et al., 2018; Saxena et al., 2013).

Active ingredients exist in different parts of plants, such as leaves, roots, stems, seeds, flowers, or fruits; but pigment molecules are more likely to exist in the outer layer of

different plant tissues. However, the concentration of phytochemicals differ from plant to the other and also dependent on the processing, cooking and cultivation conditions (Lillehoj et al., 2018; Saxena et al., 2013). The medicinal features of phytochemicals especially flavonoids, is attributed to its anti-oxidative, anti-inflammatory, immune system booster, and gut defense properties. Flavonoids is shown to modulate intestinal mucosa and epithelial cells immune response, and to affect animal hematological and biochemical indices. Phytochemicals can therefore be used in enhancing immune system together with health of broiler poultry (Kamboh et al., 2018).

The mode of action of phytogenic agents is unclear but could be dependent on the composition of the bioactive chemicals in the botanical species utilized (JE et al., 2015). The favorable effects of phytobiotics are linked to its antibiotic and antioxidant properties. Due to the direct action on pathogens, the antimicrobial effect of phytochemicals modulates the intestinal microbiome and reduces toxic bacterial by products in the digestive system. This effect can reduce the accumulation of intestinal diseases and immunological stress in livestock, thereby increasing productivity. In addition, phytochemicals can lessen oxidative stresses and increase the antioxidant activity of various organs, thereby improving animal welfare. As an immune modulator, the phytochemicals causes increased immune cells propagation, regulation of cytokines, and rise in antibody count (Lillehoj et al., 2018).

Over production of reactive nitrogen species (NOS) and reactive oxygen species (ROS) in human or animal body can cause morbidities. Foraging of these free radicals by use of phytochemicals is assumed as a good manoeuvre in reducing the degree of oxidative

stress in animals. Evidence based information on the health benefits resulting from the antioxidant properties of phytochemicals has been reported, example is the consumption of fruits and vegetables which is known to avert risks associated with many chronic illnesses (Lillehoj et al., 2018; Zhang et al., 2015).

Phytochemicals are divided into two according to their role in plant metabolism: primary metabolites and secondary metabolites. Primary metabolites include common carbohydrates, amino acids, proteins, nucleic acid purines and pyrimidines, vitamins, chlorophyll, etc. Secondary metabolites include flavonoids, alkaloids, lignans, steroids, saponins, phenolic resins, curcumin, terpenes and glycosides. Each classification of phytochemicals comprises of many other kinds of compounds that act differently. Additionally, some of these biological agents have multiple roles in plants (Koche et al., 2016).

As reported by Koche et al. (2016) phenolic metabolites are more common in plants as compared to the other agents; the rate is at 45% and is broadly distributed. The rate of occurrence of Terpenoids and steroids is (27%), and Alkaloids is (18%), other compounds account for 10% of phytochemicals. Moreover, in the phenolic classification, flavonoids, phenolic acids and polyphenols are the most common compounds. They are significant as defense compounds (Saxena et al., 2013).

Flavonoids have drawn an increased research interest due to its wide range of recent biological and pharmacological mechanism respectively. Flavonoids are known to possess various biological properties including antimicrobial, cytotoxicity, anti-

inflammatory, enzyme inhibition, as well as antitumor capabilities. Moreover, its powerful antioxidant characteristic feature makes it significant as a protective compound against free radical and reactive (ROS)-mediated disease development. Luteolin and cathechins antioxidants in flavonoids are superior to those found in vitamin C, vitamin E and β-carotene (Koche et al., 2016; Saxena et al., 2013).

As defined by Hamid et al. (2017) Alkaloids have a basic PH, natural and contain heterocyclic atoms of nitrogen. Alkaloids are derived from "alkaline" and their properties and are used to determine any nitrogenous alkali. They are naturally used by many organisms, including animals, plants, bacteria, and fungi (Saxena et al., 2013). Alkaloids have complex molecular structure, classifying them is a challenge. Nevertheless, Krishnan et al. (1983) recommended classification according to the type of hetrocyclic ring found in the composite molecule. It can also be grouped using their physiological characteristics, the various classes include; pyrrolidine, pyrrolidine-pyridine, pyridine, pyridine-piperidine , quinoline and isoquinoline (Koche et al., 2016; Rani et al., 2018). Molineux et al. (1996) contributed that, alkaloids are important to the existence of plant due to its protective nature against microbes (antifungal and antibacterial actions), herbivores and insects (prevents feeding) through allelopathy against other plants (Leoneet al., 2015). Alkaloids are documented to have a big economic value; they also possess therapeutic effects on humans by exhibiting antihypertensive, antiarrhythmic, antimalarial and anticancer effects. Other benefits reported include the stimulant properties (Saxena et al., 2013)

Terpenoidsare natural types of lipids derivatives five-carbon isoprene units. They have many cyclic structures that vary from the others depending on the functional groups and basic carbon skeletons. They are also present in all biological groups, so they are considered to be the largest natural secondary metabolite group. Economically, terpenoids are significant because they are used purposely as essences and scents in foodstuff and cosmetics purposes. Additionally, they are available widely in vegetation as part of essential oils. The basic unit is the hydrocarbon isoprene are categorized as; hemiterpenoids, monoterpenoids sesquiterpenes, diterpenes, triterpenes and tetraterpenoids (Koche et al., 2016; Leone, et al., 2015; Saxena et al., 2013). As reported by Degenhardt et al. (2003) terpenoids is significant in direct plant defense activity as phytoalexins or in indirectly as defense by producing signals to guard against herbivores. Additionally, they also possess anticancer, antimalarial anti-ulcer, liver, antimicrobial or diuretic effects (Saxena et al., 2013).

Saponins are found in many plants and easily form stable foams in soaps and other aqueous solutions, hence their name ‘saponins’. As a group, it contains compounds of glycosylated steroids, triterpenoids and steroid alkaloids. The major classifications of aglycone steroids are by-products of furostane and spirostane. Although the physiological function of saponins is not well understood, they are known to, contain anti-microbial, prevent fungi, and to defend plants from insect invasion. This nature of protection has made it to be included in the bigger class of protective molecules available in plants referred to as phytoanticipins or phytoprotectants (Koche et al., 2016; Leone, Spada, et al., 2015; Saxena et al., 2013)

Tannins are a group of heterogeneous high molecular weight polyphenol compounds that can form reversible and irreversible complexes with proteins and polysaccharides, alkaloids, minerals and nucleic acids. Based on molecular structure tannins fall in four groups namely, gallotannins, ellagitannins, complex and condensed tannins (Koche et al., 2016). Tannins have therapeutic benefits, Serrano et al. (2009) alluded to the fact that the use of tannins showed significant decrease in chronic disease prevalence. They also have properties that are beneficial in managing diarrhea, stomach and duodenal tumors: hemostatic, anti-oxidative, anti-inflammation, and antiseptic effects. This properties of tannin has generated interest lately especially due to the rising cases of communicable viral illness such as human immunodeficiency diseases in humans (Leone et al., 2015a). Among the ruminants' ingestion of tannins can exhibit toxic effects. Many research studies have previously reported that the toxicity of tannins is linked to their molecular size since the high molecular weights inhibits absorption of tannins (Frutos et al., 2004).

Natures' green belt is an exceptional fountain of various phytobiotics with highly active biochemical compounds that possess valuable medicinal properties. The favorable effects of phytochemicals in broilers especially on its growth performance and productivity, suggest that phytobiotics could be used as potential feed additive instead of AGPs (Kamboh et al., 2018; Koche et al., 2016). Dietary inclusion of one of the commonly used herbs - garlic (allacin) in a study demonstrated positive effects in broiler poultry. There was increased ration consumption, improved feed conversion ratio, protein efficacy, efficacy of energy use and performance (El-katcha et al., 2016).

Similarly, infeed herbs, lavender (Lavandula angusti-folia) has been experimented, and confirmed to improve broiler performance. Its effect on gut microbiota and intestinal histomorphology was also demonstrated where the results showed, improved ratio of feed, increased ratio intake, and weight of body (Salajegheh et al., 2018). Additionally, use of the Moringa plant as potential source of phytochemicals is also documented to control and manage diseases in humans and livestock for centuries. Use of Moringa oleifera in Africa and Asia for medicinal and food purposes is documented in various literature. It is claimed that Moringa possess vast active biochemical compounds antioxidative, free radical foraging capabilities, anti-inflammatory and anticancer effects among others (Zhang et al., 2015).

As described in the literature above, alternatives to AGPs possess properties such as; a well-defined mechanism of action that is pathogen targeted has no potential drug resistance, cheap and eco-friendly etc. However, none of these substitutes singly meets the basic requirements to substitute the effectiveness and cost efficacy of AGPs. All the additives discussed have reported contradicting results but some research suggest use of symbiotic intervention, which may be more useful. Adherence to the selection criteria and proper dosage is important in designing symbiotics. Other combinations can also include phtyogenics and organic acids and prebiotic and enzymes as suggested by recent studies on the effect of other combinations on broiler productivity (Suresh et al., 2018)

2.7 Broiler haematological and biochemical parameters

Poultry blood studies have not been done often as compared to other animals in veterinary medicine, it has since generated interest and has developed substantially in the recent past (Shefaa et al., 2017). The focus has especially been in the evaluation of normal ranges of blood parameters and the screening of various diseases under varied environmental conditions (Surai, 2016). Haematology plays an important role in clinical pathology and disease diagnostic process, and relatively reflects the general health of poultry (Al-Nedawi, 2018a).

Determination of haematological and biochemical values involves the study of blood including the examination of cellular and serum components. In addition, haematology also studies the tissues where blood cells are made, stored, and circulated (Tijani et al., 2016).

Both haematology and biochemical parameters are observed as indicators of overall health of an animal; such that, their variances are vital for the evaluation of animal responses with respect to existing conditions of physiology (De Oliveira et al., 2019). Blood profile of healthy chicken may be affected by dietary compositions. It may be difficult to evaluate the present health status of animals without detailed examination of the blood (Bettencourt et al., 2015). One among the effective approaches in evaluating health and nutritional levels of livestock on experimental feeds could be the haematological analysis (Oleforuh-Okoleh et al., 2015).

Blood transports nutrients together with other materials to various tissues of the body. It has been reported that if the blood profile value of an animal is within the normal range, it means that the diet has no negative effect on the blood profile during the experiment, however, when the value is below the expected normal values, anaemia is conclusive (Russell et al., 2016). Hematological analysis provides an opportunity to check the presence of metabolites and other compounds in the body, and is also important for the overall health of the animal (Andrade et al., 2016). Low values for haematological parameters may be as a result of the harmful effects of high dietary contents (Etim, 2014).

As discussed by Samour (2005) in the Clinical Avian Medicine book published by Harrison and Lightfoot (2005) avian haematology and biochemistry sample processing methodology was adopted from the procedures used for analysing human blood but, compliant with the recommendations of the International Committee for Standardization in Haematology (International Committee for Standardization in Haematology, 1978). However, sample processing has been improved in the recent past and avian veterinary medicine requires quality control to ensure sound and credible laboratory results (Samour, 2005).

Evaluation of blood provides a clinical basis for analysis of metabolites and other components in livestock. It is important for measuring nutritional, physiological, and pathological conditions. In fact, blood composition changes according to the physiological state of the animal. Reference values for blood parameters are important in establishing the health status of chicken (Etim et al., 2014). Indices of blood that are

normally evaluated are; Haemoglobin level, Packed cell volume, Mean Corpuscular Values, Total protein, White and red blood cell count, Lymphocyte count, Heterophils count, Eosinophils count, Monocytes and Heterophil/lymphocyte ratio (Makeri et al., 2017).

Haematological and biochemistry parameters are influenced by numerous variables including; age, chicken infections, production systems, chicken breed, nutrition, feed additives, feeding habits among others. Poultry infections including Newcastle disease, infectious bronchitis, Gumboro infectious bursitis and infectious avian nephrosis have been reported in many research articles to highly affects biochemical parameters (Ogbuewu et al., 2017). Dietary composition and inclusion of alternative infeed diet in poultry production has also been reported to alter serum biochemistry and blood values (Shefaa et al., 2017).

A study done by Shefaa et al. (2017) on unvaccinated broiler chicken infected with Newcastle disease revealed a statistically significant reduction of red blood cells (RBCs), haemoglobin (Hb), pack cell volume (PCV), MCHC indices while mean copular volume (MCV) was noticeably increased, displaying macrocytic hypochromic anaemia. Effect of environmental conditions on blood and biochemistry parameters in a study done by (Makeri et al., 2017) showed presence of circadian rhythms in conjuction with acrophages in the blood measurement of broilers raised under normal photoperiod and tropical conditions.

The use of botanical feed additives as an alternative to AGP in various research studies, demonstrated effect on both haematological and biochemistry parameters of broiler chicken. A study done by El-Katcha et al. (2016) using garlic extract additives at different graded level in broiler chicken feed revealed changes in haematological parameters. The count of Red Blood cells, levels of haemoglobin and the packed cell volumes increased while a reduction in serum triglycerides was also noted. Additionally, there was improved kidney and liver functions demonstrated by a decrease in blood serum creatinine, liver function tests compared to the control. However, increased allicin levels in feed showed adverse effect. The birds' immune response was notably improved due to increased neutrophil indices (El-katcha et al., 2016).

Studies to determine the effects of *Moringa* leaf powder (MOLM) on the serum hematological and biochemical characteristics of broiler chickens have shown that the compacted cell volume changes. Results of the serum biochemistry also revealed a significant decrease in protein indices. It concluded that, MOLM could be added at 15% graded level in feed in broiler diet without resulting to any negative variation in haematological and serum biochemical values of broiler chickens (Tijani et al., 2016). Another study investigating *Moringa oleifera* leaves added in feed in broiler production chain revealed reduced total levels of cholesterol, concentrations of serum triglycerides and low-density lipoproteins. Additionally, atherogenic index were also reduced (Oumbortime et al., 2020).

In a review article by Modisaojang-Mojanaga et al. (2019) the useful properties of *Moringa oloifera* on haematological and biochemistry parameters in broiler chicken was

attributed to the nutritional and phytochemical value of the plant. Additionally, positive effects of Moringa oleifera on haematological and biochemistry value of broiler chicks used in the experiment conducted by (Onunkwo & George, 2015). The study revealed rise in Haemoglobin (Hb), globulin and plasma total proteins level with increase in MOLM levels, inversely, Heterophil /Lymphocyte (H/L) ratio was lowered by increase in the level of MOLM. Biochemistry analysis showed a rise in Thyroid hormones (T_3 and T_4), reduced AST but ALT was not altered with increased amounts of in feeds antibiotics (Onunkwo & George, 2015). Little has been done on *Moringa stenopetala* on blood parameters but, it is evident that *Moringa oloifera* of up to 10% can be used in broiler feed to improve poultry performance. Feeding cost could substantially be reduce thereby reducing the cost of broilers production (Tijani et al., 2016).

2.8 Broiler gut microbiota

Over the past years, attention on intestinal health has risen due to attempts to implement new and innovative feeding strategies to deal with the extensively evolved landscape in the nutrition of poultry. These changes not only results from the genetic modification aspect of poultry but also from quite a number of legislative strategies that have focused attention on the environmental impacts of poultry, user negative perception on the use of antibiotics in feeds and resultant effects on feed costs among others (Lilburn & Loeffler, 2015).

Intestinal wellbeing is essential to sustain effective and viable digestive system physiology (Salois et al., 2016). Poultry gut has several microbial species, majority of those found at the level of phylum include; Bacteroidetes, Firmicutes, Actinobacteri and

Proteobacteria. The presence of bacteria differs throughout the gastrointestinal tract of chicken, with particular microbial profiles being found in the culture of ileum, gizzard, large intestine and appendix of broilers (Carrasco et al., 2019).

The gastro intestinal tract of chickens comprises of the gullet (oesophagus), crop, proventriculus, ventriculus (jejunum, gizzard, duodenum, colon, ileum, cecum, and cloaca (Jacob & Pescatore, 2013). The gut is much shorter as compared to other animals in relation to their body length. Due to the small anatomical nature of poultry gut, coupled with a somewhat poor digestion, microbiota proliferation requires exceptional adaptations to the intestinal environment. The hind gut is characterised by a slow transit rate of food that favours growth of different groups of micro-organisms (Svihus and Itani, 2019) therefore influencing the use of nutrients and the general wellbeing of birds (Yadav & Jha, 2019).

Intestinal health is essential to sustain effective and viable digestive system physiology (Salois et al., 2016). The physiology of intestinal microbiota is vital because the acquisition and maturation of microflora throughout the growth stages of poultry affect development of the intestinal epithelium (Souza et al., 2020). The gut normal flora act by producing short-chain fatty acids (SCFAs): butyrate, propionate, acetate, and lactate, which are generated from intestinal bacterial fermentation of indigestible foods, SCFAs provide energy through glucogenesis for intestinal epithelium and contribute to the reduction of pathogenic bacterial in the hindgut, making them important for gastrointestinal health. SCFAs also facilitates intestinal epithelial cell growth,

differentiation and grows the villus size, thus increasing the absorptive surface area (Yadav & Jha, 2019).

Poultry gut consists of several microbial species. According to Slawinska et al. (2019), 16s rRNA sequences run on Sanger sequencing revealed that poultry digestive tract comprises of 70% Firmicutes (they include 8% Lactobacillus), 12.3 percent of Bacteroidetes, as well as a proportion of 9.3% Proteobacteria. Bifidobacteria from Actinobacteria accounted for 1% of sequencing (Slawinska et al., 2019). The presence of bacteria in the gastrointestinal tract of chickens varies, and microbial characteristics have been identified in broiler, ileum, stomach, appendix, large intestine and crop (Carrasco et al., 2019). Lactobacilli for example, colonize the small intestine and have unique complex nutrient requirement similar to the host. Additionally, this bacteria cause competition to amino acids because the lactobacilli are unable to synthesise amino acids for their own anabolism therefore relying highly on the available amino acid in the intestinal lumen (Apajalahti & Vienola, 2016). In an experiment done on the rate of nutrient assimilation by lactobacilli, it was reported that the bacteria may take up to approximately 3–6% of total nutritional amino acids (Svihus & Itani, 2019).

Even with the benefits of gut commensal microbiota, research has shown that the toxic compounds produced as byproducts of digestion can also cause adverse effect on intestinal health. When the actual feed protein, protein byproducts (enzymes, epithelial cells, and antibodies, mucin, and microbial) usually bypass the small intestines unassimilated to the large intestines, the commensals in the hindgut ferments and it generates toxic metabolites (phenols, indoles, ammonia, amines, and cresol). This affects

the proliferation of intestinal cells and growth rate performance of the poultry (Apajalahti & Vienola, 2016). While amines are vital for gut growth, production in high concentration leads to ventricular erosion, deaths and retarded growth rate in chicken. Reduction in undigested protein decreases fermentation processes in the large intestines (Qaisrani et al., 2015b).

Just like other animals, bile acids have a crucial role in the emulsification and absorption of lipids in poultry (Lai et al., 2018). Presence of gut commensals can impede fat assimilation of fats by deconjugating bile acids subsequently restricting poultry growth. Additionally, these commensals promote increased rates of turnover of enterocytes and cells called goblets which results to tremendously increase metabolic rate and synthesis of proteins. Thus, generating numerous immature cells which are inefficient in the uptake of nutrients have loosened junctions that would otherwise provide attachment point to the commensal bacteria responsible for protecting against the non-indigenous pathogenic microbes (Yadav & Jha, 2019)..

Due to the key role of the gastro-intestinal system in poultry, several studies on poultry production have focused on reviewing and researching on the effects of possible physiologic modification in poultry. Disturbance of poultry intestinal health inhibits the digestive, absorptive, metabolic, immunological and endocrinological functions. Interest in these research areas has increased due to the rising demands for commercial value, animal wellbeing, food safety, decrease in environmental effects, and the prohibition in the use of growth antibiotic growth promoters (Morgan, 2017). Even though evaluation of dietary effects on the intestine have become popular, other methods including

histology can be used to measure intestinal morphology of birds (Lilburn & Loeffler, 2015)

Feed is a key element that subjects internal organs of the poultry through the digestive system to external environment. Advanced molecular studies that describe bacteria without use of culture method have unlocked the prospects to study the effects of diet on intestinal microbiota. Prior research studies have shown effects resulting from interaction of feed with microbial such as; bacteriological communities' modification, change in microbial energy source and discriminatory growth of target bacteria. Although the gut normal flora plays an essential role in host health and digestion, pathogenic microbial causes deleterious effects. Therefore, when selecting feed elements, intestinal conditions and a balance between the host, environment and microbiota should be considered (Yadav & Jha, 2019). Though there are commercial products available, paucity of information on their likely impact on poultry health and efficiency exists (Gaucher et al., 2015). Therefore, to attain microbiota needed for optimal broiler growth, better health and realization of economical feeding program, there is a need for studies on microbiota manipulation using approaches that involve use of feed additives enhancements separately or mixed in feeds (Yadav & Jha, 2019).

2.9 *Moringa oleifera* and *M. stenopetala* as feed additive

In this post antibiotic time, use of safe, efficacious and cost-effective alternative feed additives is an effective and sustainable approach to modulating the digestive tract microbiota in broiler. The alternative dietary supplements discussed above, such as

prebiotics, probiotics, phytochemicals, organic acids, symbiotics, and exogenous substances have been used commercially and have been proven to effectively regulate the gut microbiota to achieve optimal Poultry health and growth performance (Yadav & Jha, 2019).

Phytogenic feed additives (PFA) which are plant derivatives are used to increase livestock performance. Various studies have shown that phytobiotics have effects on growth, immune regulation, and antioxidant responses, and they are recommended as good alternatives to growth-promoting antibiotics (Mehdi et al., 2018). Some medicinal plants such as the *Moringa* tree hoards many dietary and therapeutic properties that is consumed as food and traditional medicine by humans as well as livestock (Hagan et al., 2018). Understanding the role of *Moringa* plants in modulation of gut microbiota is important in improving broiler performance and production. *Moringa* tree is a drought-tolerant, multi-purpose fast-growing and one of the most useful trees in the world as a result of its medicinal and nutritional properties (Daba, 2016).

2.9.1 Origin and distribution of *Moringa* tree

Since ancient times, the *Moringa* tree also called the drumstick, horseradish tree, radish tree or West Indian Ben has been used as food and livestock fodder. The *Moringa oleifera* species have pods that looks like a slender and bowed stick used for beating the drums. Use of the name radish tree is thought to originate from the siliqua of the radish tree, which has dropping, slandered as well as thinly shaped fruits when immature. The tree is native to northwest India and Tamil, it grows along the Chenab River in the

Himalayan region, extending eastwards to the Sarda and Tarai zones of Uttar Pradesh in India (Ramachandran et al., 1980).

Globally, *Moringa* is geographically distributed vastly, in the Middle East and Asian countries. Moringa is found in; Malaysia, Oman, Qatar, Saudi Arabia, United Arab Emirates, Yemen, Afghanistan, Bangladesh, Haiti, Indonesia, Iran, Vietnam Kiribati, Northern Mariana Islands, Pakistan, Philippines Thailand, Myanmar and Nepal.

In Africa, it is distributed in; Ethiopia, Benin, Mauritania, Burkina Faso, Cameroon, Chad, Eritrea, Gambia, Ghana, Guinea, Kenya, Liberia, Mali, Niger, Uganda, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Zanzibar. Others include the Marshall Islands in central Pacific Ocean among many other parts of the word. (Biovision Foundation, 2020).

Moringa has been naturalized in Africa, different species of *Moringa* trees are distributed in various countries; *Moringa* arborea is natively found in Kenya. Also *Moringa* rivae is native to Kenya. On the other hand, *Moringa* borziana is indigenous to Somalia and Kenya, *Moringa* pygmaea is locally available in Somalia. Other species including; *Moringa* longituba is native to Kenya, Ethiopia and Somali. Further, the *Moringa* ruspoliana species is found in Ethiopia, *Moringa* ovalifolia in Namibia and Angola, *Moringa* drouhardii and *Moringa* hildebrandi native to Madagascar and *Moringa* peregrine originated from the Red sea and Horn of Africa. The *Moringa* concanensis, and *Moringa oleifera* species which were originally found in sub-Himalayan regions of Northern sections of India have spread to Nigeria , Kenya among

many other parts of Africa (Leone et al., 2015). *Moringa stenopetala* is common in Djibouti, Uganda, Sudan, and mostly in the northern part of Kenya and also southern Ethiopia specifically around the Kaffa, Gamo-Gofa and Sidamo region of the country (Slow food Foundation, 2020)

In Kenya, *Moringa oleifera* is known as Muzungwi in Chonyi and Giriama , in swahili it is Mlonge, Mzunze, Mjungu moto, or Mboga chungu and in Tharaka known as Muguunda in (Biovision Foundation, 2020). It is generally distributed in Laikipia, Kilifi, Baringo, and Makueni counties (Ndung'u et al., 2018) while stenopetala also known as the cabbage tree or African *Moringa* (Leone et al., 2015) is found in northern Kenya (Gay and Kai, 2019) Baringo and Turkana counties (Abuye et al., 2003). "In English, *Moringa oleifera* Lam. is called drumstick tree possibly because of its pod shapes and it does not die as it can withstand drought conditions" (Leone et al., 2016). *Moringa stenopetala* Bac. is native to Northern Kenya and Southern Ethiopia (Udofia et al., 2020). Water is reserved in the tuberous roots which enable the plants to withstand droughts.

Since the first international conference about *Moringa oleifera* that took place in Tanzania in 2001, many scientific symposiums and research publications disseminating evidence-based information about the valuable *Moringa oleifera* properties have increased. Today, *Moringa oleifera* has been nicknamed "natural gift", "miracle tree", or "mother's best friend**"** (Leone et al., 2015).

2.9.2 Taxonomy of *Moringa*

The tree belongs to the division of magnoliophyte, magnoliopsida class and the order is violes. It falls under the monogeneric genre, *Moringa* of Moringaceae family, where there are thirteen (13) species of *Moringa* distributed from Asia to Africa (Palial et al., 2011). These species comprise of *Moringa* longituba, *Moringa* drouhardi, *Moringa* arborea, *Moringa* rivae, *Moringa* borziana, *Moringa* ruspoliana, *Moringa* pygmaea, *Moringa stenopetal, Moringa* ovalifolia, and *Moringa* hildebrandi, *Moringa* peregrine originates from the *Moringa* concanensis, and *Moringa oleifera* (Leone et al., 2015).

The most widely cultivated species is the *Moringa oleifera* and *Moringa stenopetala* species, which are cultivated for multipurpose in the tropical countries. The *Moringa* tree extracts can be used in various ways including: herbal medicines, human food, water treatment, livestock feedstuff, Alley cropping, manuring, live fencing, as home washing agent, firewood, timber among other uses (Daba, 2016). *Moringa stenopetala* is also known as 'cabbage tree' because it is consumed just as cabbage and spinach (Kumssa et al., 2017). According to Nadeem and Imran (2016), there is documented evidence-based information on *M. oleifera* and *M. stenopetala* species because of their amazing nutritive and therapeutic properties. Substituting antibiotic growth promoter (AGP) with this two species has been proven to possess valuable consequences on rate of growth performance and meat production of broiler poultry (David et al., 2015).

2.9.3 Botanical Description of *Moringa*

The Moringa oleifera tree is a slender and soft wood in nature, with a height of 1.5 to 2.0 meters before it begins branching but can extend to 3.0 meters; it grows very fast and

branches freely. The stem is usually straight but can be deformed sometimes. It is characterised by thickened, softened, a whitish-gray, fissured and warty or corky bark that is almost rough. When wounded, the bark oozes gum like substance, which is white in color initially then turns to reddish brown or brownish black color after a period of time. The wood is also light and characterized by density ranging from 0.5 to 0.7 g/cm3 (Parrotta, 2005). The twigs grow in a disorganized manner and forms a canopy (Palial et al., 2011).

Figure 2.1

Aerial parts and stem of Moringa tree

Source: (Parrotta, 2005)

The leaves are 24-45 cm long, spirally, and alternately organized, and at the distal end of each branch, the leaves are crowded. Leaves are of two or three petals, the pinnae is opposite to the pinnule; the leaflets are 1.2-2.0 cm long, 0.6-1.0 cm broad. Lateral lobes are elliptical, with obovate ends (Parrotta, 2005).

Figure 2.2

Moringa leaves

Source: *Moringa oleifera* (plantregister, 2017)

The *Moringa oleifera* tree is free flowering plant and normally occurs 4 to 12 months after planting (Palial et al., 2011). The flowers are 0.7- 1 cm long, scented, bisexual, oblique, stalked, united into erect, axillary, many-flowered panicles, densely pubescent and jointed beneath the apex. Additionally, the pods contain maximum of 26 seeds. Usually, they are dark green when developing, consumes almost 3 months to fully mature after flowering. When they mature, they turn brown and open longitudinally at three corners, forming spherical, triangular, dark brown seeds. The seed is 1 cm in

diameter and has 3 light white translucent wings on the corners. Width 0.40.7 cm, the weight of seeds varies from species to species, but ranges from 3,000 to 9,000 seeds per kilogram (Leone et al., 2015).

The difference between *Moringa stenopetala* and *Moringa oleifera,* is that, *Moringa stenopetala* is composed of leaves which are 3.3 to 6.5 cm long and have a sharper apex, bears bigger pods. The pods twist when the fruits are fresh, with seeds that are ellipsoidal rather than globular. The seed are normally cream in colour instead of dark brown in the oleifera tree (Slow food Foundation, 2020).

Figure 2.3

Showing the pods, flowers and seeds of M. oleifera

Source:(Kuli Kuli Foods, 2018)

2.9.4 Cultivation and production of *Moringa*

The *Moringa* tree is drought resistant and grows well in different type of soil except in water-clogged environments. The *Moringa oleifera* and *Moringa stenopetala* species are largely cultivated in most subtropical and tropical geographical regions in the world

having a temperature range between 25–35 °. The tree performs well in loamy or sandy soil having a slightly acidic to slightly alkaline pH of 4.5-9 (Kumssa et al., 2017). It requires an average rainfall range of 250–3000 mm. Soils with a good drainage favours cultivation, the seeds and leaves part of *Moringa* tree are quiet significant (Leone et al., 2015). *Moringa stenopetala* is more drought tolerant as compared to *Moringa oleifera* (Gay & Kai, 2019).

Planting of *Moringa oleifera* can be either through sowing or through cutting, but seeding necessitates good selection of the seeds. When sowed, seeds sprout within a fortnight, with a maximum 2 cm depth. Seedlings are transplanted when they are approximately 30 cm (3–6 weeks) after development (Leoneet al., 2015). During transplanting, one should be careful not to break the tap roots of the seedlings, as they are very tender. Using cuttings 1m long and 4-5cm in diameter is recommended, but *Moringa oleifera* may not have a good deep root system. The *Moringa oleifera* trees are normally affected by drought and winds (Gopalakrishnan et al., 2016).

Depending on the scale of production, the recommended spacing for maximum production of leaves are as follows; Large-scale farming, spacing varies from 10 cm × 10 cm to 20 cm × 20 cm; medium scale production is done within a range of 50 cm × 100 cm. If the trees have to be assimilated into the agroforestry, a spacing of 2-4 meters between rows is required. The yield of *Moringa oleifera* varies in the quantity per kilogram ranges between 3000 to 9000 seeds. In favorable storage conditions, the normal propagation rate is 80%–90%. Nevertheless, the viability reduces if seeds are

stored in slightly high temperature and high relative humidity storage conditions. The sprouting of seeds reduces at a rate of 7.5% after three months (Leone et al., 2015).

The adaptation of *Moringa stenopetala* is wide, varies from the arid to humid climates with altitudes ranging from 390 m to about 2200 meters above sea level. The species can also tolerate light frosts condition, although severe frost may cause ground level death. It also requires annual rainfall ranging from 250-1400 mm. The seedlings normally sprout within a period of 1-2 weeks. For easier harvesting the seedling can be left to grow in the shade until they are 6-15 m/18-45 ft, or lower at about 1-1.5 m/3-4.5 ft for easier harvesting (Gay & Kai, 2019).

When propagating *Moringa stenopetala,* seeds are sown in polythene bags; this is the best and suitable kind of material to plant. The recommended seeds are those that are aged a year and below because it guarantees a 100% germination. When sowing, the seed inserted, one centimetre (1 cm) deep into the soil, which should be an aggregate of loam and sandy soils, enriched with manure or fertilizer. Seeds sprout speedily and optimally in temperatures between 25–30°C. The bags have to be exposed to partial slight and watered daily. Prior to transplantation, the branches and roots are severed, and seedlings left to dry for a week with the roots covered with ashes and upper part covered with dung traditionally. The height of seedlings during transplantation is 20 cm tall or 6 months old, and has to be well watered , approximately 25 litres of water every 3–4 days) if all the trees have to survive (Abuye et al., 2003).

It is estimated that, one *Moringa stenopetala* tree can produce up to 2000 fruits or 6 kilograms of seeds in favourable environments. In the rift valley region of Kenya where

the altitude is 1200 m, medium to high yields are reported. Areas with altitudes of over 1650 m , production is poor (Kumssa et al., 2017).

Farming of *Moringa* has its own challenges. In a study conducted among *Moringa* growers in Kenya and Ethiopia, majority of the farmers interviewed cited infestation of trees by pests and diseases, rotting of the trees in flood prone areas, competition from parasitic plants and uncertainty about the dietary and therapeutic values of *Moringa* among other reasons as challenges in cultivation (Kumssa et al., 2017)

2.9.7 Therapeutic properties of *Moringa oleifera* and *Moringa stenopetala*

Usage of *Moringa oleifera* and *Moringa stenopetala* as a medicinal plant by Africans and Asians dates back to centuries ago, the leaves and immature fruits were used also used as food in human sustenance (Tamilselvi & Arumugam, 2019). Apart from the abundant dietary value, *Moringa* plant has a panacea of medicinal and preventive properties. Many research publications have revealed that hydroalcohol, aqueous, or alcohol extracts of *Moringa oleifera* leaves contain multiple biological properties such as antimicrobial, antioxidant, anti-inflammatory, vital organs protective, pain-relieving, anti-peptic ulcer, antihypertensive, anticancer, anaphylactic**,** and other immune boosting actions (Jacques et al., 2020; Stohs & Hartman, 2015). Additionally, the use of Moringa oleifera species was recommended by World Health Organization [WHO] as a substitute to feed source in the management of malnutrition (Daba, 2016).

Multiple researches conducted have also demonstrated that the leaf extracts from *Moringa* trees have antimicrobial activities over some disease-causing microbes that cause water associated morbidities like Staphylococcus aureus, E. coli, Salmonella

typhi, Shigella species in humans. This capability linked to the benzyl isothiocyanate compound that is an active bactericide and fungicide properties. The leaf extracts of *Moringa stenopetala* exhibit considerable antimicrobial and antifungal action, has shown to treat leaf blight in sunflower plants. Such kind of evidence based information suggests that *Moringa oleifera* and *Moringa stenopetala* possibly possess a potential for formulation of antibiotic preparations (Abrar et al., 2017; Elangovan et al.,2014).

Further, various research reports indicate that *Moringa oleifera* possess multiple properties of preventive action against many microorganisms. The seeds of *Moringa oleifera* for example might be used as antimicrobial because of the presence of isothiocyanate in them. It also contains glucomoringin which an active antimicrobial and fungicidal agent. Additionally, *Moringa oleifera* leaves extracts has been documented to be active against bacterial such as the Staphylococcus aureus strains (Jacques et al., 2020). In a study conducted by Hafsa et al. (2019) *Moringa oleifera* leave additives fed to broiler poultry revealed reduced hindgut count of E. coli, Salmonella and Staphylococcus spp, while the Lactobacillus spp increased. It also concluded that *Moringa oleifera* additives could be utilized in feed to promote growth performance and antioxidant activities. Furthermore, it can also modulate gut microbiome and protect against pathogenic microbe, with no adverse effects on the broiler chicken(Kumar et al., 2018).

In an inflammatory process, antibodies that are specific to tissue and cellular antigens are deposited in tissue thereby causing injury, which may induce phagocytosis and subsequent cell damage or disruption of cell physiology. The cardinal signs of inflammation include pain, oedema, redness and warmth (Abbas et al., 2020). The

effectiveness of *Moringa oleifera* as an anti-inflammatory agent when administered orally with approximate dosages at 200 mg/kg was demonstrated when the results showed reduced swelling in mice. The analgesic test depicted that *Moringa oleifera* extract could be used as analgesia. This was seen when the extract considerably decreased the number of wriggles in mice at 100 and 200 mg/kg, it was an equivalent of 10 mg/kg of indomethacin (Adedapo et al., 2015). Therefore, *Moringa oleifera* has potential to provide natural antioxidants with resultant anti-inflammatory activity (Adedapo et al., 2015; Tamilselvi & Arumugam, 2019)

According to a study done by Waterman et al. (2014), extracts derived from *Moringa oleifera* leaves reduced gene expression and facilitated release of inflammatory markers (iNOS and IL-1b) in macrophages. The research findings recommended that *Moringa oleifera* isothiocyanates could be utilized to relieve low-grade inflammation linked with chronic morbidities (Saini et al., 2016).

As an immune regulator, *Moringa oleifera* extracts have been studied extensively and documented to be very effective (Jacques et al., 2020). Research conducted to assess the influence of *Moringa* on serum biochemical profile showed elevated leucocytes, interestingly, incremental levels of the extract also increased the white blood cells count. *Moringa oleifera* and *Moringa stenopetala* are therefore useful to birds in developing antibodies and strengthening the body's defense system. Phytochemicals such as flavonoids added to feed have antioxidant properties, which boost the immunity in animals. Additionally, phytochemicals modulates detoxification of enzymes, foraging of ROS and regulation of gene expression in cells (Sebola & Mokoboki, 2019**).** Abbas et al. 2020, described leucocytes as the defense cells of the body, and so *Moringa*

contributes significantly in reinforcing the immune system thereby giving the poultry the ability to combat disease. The study concluded that *Moringa oleifera* could be utilized infeed in poultry devoid the possibility of any adverse effect or decreased growth performance (Sebola & Mokoboki, 2019).

The ethanolic extracts from leaves and seeds of *Moringa oleifera* have also been reported to exhibit hepatoprotective action (Adedapo et al., 2015). The Liver function tests (LFTs) also known as hepatic panels are useful biomarkers in liver damage. Johnkennedy et al. (2010) authored that, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alpha GlutamylTransferase (ALP), are most sensitive biochemical markers used in the diagnosis of liver diseases in poultry. This is due to the inappropriate use of antibiotics as growth promoters in the poultry farming (Rehman et al., 2018). Evidence on the hepatoprotective nature of *Moringa* plant was illustrated in a study that compared effect of canola and *Moringa oleifera* on LFTs. The results were similar with that of Adedapo et al. (2015) where, the reducing tendency of numerical values of ALT concentration was observed when the chicken were fed in incremental levels of aqueous extract of *Moringa oleifera* in their feed (Rehman et al., 2018).

Similarly, Sebola and Mokoboki (2019) made the same observation; they reported that, decreased levels of AST and ALKP were detected with added levels of Moringa oleifera. The results showed that *Moringa oleifera* had no adverse effect in the liver parenchyma of the chicken thereof, resulting in enhanced immunomodulation of poultry. Thus, the study suggested utilization of *Moringa oleifera* leaf extract as additives in broilers feed (Rehman et al., 2018; Sebola & Mokoboki, 2019)

The antifungal and antiradical effects of *Moringa stenopetala* was reported in a research done by Kekuda et al. (2016) the output of their research revealed that *Moringa stenopetala* extract was active in preventing the growth of aspergillus species especially. They observed that the activity was concentration dependent and demonstrated the presence of notable antioxidants (DPPH and ABTS). The findings suggested that *Moringa stenopetala* was a potential medicinal plants with antifungal and antiradical properties (Kekudaa et al., 2016) .

Even as *Moringa stenopetala* and *Moringa oleifera* is widely consumed and is thought to be safe on oral administration, information on its safety and efficacy is limited (Okumu et al., 2016). Human clinical trials on the efficacy and safety of *Moringa oleifera* conducted previously documented no side effects or toxicity to date. Additionally, use of *Moringa oleifera* and *Moringa stenopetala* as food and medicinal formulation has never been reported to be toxic by the consumers globally. Research using animal models to evaluate the possibility of toxicity of graded levels of *Moringa oleifera* leave extracts have shown a high levels of safety and efficacy but limited (Stohs & Hartman, 2015). One of the studies evaluating the acute toxicity of orally administered *Moringa* leaf powder in rats showed no alterations in clinical symptoms or overall pathology (Moodley, 2017).

Another research undertaking by Okumu et al. (2016) divulged that, 2000 mg/kg of oral aqueous-methanol *Moringa oleifera* leaf extract administered over two days, yielded no notable toxic signs and symptoms in the rats neither did it cause death. There were no changes in the vital signs, secretion and excretion functions. Additionally, no death or diseases in the rats were reported in the entire period of the experiment. However, liver

examination showed acute signs of mild liver damage; this suggested that *Moringa oleifera* in high doses might possibly be harmful due to the phytochemicals found in the extract.

Not much has been studied on the safety of *Moringa stenopetala* (Bayu et al., 2020). However, the use of *Moringa stenopetala* crude extracts and leave portions could potentially be harmful to animals as suggested by some studies. One of such studies examined the liver and kidney of rats. Although the experiment did not yield any sign of acute toxicity, the biochemistry results showed increased liver function makers, which, notably were dependent on incremental dose of the extract. However, the kidney function tests were normal and findings from this research were concurrent with the ones published by (Bayu et al., 2020). The study concluded that *Moringa stenopetala* extracts are toxic to the liver but more studies needed to be executed to confirm the findings (Geleta et al., 2015). Su and Chen (2020) reported that if in excess, raw *Moringa* when added to chicken feeds, could become harmful due to high bioavailability of protein; thus, it is important to avoid excessive protein intake. According to Udikala et al. (2017), *Moringa* product administered in overdose may be responsible for high amounts of iron accumulation in animals. Studies such as Alabi et al. (2017); Gakuya et al. (2014); Hussein and Jassim (2019) agree that using Moringa leaf powder up to 10% has no side effects in regards to broiler growth performance, but a value higher than 10% will have a negative impact.

Figure 2.4

Moringa oleifera tree (Captured by Ebenezer Udofia)

2.10 *Moringa oleifera* and *stenopetala* effect on growth performance of broilers

The phytobiotic compounds derived from *Moring oleifera* leaf powder has antioxidant properties and bioactive immunomodulatory and antibiotic mechanism (Rehman et al., 2018). When added to broiler feed, various experimental

studies have demonstrated its significant effect in weight of body, feed intake, and ratio of feed conversion ratio relevant for the performance and productivity of chicken. In an experiment by Voemesse et al. (2018) varying levels of *Moringa oleifera* leave meal fed on one day old chicks showed a significant deviation at ($p<0.05$) between the treatment

groups and the control. There was a significant increment in gizzard, weight gain per day, feed conversion ratio, overall body weight and relative weight in the treatment group.

Similarly, effect on feed conversion ratio was seen in chicks feed on $AMOLE_{90}$ and $AMOLE_{120}$ (Aqueous *Moringa oleifera* (Lam) Leaf Extracts). The findings revealed that the chicks had a better performance as compared to the ones subscribed in positive control treatment. Those on $AMOLE_{60}$ and $AMOLE_{150}$ depicted more weight on the large intestine and lung respectively (Alabi et al., 2017). Further, aqueous and ethanolic leaf extracts and leaf meals of oleifera in a study done by (Hussein & Jassim, 2019) depicted significant effects ($p<0.05$) as per the summative weight, increase in weight, feeding, FCR and dressing percentage. The two studies concluded that *Moringa oleifera* (Lam) Leaf Extracts could be used as a NADP in broiler production.

Not so much has been done on MSL but compared to oleifera, findings by Tamiru et al. (2020) on *Moringa stenopetala* leaf meal showed different effect on broilers. According to study, the day- old Cobb 500 broilers that had feed supplemented with the leaf meal of up to 2% demonstrated no major deviations in weight gain, intake of feeds, and FCR. In addition, compared with the control feed, *Moringa oleifera* leaf powder significantly ($p<0.05$) increased the overall weight gained by of poultry. The FCR was also higher and showed no adverse effect on broilers health. The study concluded that MSL was a viable dietary supplement that can be included in the chicken diet as an alternative to up to 6% of expensive protein sources (Melesse et al., 2011). Furthermore, as shown by Adedapo et al. (2015); Ogbe and Affiku (2019) *Moringa oleifera* and *Moringa*

stenopetala with its high potential to improve growth performance of broilers could be used locally to replace AGP which has been demonstrated to be deleterious to the health of the populace.

2.11 Effects of *M. oleifera and stenopetala* on haemato- biochemical parameters

The useful properties of *Moringa oloifera* on haematological and biochemistry parameters by (Modisaojang-Mojanaga et al., 2019; Onunkwo and George, 2015) in broilers was shown earlier in this study but evidence on *Moringa stenopetala* on blood parameters is scanty. The studies have revealed changes in blood parameter with the use of *Moringa* plants in broiler feed (Onunkwo & George, 2015). According to study done by Voemesse et al., 2018, inclusion of *Moringa oleifera* at different graded levels significantly increased ($p<0.05$) regarding albumin amount, total protein, calcium, magnesium and iron levels compared to the controls. Similarly, all blood biochemistry indices were significantly influenced ($p<0.05$) in all *Moringa* treatments vis-a-vis the control (Hussein & Jassim, 2019).

The influence of graded levels of *Moringa oleifera* on broilers was also shown in a study by (Hassan et al., 2016)the findings revealed that with an increase in *M. oleifera* levels, haemoglobin was raised, total protein, Thyroid hormones (T_3 and T_4) and AST increased significantly ($p<0.05$). On the contrary heterophil/Lymphocyte (H/L) ratio reduced and ALT was not affected by adding MOLM levels. To further support the efficacy of oleifera, Abbas et al. (2018) reported that feeding broiler with different levels of *Moringa* significantly raised the red blood cells count and haemoglobin ($P\leq0.05$) but the packed cell volume, leucocytes, lymphocyte, hetrophil and heterophil/lymphocyte ratio

was not affected. In addition, biochemistry parameters revealed remarkable decrement ($p \leq 0.05$) in total protein, albumin, globulin, ALP and uric acid. The study recommended inclusion of 0.75% of *Moringa oleifera* leaf powdered meal as part of broiler diet.

Research reports by Modisaojang-Mojanaga. et al. (2017) on the use of *Moringa stenopetala* supported the fact that the plant could be used to enhance productivity in broilers. The study revealed that treatment groups that had been supplemented with *stenopetala* decreased the total cholesterol than the control. Similarly, in another study, chicken fed with *stenopetala* leaf meal showed total protein increased remarkably at ($p>0.05$) while ALT and of urea were low ($p> 0.05$) in those fed with *stenopetala* diet (Melesse et al., 2013). Both studies concluded that *Moringa stenopetala* could be used in the production of broilers effectively.

More reports like that of Tijani et al. (2015) have depicted varied implications from oleifera on broiler blood composition. The findings revealed significantly increased($p<0.05$) white blood cells altogether with packed cell volume with increased levels but reduced in broilers fed on higher (20%) oleifera level. Significant reductions in albumin, total protein, uric acid, aspartate aminotransferase and alanine aminotransferase levels were observed in birds fed a 20% diet ($p< 0.05$). However, creatinine was greater in birds feeding on 20% oleifera diet ($p<0.05$). This corroborates the use of *Moringa oleifera* to improve performance and health of chicken. As much as studies depict *Moringa* to be safe than AGPs, a study done in Mexico using oleifera on ross-360 broilers revealed possible damage to the liver and kidneys due to the differences in albumin, alkaline phosphatase and glutamyl transpeptidase levels. The

findings were not confirmed through histopathology, however different levels of *oleifera* need to be studied to ensure accurate supplementation in poultry diets (Fuentes et al., 2019). No studies have reported on the toxicology of *Moringa stenopetala* necessitating more research to establish safety in broilers.

2.12 Effects of *M. oleifera* and *stenopetala* on the gut microbiota

The use of *Moringa* plant to modulate gut microbiota has been demonstrated in many studies. Gut microflora prevents inhabitation of the poultry intestinal lumen by harmful bacteria and other non-commensal microorganisms by outcompeting them and at the same time producing substances that are bactericidal (Clavijo & Flórez, 2018). One of the studies on Lactobacillus revealed that it was effectual for preventing proliferation of Salmonella, Shigella, Clostridium, and Listeria bacteria. The commensal microflora acts by attaching itself to the brush border of epithelial cells thereby blocking pathogenic bacteria from attaching itself to the cellular walls and gaining entry. The gut normal flora inhibits the proliferation of pathogens by also producing organic acids and bacteriocins that enable stimulation of the immune system and competition in absorption of nutrients and attachment on the cells (Yadav & Jha, 2019).

As reported by Adamu and Boonkaewwan (2014) inclusion of *M. Stenopetala* in broiler diet was seen to have protective result against E. tenella infection. Further they suggested that even though *M. stenopetala* had protective influence it did not counter mortality rates in broilers. Similarly, use of 15% *M. oleifera* exhibited considerable improvement in the mortality and antibody against new castle disease. This shows that *Moringa* could be used for immune modulation as well (Hassan et al., 2018).

From literature, the efficacy of in feed Moringa oleifera leaves on ileal microbials of broiler chickens was also supported by (Abu Hafsa et al., 2020). The study revealed that with administration of oral oleifera the total intestinal count of Staphylococcus spp, E. coli, and Salmonella reduced while count of Lactobacillus spp. was increased in broilers. This evidence presented is also supported by Moreno-Mendoza et al. (2021) in their experiment they singled out Moringa leaf powder to be capable of enhancing gut health. The studies deduced that oleifera supplement enhanced growth performance and ant- oxidation, changed the intestinal microbials, and was protective against harmful gut microbiota with no adverse effect on the broilers. More studies need to be done on *M. stenopetala* to establish its effect on gut microbiota

2.13 Phytochemicals and nutrient in *M. oleifera* and *stenopetala* extracts

The *Moringa* tree is laden with fiber, minerals and proteins elements, which is vital when included in feed for livestock production. The leaves of *Moringa oleifera* has been reported in many research studies to contain high protein content as compared to other leafy vegetables consumed by humans (Daba, 2016). *Moringa oleifera* for instance, is known to be rich in nutritive properties because of the different phytochemicals found in the leaves, pods and seeds (Tshabalala et al., 2019).Plants have many compounds such as phytochemicals (phyto, in Greek meaning plants) that have the capability of treating many diseases (Ndung'u et al., 2018). Phytochemicals are naturally occurring organically active compounds found in plants that provide protective or preventive properties and nutrients (Altemimi et al., 2017). Research established that plants produce phytochemicals for defence; moreover, these compounds protect humans

against morbidities too. Recently, studies undertaken have also demonstrated that phytochemicals can be used to improve growth performance in poultry production (Abrar et al., 2017).

2.13.1 Nutrient properties of *Moringa oleifera* and *Moringa stenopetala*

The whole plant of *Moringa oleifera* is a hive of vital nutrients. Its leaves contain many minerals like magnesium, potassium, zinc, iron, calcium and copper (Wang et al., 2018). It also contains pyridoxine, beta-carotene, folinic acid, nicotinic acids, vitamins E, D and C. A significant amount of terpenoids, tannins, sterols, flavonoids, anthraquinones, saponins, alkaloids (Mahfuz & Piao, 2019a; Shousha et al., 2019). Phytochemicals are also present in the *Moringa oleifera* tree. In humans, use of *Moringa oleifera* may help in reducing glucose levels and also fight cancer due to the presence of glucosinolates, isothiocyanates, compounds of glycoside and glycerol-1-9-octadecanoate respectively (Gopalakrishnan et al., 2016).

The seeds contain oleic acid, an antibiotic called pterygospermin, as well as fatty acids such as linoleic acid, linolenic acid, and behenic acid, tannins, saponins, phenolic compounds, phytates, flavonoids. Phytochemicals such as terpenoids and lectins are used to make seeds. It also contains fat, fiber, protein, minerals, vitamins, such as A, B, C and amino acids (Zaku et al., 2015). The roots and bark contain alkaloids such as moriginine, morphine, minerals such as calcium, sodium, and magnesium. The flowers contain calcium and potassium, amino acids and nectar, and the pods are also laden with fibrous materials, carbohydrates which are unstructured, ash, and proteins. It also contains linoleic acid, oleic acid, linolenic acid palmitic acid (Leoneet al., 2015).

Table 2.2

Composition of dried leaf powder of Moringa oleifera per 100 g of edible portion of leaves

(Gandji et al., 2018; Zaku et al., 2015)

Nutritional value per 100 g	Values
Moisture (%)	7.5
Calories (Kcal)	205
Proteins. (g)	27.1
Fats. (g)	2.3
Carbohydrates. (g)	38.2
Fibers. (g)	19.2
Calcium (mg)	2,003
Magnesium (mg)	368
Phosphorus (mg)	204
Potassium (mg)	1,324
Iron (mg)	28.2
Sodium (mg)	870
Vitamins A-Beta Carotene (mg)	16.3
Vitamin B1 - thiamin (mg)	2.64
Vitamin B2 - riboflavin (mg)	20.5
Vitamin B3 - nicotinic acid (mg)	8.2
Vitamin C ascorbic acid (mg)	17.3
Vitamin E tocopherol acetate mg)	113
Amino Acids (g)	27.1
Oxalic acid (mg)	1.6%

According to report by Shousha et al. (2019), “The calcium in *Moringa oleifera* leaves is equivalent to 4 cups of milk, the iron content is 3 times that of spinach, the vitamin A in carrots is 4 times less, and the protein content in milk is 2 times."

In addition, *Moringa oleifera* leaves are said to contain several classes of antioxidant compounds, including ascorbic acid, carotenoids, phenolic compounds and flavonoids (Shousha et al., 2019; Fej et al., 2019). In addition, *Moringa* has low toxicity in animal nutrition which has increased interest in its utilization as a protein source for livestock (Abd El-Hack et al., 2018).

The *Moringa stenopetala* species are naturalized in the tropics and are currently seen as promising therapeutic plants besides *Moringa oleifera* which are also termed as ‘ a gift of nature’ (Hagos et al., 2018). Many research studies have reported *Moringa oleifera* to possess rich properties that are basic pillars of matter (Daba, 2016). Even though the entire tree parts have beneficial effects, the leaves especially, are reported to contain valuable source of protein and energy that can be used by livestock and humans. Moreover, the leaves may also be fed to monogastrics animals like poultry but in small quantities due to the high amount of phytate and tannin (Mikore & Mulugeta, 2017). Mikore and Mulungeta (2017) in their research report established that, mineral elements found in the leaves of *Moringa stenopetala* Bac are high in nutritive properties that may be utilized as feed additives. In Ethiopia, green leaves, flowers and fruits are often eaten as vegetables because they are enriched with protein and calcium, and phosphorus (Hagos et al., 2018).

In one of the studies that was doneto evaluate extracts of the leaves of *Moringa stenopetala*, the report concluded that, as compared to kale and Swiss, the raw leaves of *Moringa stenopetala* had an elevated crude protein content on a dry matter basis (9%) and a bigger percentage of carbohydrates, crude fiber, and calcium. Additionally, the vital vitamins C is approximately 28 mg/100 g and 160 µg/100 g of beta-carotene (Abuye et al., 2003).The leaves also contain an essentially good amount of crude lipid, moisture, ash, crude fiber, energy, and crude which have nutritive properties. Thus, *Moringa stenopetala* leaves could be used as feed additive for humans as well as livestock (Hagos et al., 2018; Mikore & Mulugeta, 2017).

The ash value found in *Moringa stenopetala* implied that the leaves are also rich in inorganic minerals (Fikremariam et al., 2019). Proximate composition such as potassium, iron, Zinc, phosphorus, and Calcium are present in significant levels with values ranging between 3.08 mg/100 g for iron and 792.8 mg/100 g for calcium. The raw leaves comprise of 9% crude protein on a dry matter basis (Abuye et al., 2003).

The age of *Moringa stenopetala* tree is documented to affect the nutritive, anti-nutritive and mineral leaves content. One of the studies undertaken in Ethiopia that evaluated the nutrient content of leaves in trees of different ages, results showed that 4 and 5- year- old trees had higher nutrient composition in comparison to those of three, six and seven years. Further, tannin and phytate were found to be least in trees of five years compared to those of three, four, and six and seven years. The proximate composition was in sufficient amount in trees of three and five years, respectively. In summary, studies have shown that five-year-old trees have better anti-nutrition, nutrition, and proximal components than trees of other ages (Fikremariam et al., 2019**).** Similarly**,** soil and

climatic conditions, could also contribute significantly in disparity of proximate composition and mineral composition of *Moringa stenopetala* (Hagos et al., 2018)

Table 2.3

Proximate composition, mineral and vitamin contents of raw leaves of Moringa stenopetala

Variables	**(mg/100g DM)**
Na	403.5 ± 21
K	453.0 ± 11
P	65.6 ± 13
Ca	792.8 ± 92
Fe	3.08 ± 0.8
Zn	0.53 ± 0.8
Mg	4.6 ± 1.1
Mn (mg/kg DM) -	86.1 ± 6.96
Vitamin C (mg/100 g)	28.07 ± 7.3
b-carotene	(mg/100 g) 160
a-carotene	(mg/100 g) 54
Retinol equivalent (RE)	34.04
Carbohydrate	51.8 ± 2.6
Protein	9.0 ± 0.7
Fat	5.8 ± 1.4
Crude fiber	20.8 ± 3.3
Ash	12.6 ± 1.1
Energy (Kcal)	295.4

(Abuye et al., 2003)

2.13.2 Phytochemicals in *Moringa oleifera* and *Moringa stenopetala*

Phytochemicals exhibit anti-inflammatory effect that makes it fundamentally important in other biological activities and therapeutic uses (Leone et al., 2015). The antioxidant phytochemicals contained in medicinal plants, contribute significantly in the control and management of chronic morbidities resulting from high levels of oxidative stress. Research undertaking to investigate the antioxidant implications of *Moringa oleifera* leaves methanol extract reported that the in vitro antioxidant effect of crude methanol extract were markedly revealed. Just as reported in other research studies, n-butanol (89.9%) and ethyl acetate (88.9%) fractions, exhibited the strongest in vitro free radicle scavenging action against 2,2,1-diphenyl-1-picrylhydrazyl [DPPH] (Atta et al., 2018).

Different sections of *Moringa* tree have been determined to contain uniquely exceptional phytochemicals (Saini et al., 2016). It contains a variety of phytochemicals including glucosinolates, phenolic acids, steroids, saponins, flavonoids, alkaloids, tannins, and terpenes (Leone et al., 2015a; Rani et al., 2018; Saini et al., 2016). Compared with other parts of the *Moringa* tree, the leaves contain the highest levels of flavonoids, which can be used as functional and nutritional ingredients (Leone et al., 2015b; Nouman et al., 2016). The antioxidant properties of Moringa are because of the abundant of flavonoids present. Rutin**,** kaempferol, quercetin, rhamnetin, myricetin, and apigenin are the available types of flavonoid, and are commonly found in flavanol and glycoside form (Rani et al., 2018). Flavanoids in *Moringa oleifera* and *Moringa stenopetala* have anti-inflammatory, immunomodulation, and gut defense effect (Kamboh et al., 2018).

The glucosinolates, 4-O-(α-L-rhamnopyranosyloxy)-benzyl glucosinolates is also referred to as glucomoringi. The agent is mainly found in the seeds and leaves of

Moringa oleifera (Koche et al., 2016; Rani et al., 2018). Phenolic acid found in the leaves contains gallic acid in high quantities. Others include; ferulic acid, chlorogenic acid, caffeic acid, o-coumaric acid and ellagic acid. Additionally, it contains marumoside A and marumoside B, together with pyrrolemarumine-4″-O-α-L-rhamnopyranoside type of alkaloids (Kumar & Goel, 2019).

The seeds generally contain alkaloids, resins, tannins, flavonoids, glycosides among others. However, regardless of the extraction method used, several studies reported that the seeds contained methionine, cysteine, 4(α-L-rhamnanopyranosyloxy)-benzyl glucosamine, and Moringa Glycosides, niazimicin niazirin, and benzyl gluconate. The 4-(alpha-L- rhamnopyranosyloxy) benzylglucosinolate agent has antimicrobial effect and has been described to cause gram-negative and gram positive bacteria in vitro bactericidal action in untreated water used (Idris et al., 2016; Rani et al., 2018).

The pods contain isothiocyanate, thiocarbamate, nitrite, O(1-heptenoxy) undecanoate propyl, O-ethyl-4(α-L- rhamnosyloxy) benzyl carbamate Esters, methyl hydroxybenzoate and β-sitosterone. The bark contains 4-(alpha-L- rhamnopyranosyloxy) and benzylgiucosinolate. Analysis of the flowers revealed that it contains D-glucose, kaemopherol, kaempferitin, quercetin, isoquercetin, D-mannose and protein, ascorbic acid. The roots contain trace levels of moringine, spirachin, moringinine, 1, 3-dibenzyl urea, Deoxy-niazimicine, alpha- phellandrene, p-cymene, 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolat. Lastly, the stem contains 4-hydroxyl mellein, vanillin, octacosonoic acid, beta- sitosterone and beta- sitosterol. The tannin levels in *Moringa oleifera* is however lower than that in *Moringa stenopetala* and *Moringa*

oleifera does not contain α-carotene, of which, is normally found in most green leafy plants (Abrar et al., 2017; Hagos et al., 2018; Paikra et al., 2017; Rani et al., 2018). The leaves of *Moringa oleifera* is the most studied and utilized parts of the plant (Rani et al., 2018). Even though the leaves are reported to hoard high phenolics and flavonoids, the content is reliant on several elements such as geographic location where the *Moringa* tree is propagated, variety of water, fertilizers and soil used, the type of procedure used to extract and storage (Valdez-Solana et al., 2015). Several studies have documented that the leaves have a plethora of phytochemicals used for various medicinal and therapeutic purposes (Zaku et al., 2015).

In a study done in Chad, Haiti and Sahrawi, to determine the phenolic profile of the tree *Moringa oleifera* found existence of substantial amounts of phenolic compounds in leaves. Ferulic acid and Salicylic acid were also discovered in different level ranging between 0.14 mg/100g to 0.33 mg/100g and between 6.61mg/100g to 9.69 mg/100 g, respectively (Leone et al., 2015ab). In India, Puducherry region, evaluation of *Moringa oleifera* leave extracts revealed presents of alkaloids, triterpenoids, flavonoids, tannins, saponins, glycosides and carbohydrates in the leaves extract (Shanmugavel et al., 2018). In Nigeria, the phytochemical components of *Moringa* are found to be rich in tannins (21.19%), followed by phytate (2.57%), inhibitor of trypsin (3.0%), saponins (1.60%), and oxalate (0.45%) and cyanide grade is the lowest (0.1%).The study suggested use of *Moringa oleifera* as feed additives to enhance growth and health status of chicken (Ogbe & Affiku, 2019). Similarly, Adedapo et al. (2015) profiled flavonoids, terpenoids, glycosides, tannins and saponins in phytochemical analysis study on, *Moringa oleifera*

leave extracts. They concluded that *Moringa oleifera* could be used for therapeutic and dietary purposes.

As compared to *Moringa oleifera, Moringa stenopetala* has not been researched on extensively in Kenya, yet the potential use of its extract is evident. Available information is on the nutritive and medicinal value, but minimal on phytochemical composition of *Moringa stenopetala.* Some research conducted in Ethiopia reported the presence of some phytochemical compounds such as tannins, saponins, glucosinolates, alkaloids etc. The phytochemical analysis report of *Moringa oleifera* crude leaf extract recorded the presence of saponins, alkaloids, polyphenols, coumarins, tannins, flavonoids, cardiac glycosides terpenes, phytosterols, and saponins (Meresa et al., 2017).

According to previous research report of an evaluation done on leaf extracts from *Moringa stenopetala* harvested in South Ethiopia, the results showed that it contained, o-(rhamnopyranosyloxy) benzyl glucosinolates (5.70±0.77 mg/g DW. Glucosinolates compounds were detected in large quantities. Interestingly, glucoconringiin found in the seeds was not identified in the leaves extract, on the contrary, this compound is found in the leaves of *Moringa oleifera* (Mekonnen & Dräger, 2003). Even though *Moringa stenopetala* products are used in Kenya and Ethiopia, consumption of *Moringa stenopetala* leaf powder in the European Union as traditional food raised safety objections and is prohibited as a commercial commodity (EFSA, 2019). As a way of getting maximal benefits from *Moringa stenopetala*, studies have suggested in-depth studies on phytochemicals compounds factors of fresh *Moringa stenopetala* leaves (Gore, 2018; Mikore & Mulugeta, 2017; Seifu, 2014).

The ban imposed on AGP in poultry farming calls for new and effective strategies to manage avian enteric infections. Dietary phytonutrients have been shown to have a positive impact on gut health by reducing the negative effects of necrotizing enterocolitis (NE). *Moringa oleifera* and *Moringa stenopetala* has been recommended as a potential application to modulate gut microbiota, it is a promising medicinal plant alternative in promotion of poultry performance and productivity (Lillehoj et al., 2018).

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

This chapter elaborates details regarding the site of the study, designs and experimental procedures for data collection and analysis. Additionally, the ethical considerations in this study are also highlighted.

3.2 Site description

The present study was conducted in Thika, an industrial town in Kiambu County, Kenya. It is situated about 42 kilometres Northeast of Nairobi, off the A2 road near the confluence of the Thika and Chania Rivers at in Latitude: 1.03876 South and Longitude: 37.08338 East. It is a home to 284,776 persons according to the 2019 Kenya Population and Housing Census statistics (Republic of Kenya, 2019) and the population is rapidly growing. The elevation of Thika is approximately 1,631 metres from sea level. The average annual precipitation in the area is 840 mm, and the average annual temperature is 19.8 °C.

3.3 Experimental chicken and management

In this study, 300 Cobb-500-day-old chicks of mixed sex bred at Kenchic Ltd, Nairobi, Kenya, were used (Appendix 2). They were housed in an open sided poultry pen that was partitioned into sixty units, each measuring 0.5 m^2, at 35°C (brooding temperature). The chicks were offered standard commercial broiler starter pellets for seven days as they acclimatised to the experimental setup environment prior to introduction of the experimental diets. After an acclimatization duration (seven days) (Appendix 3), the

chicks were individually weighed and randomly divided into twenty (20) treatments, 5 chicks in every group using a completely randomized block design (CRBD). Before introducing the chicks into their respective treatment units, the pens were thoroughly washed, disinfected, and equipped with feeder trays and drinkers. The pen units were furnished with soft wood shavings as bedding material to provide warmth, and regularly replaced with clean material. The room temperature was gradually reduced by 2°C per week to 23°C with the help of infra-red bulbs, with continuous lighting (electric bulbs and sun light). Afterwards, the chicks were weighed weekly to monitor their weight gains throughout the experimental period. Vaccines were administered appropriately, based on the vaccination schedule in the area. The chicks were monitored throughout the experimental period.

3.4 Plant materials and extracts

The plant material that was used in the study were leaves of *Moringa oleifera* and *Moringa stenopetala*

3.4.1 Collection of plant materials and preparation of powders

The plant samples used throughout this study were taken from naturally growing *M. oleifera* and *M. stenopetala* in Isiolo and Kilifi counties respectively. Their collection in the same area was to avoid any soil micronutrient content variations.

The healthy and mature *M. oleifera* and *M. stenopetala* trees were identified by a reputable botanist and authenticated by a taxonomist based at East African herbaria at the National museums of Kenya, where voucher specimens (MOEU2019 for *M. oleifera* and MSEU2019 for *M. stenopetala)* handed in. After that, fresh and green leaves were

harvested using a pair of secateurs into clean woven bags and transported to the Pharmacognosy Laboratory at Mount Kenya University, main Campus-Thika. The leaves (Appendix 1) were separated from the twigs and spread on clean laboratory benches to dry for two weeks under ambient room temperature and aeration. The dried leaves of the two plant species were ground into uniform coarse powders using an electric plant mill. The powders were stored in clean, dry plastic containers that were sealed and kept on a laboratory shelf awaiting use.

3.4.2 Preparation of the study extracts

To obtain the ethanolic leaf extracts of *M. oliefera* and *M. stenopetala*, the cold maceration procedure described by Bibi et al. (2012) was followed with slight modifications. Briefly, 600 g of each powdered plant powders were accurately weighed separately using an electric analytical balance and placed into two different labelled 2-litre conical flasks into which 1.2L of analytical grade ethanol were added. The contents were stirred, and flasks shaken to facilitate the merc-menstruum interaction. The setups were covered with an aluminum foil paper left and occasionally agitated using a mechanical shaker, for 48 hours. Thereafter, the merc-menstruum mixtures were separated by filtration through Whatman No.1 filter papers into separate, well labelled clean flasks. The merc materials were extracted two more times to exhaust extraction. And the cumulative filtrates were concentrated using a rotary evaporator under vacuum with the hot water bath set at 40 °C The concentrated ethanolic extracts were then transferred into clean, pre-weighed glass sample bottles and completely dried in the hot

air oven at 35 °C for 96 hours. The completely dried samples were capped and sealed with the Para film and stored in 2-8°C refrigerator waiting analysis.

Besides, the aqueous extracts of *M. oleifera* and *M. stenopetala* were prepared by hot maceration in accordance with the method prescribed by Bibi et al. (2012) though with minor amendments. Momentarily, a 50g load of course powdered plant materials of each plant were separately weighed out using analytical balance and placed into a 500 ml conical flask. Then it was mixed with 250 ml of distilled water and shaken. The plant materials were then boiled in a hot water bath for 30 minutes. The contents of the flask were allowed to cool until they attained room temperature followed by filtration through No. 1 Whatman filter paper. The resultant filtrate was crystallized through freeze drying and stored at a refrigeration temperature of (2-8 °C), awaiting experimentation.

3.5 Dosage and extract administrations

According to the guidelines of the Organization for Economic Corporation and Development (OECD), the dosage of herbal extract (mg) should be an appropriate volume not exceeding 10 ml/kg (1 ml/100 g) of body weight of experimental animals when administered orally. However, in an aqueous solvent, it can be considered as 20 ml/kg (2 ml/100 g) body weight (OECD, 2000). Large doses (40 ml/kg of body weight) can cause unnecessary discomfort in the animal by overloading the stomach, increasing gut motility, or causing passive gastric reflux and irritation of the oesophagus and stomach (Bonnichsen, Dragsted & Hansen, 2005).

Based on a chosen volume of 10 ml/kg, the dosage volume required for 100 grams of chicken can be calculated as follows.

$$Administrationvolume = \frac{Bodyweightoftheanimal}{1000\ g} \times\ 10\ ml$$

This study used the extract concentrations of 1mg/kg, 5 mg/kg, and then 25 mg/kg as lower, medium and higher dosages, respectively. Appendix 5 shows a photograph of the extract administration procedure.

Dosage calculation was done according to the OECD's guideline:

$$Administrationvolume = \frac{Bodyweightoftheanimal}{1000\ g} \times\ intendeddose\ (mg)$$

3.6 Feed preparation

Ground maize, fishmeal, soya bean, limestone, salt, wheat bran, broiler premixes, methionine and lysine were obtained from a local agro vet store. Proximate analysis of M. oleifera Lam leaf powder (MOP) and *M. stenopetala* Bac. (MSP) was conducted at the Pharmacognosy Laboratory of Mount Kenya University, at Unga Feeds and Kenchick Ltd laboratories, before the feed formulation (Appendix 16). Based on the proximate composition of the two powders, the feeds were formulated as per the guideline of the NRC (1994) and piloted on 20 chicks to ascertain acceptance of the feed. The composition of the diet was as tabulated in table labelled 3.1.

Table 3.1

Composition of Broiler experimental diet (%)

	Starter					Finisher				
Ingredients (%)	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Maize	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Soya beans cake	23.00	23.00	23.00	23.00	23.00	18.00	18.00	18.00	18.00	18.00
Wheat offal	7.20	7.20	7.20	7.20	7.20	12.20	12.20	12.20	12.20	12.20
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lime stone	1.50	1.50	1.50	1.50	1.50	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.50	2.50	2.50	2.50	2.50
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Tetracycline®	0.25	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
MSP/MOP	-	0.25	0.45	0.65	0.00	-	0.25	0.45	0.65	0.00
Total	100	100	100.2	100.4	99.75	100	100	100	100	99.75

Note: MSP is *Moringa stenopetala* powder; MOP is *Moringa oleifera* powder; Tetracycline® is the antibiotic supplement.

3.7 Experimental design

The applied experimental design throughout this study is a completely randomized block design. Three hundred Cobb 500 broiler chicks from Kenchic Ltd, Kenya were randomly distributed into twenty treatments and assigned to three replicates of 5 chickens each per unit. The experimental feeds contained 250 grams, 450 grams and 650 grams of M. oleifera Lam leaf powder mixed in 100 kg of feed for treatment 1 (T1), 2 (T2) and 3 (T3) respectively. Furthermore, the experimental feeds for treatments 4 (T4), 5 (T5) and 6 (T6) contained 250 g, 450 g, and 650 g, respectively, of *M. stenopetala Bac*. leaf powder incorporated into 100 kg of feed. The chicks in treatments T 7, T 8 and T9, respectively, received ethanolic extracts of *M. oleifera* ethanol extracts at doses of 1

mg/Kg; bw 5 mg/Kg bw and 25 mg/Kg bw, respectively, while those in treatments T 10, T11 and T 12, received ethanolic extracts of *M. stenopetala* Bac. at doses of 1 mg/Kg bw; 5 mg/Kg bw and 25 mg/Kg bw, respectively. Besides, chicks in treatments T 13, T14 and T15, and those in treatments T16, T17 and T 18, received aqueous extracts *M. oleifera* and *M. stenopetala* Bac., respectively, at doses of 1 mg/Kg bw; 5 mg/Kg bw and 25 mg/Kg bw, respectively. Chicks in treatment 19 (T 19) were offered a basal poultry diet with antibiotic supplements 250g in 100kg (Tetracycline®) as positive control, while those in treatment 20 (T20) received a basal diet with neither antibiotic nor *Moringa* supplements as negative control.

Table 3.2

Experimental layout

Replicate I	T19 T 20	T16 T18 T17	T1 T3 T2	T8 T7 T9	T4 T6 T5	T10 T12 T11	T13 T15 T14
Replicate II	T10 T12 T11	T8 T7 T9	T4 T6 T5	T16 T18 T17	T1 T3 T2	T14 T15 T13	T19 T 20
Replicate III	T7 T8 T9	T6 T5 T4	T19 T 20	T3 T2 T1	T14 T13 T15	T10 T12 T11	T17 T16 T18

There were 5 birds/ cage in six categories beside the positive control (15 birds), negative control (15 birds) and each of the categories had 3 levels i.e. Total Birds: 6 x 3 x 3 x 5 =270 +15 + 15 =300.

The twenty treatments for this experiment were as follows:

i. MOP 250 g T1

MOP 450 g T2

MOP 650 g T3

ii. MSP 250 g T4

MSP 450 g T5

MSP 650 g T6

iii. MOEW 1 mg T7

MOEW 5 mg T8

MOEW 25 mg T9

iv. MSEW 1 mg T10

MSEW 5 mg T11

MSEW 25 mg T12

v. MOEE 1 mg T13

MOEE 5 mg T14

MOEE 25 mg T15

vi. MSEE 1 mg T16

MSEE 5 mg T17

MSEE 25 mg T18

vii. Positive control - Diet with antibiotic supplements (Tetracycline®) T19

viii. Negative control – Diet with neither antibiotic nor Moringa supplements T 20

Note: MOP = *M. oleifera* powder; MSP = *M. stenopetala* powder
MOEW = *M. oleifera* water extract; MSEW = *M. stenopetala* water extract
MOEE = *M. oleifera* ethanol extract; MSEE = *M. stenopetala* ethanol extract

3.8 Data collection procedure

Data for this study were collected and determined throughout the entire period of the experiment. The data collected and determined included: Bird performance characteristics; haematological and biochemical parameters; gut microbiota; and the phytochemical compositions of *M. oleifera* and *M. stenopetala*.

3.8.1 Bird performance characteristics

Growth performance was one of the parameters in broiler chicken that was determined and this was done by collecting data on feed intake; body weight gain and the feed conversion ratio.

(i) Feed intake (Grams)

The weight of the amount of feed consumed including the remnants was recorded daily so as to determine daily feed intake by subtracting the initial amount fed from the leftovers. Weekly averages were then computed by dividing the feed consumed by the number of chickens comprising each treatment.

(ii) Body weight gain (G)

At the beginning of the study, initial weights of the birds were taken. Thereafter weekly weights were recorded and then subtracted from the previous week's values to determine a weekly gain of weight. These records were gotten using a digital weighing scale and they were used to compute average weight gain of the experimental birds.

(iii) Feed conversion ratio (FCR)

Feed conversion ratio was computed by taking the ratio of feed taken against the body weight gain of birds per treatment as shown in the following formula.

$$FCR = \frac{averagefeedintake\ (g)}{averagebodyweightgain\ (g)}$$

3.9 Determination of haematological and biochemical parameters

On the last feeding trial day of the experiment in the fifth week, the fresh blood was harvested from two randomly selected chicken per replicate. The broiler chicken were restrained by gently holding their wings from the back. The branchial vein was located under the wing (Appendix 10), sterilized with an alcohol swap and then 2 ml of whole blood drawn and accurately transferred into vacutainers with an anti-coagulant (purple capped). The samples of blood were transported to the Biochemistry Laboratory located at Mount Kenya University, where hematologic parameters were determined using a hematology analyzer.

The following day after the last day of the feeding trial, two healthy broilers from each replicate, summing up to six broilers per treatment were selected randomly. Each of the broiler was restrained and about 3ml of the blood aseptically drawn from the branchial vein under the wing and then transferred into the plain vacutainers (red capped). The total blood samples were then transported to the Mount Kenya biochemistry laboratory and sera for all the samples prepared following the standard serum preparation procedures. The prepared sera were accurately transferred into the serum vials and used

to determine the biochemical parameters (urea, glucose, cholesterol, calcium, creatinine, triglycerides, total protein and albumin). These biochemical parameters were determined spectrophotometrically following the protocols provided in each of the commercially available kit (Appendix 6).

3.10 Effects of *M. stenopetala* and *M. oleifera* leaf extract gut microbiota

On the last feeding trial day of the experiment in the fifth week, two broiler chicken from each replicate, summing up to six broilers per treatment, were selected randomly and transported to Kenchic Laboratory in Nairobi. The chicken were slaughtered and content in the cecum and rectum were collected for determination of Coliforms' total viable count.

3.10.1 Media preparation

The violet-red bile agar (Appendix 12), used in this study for the determination of the Coliforms' total viable count, was prepared according to the instructions from the manufacturer. This involved suspending 41.3 g of the violet-red bile agar powder into 1000 ml of the distilled water. Then the flask content was boiled to completely dissolve and then sterilized.

3.10.2 Buffered peptone water preparation

Buffered peptone water was prepared according to the manufacturer's instructions. This involved suspending accurately weighed (20 grams) of the buffered peptone powder in 1000 ml of distilled water and heating on a hot plate to completely dissolve. The well dissolved buffered peptone water was redistributed into clean universal bottles, each

carrying 9 mls, and then sterilized by autoclaving at 15 bars of pressure, 121 ^{0} C temperature, for a duration of 15 minutes.

3.10.3 Sample preparation

The broiler chicks' gut content (from cecum and rectum) samples were received into the Kenchic laboratory and accurately booked in the laboratory register. The laboratory benches were then sterilized using 70 % ethanol solution and the sterility of the working area maintained using an ethanol lamp to avoid contaminations. The samples were prepared by dissolving in 9 ml of the sterile peptone water. Each sample (1 g) was aseptically weighed and transferred into 9 ml of sterile peptone water using a sterilized spatula, in a universal bottle, capped and then shaken to mix (Appendix 15). Each of the samples was then serially diluted to obtain 9 dilutions. This was done by pipetting out 1 ml of the dissolved sample and then toping it up to the ninth mark with sterile peptone water. This dilution procedure was repeated until the last dilution was obtained. The sample dilutions were then accurately labelled and kept in aseptic conditions until the plating time.

3.10.4 Determination of the total coliform count in the gut content

The total coliform count was determined as per the ISO 4832procedures. The pour plate method was used in plating the samples in the media, and it involved mixing of the samples with the agar. The petri dishes were labelled as per the sample and the dilutions. Dilutions 5,6,7,8 and 9 were used to determine the coliform count.

The samples were poured on to the respective labelled petri dishes and then about 20 ml of the Violet red bile agar added. The media was swirled to ensure thorough mixing of

the sample with the media and then left to solidify. The well solidified plates were placed in an incubator at 37°C for 24 hours, then results were read and recorded. All the experiments were conducted in triplicates. The sterility of the media was checked by incubating the uninoculated media. The agar was inoculated with E. coli and S. aureus to check the ability of the media to grow micro-organisms and to differentially grow coliform bacteria.

3.11 Evaluation of phytochemical compositions of *M. oleifera* and *M. stenopetala*

Moringa tree has many uses with nutritional and medicinal properties benefiting both humans and animals. These properties varies as a result of changes in agro-climatic conditions, it is therefore important to carry out phytochemical screening to determine their phytochemical profiles.

3.11.1. Qualitative phytochemical screening of *M. oleifera* and *stenopetala*

Powdered from leaf powders of *M. stenopetala* and *M. oleifera* were qualitatively screened for the presence of tannins, alkaloids, glycosides, steroids, flavonoids, phenols and terpenoids. Standard procedures for the qualitative determination of the phytochemicals as described by (Savithramma, 2011; Khandelwal & Vrunda, 2015). Detail description of these methods are presented in Appendix 7.

3.11.2 Quantitative phytochemical screening of *M. stenopetala* and *oleifera*

The quantitative determination of flavonoids, alkaloids, saponins and phenols were done following standard procedures (Appendix 8).

3.12 Proximate analysis

Proximate analysis was performed on powders to determine the composition of nutrients, to assess if the powder contains the required and recommended amounts. Proximate analysis helps assess the combustibility of materials (in this case *Moringa*), which helps determine their digestibility and nutritive benefits

3.12.1 Determination of moisture content

Two grams of *M. oleifera* leaf powder was measured and put into a cleaned and dry crucible. The same was done to *M. stenopetala* leaf powder sample. Both were heated at 105°C inside a hot air oven till a constant weight was attained. Quantification of the moisture content was by computing weight lost from the original sample which was them done as a percentage (FAO, 1980).

3.12.2 Determination of crude protein

The quantity of crude protein was computed using the method of Kjeldahl method though slight modifications using the following steps (Appendix 22) (AOAC, 2004).

Digestion: a gram of each ground sample was weighed and placed into separate digestion flasks with lysine hydrochloric acid and reagent blank included to verify the accuracy digestion parameters. Next was addition of 0.04 grams anhydrous copper sulfate 15 grams Potassium sulphate, and 0.8 alundum granules. Then 20ml concentrated sulphuric acid was added before heating the mixture using heating mantles until white fumes were no longer observable in the bulb of the flask. The mixture was then gently stirred, then heated in the fume chamber for another 90 minutes, and then cooled to room temperature.

Distillation: An accurate mixture of 70 mL water (V/V HCl) and 15 mL of hydrochloric acid was composed to create a standard solution which was transferred into a titration flask. In the case of reagent black. One mL of acid was added into 85mL of water in sequence with methyl red solution indicator. At the bottom of flask, 100 ml containing 45% sodium hydroxide was steadily added as a way of increasing pH of the mixture. After that, distillation was made until a minimum volume of 150 mL was collected into the titration flask.

Titration: The acid that was excess underwent titration through the use of 0.1 M sodium hydroxide standard solution until the orange end point (change of colour is red, orange and then yellow). The volume was noted to a calculated accuracy of the nearest 0.01 ml (VNaOH). A similar titration process was conducted on reagent blank (B).

The required calculations were done using this equation below.

$$\% N(DMbasis) = \frac{[(VHClxNHCl) - (VBKxNNaOH) - (VNaOHxNNaOH)]}{1.4007} xWx \frac{LabDM}{100}$$

Whereby; DM is dry matter; V NaOH = the volume of standard NaOH required for complete titration of the sample; V HCl = the volume of standard HCl transferred into the titration flask (ml); N NaOH = normality of the NaOH in use; N HCl = normality of the HCl in use; V BK = the ml standard NaOH required for titration with 1 ml standard HCl minus B. In this case, B = the standard NaOH ml required for titrating the reagent blank obtained by this procedure, and distilled in 1 ml standard HCl; 1.4007 = milliequivalent nitrogen weight times 100; W = the weight of the sample (g).

Computing the percentage related to crude protein was done as per equation (2)**:**

$$CP(DMbasis) = \%\, N(DMbasis)XF$$

Where F = 6.25 (AOAC, 1990; 2004).

3.12.3 Determination of crude lipid

Using the Soxhlet extraction method (Appendix 23) for estimation, a measured sample by weight (10g) of *M. oleifera* powdered leaves was wrapped with filter paper and then put inside a thimble. Same was done with *M. stenopetala* and both were covered using cotton. Both samples were put into extraction column connected with Liebig condenser so that N-hexane at a volume of 200 mL could be used in the process of extracting lipids from the aforementioned samples (AOAC, 2004).

3.12.4 Determination of crude fibre

Powdered leaves of *M. oleifera* and *M. stenopetala* were weighed at 5 grams and separately mixed with 200 mL by volume of 1.25% sulphuric acid. This mixture was heated in a hot water bath for a duration of 30 minutes and then filtered using a Buchner funnel. Each residue was cleansed thrice using water that is distilled so as to clean them off the acid. These residues underwent boiling inside 200 ml 1.25% NaOH for 30 minutes, then filtered and debased with distilled water 3 times, then washed one time 10% HCl in sequence with another two times washing using absolute ethanol. Creating fat free residues was done by washing them three times with petroleum ether. The residues formed were put into a marked crucibles and dried overnight in a drying oven with hot air at 105°C. Further the residues were placed into in a muffle furnace at 550°C

and allowed to burn for one and half hour (90 minutes). They were allowed to cool until they achieved room temperature so that the remaining ash could be weighed (AOAC, 2004).

$$\% \, Crudefibre = \frac{W2 - W1}{W0} \times 100$$

Where W_1 = is the weight of an empty crucible; W_2 = W_1+ ash and W_0= is the measured weight of sample.

3.12.5 Determination of ash content

Five grams of powdered M. oleifera leaves was weighed and placed into a labelled crucible. A similar amount of *M. stenopetala* leaf powder was weighed into a labelled crucible and a muffle furnace was used to heat them for 6 hours at 550 ^{o}C. A desiccator was used to allow the resulting ashes to cool up to room temperature before taking their weight. Percentage of water-soluble ash and corresponding acidity was determined as per the below formula (AOAC, 2004). The weight difference was then used to calculate the percentage ash content.

$$\%Ashcontent = \frac{W2 - W1}{W0} \times 100$$

Where W_1 = is the weight of an empty crucible; W_2 = W_1+ ash and W_0= is the measured weight of sample.

3.12.6 Determination of carbohydrate

Carbohydrate content within *M. oleifera* and *M. stenopetala* sampled leaves was computed by getting the difference between the sum of percentages of lipid, ash contents, moisture, and fibre from 100 (Idris et al., 2019).

3.12.7 Determination of amino acids in *Moringa oleifera* and *stenopetala* leaf

Concentration of amino acids in leaf powders of *M. oleifera* and *M. stenopetala* were derived through slightly amended methods described by previous (Okoronkwo et al., 2017). Five grams of leaf powders was weighed, macerated at 50 ºC for a time of 30 minutes in 50 MmL water bath, and finally filtered using Whatman filter paper No. 1. Aliquots (5 ml) were placed in separate and clean flasks followed by addition of 1 % Ninhydrin solution (5mL), 0.25 % sodium carbonate (2.5 mL), which was prepared in 95% ethanol. The mixture was heated to 95 ° C. in a hot water bath for 5 minutes and allowed to cool up to room temperature. A UV-Vis spectrophotometer configured at 204 nm to 350 nm was used to measure absorbance with distilled water serving as blank value (Okoronkwo et al., 2017).

3.12.8 Determination of vitamins (A and B) in *M. oleifera* and *M. stenopetala* leaf

In accordance with Bhatnagar Panwar et al. (2013) method, which was modified slightly for this study, the content of β-carotene (vitamin A) in the tested plant materials was analyzed. Separate 10 mg of dry powder from *M. oleifera* and *M. stenopetala* were placed in test tubes containing 10 ml ethanol supplemented with 0.1 grams butylated hydroxytoluene (BHT). The experimentation mixtures were incubated in a 70°C water bath times for 15 minutes. The samples were saponified by adding 180 µl at 80% KOH.

Vertexing was performed on each formulation in the test tube for 30 minutes for maximal recovery of carotene and its esters. Resulting samples were directly put in an ice filled bath, then 2.5 ml deionized water added into 2.5 ml hexane/toluene at a ratio of 10 was added. Tubes were vortexed once more and centrifuged for 5 minutes at 2100 rpm. A upper layer comprising hexane/toluene fraction was extracted three times and transferred into distinct test tubes. They were dried in a Speed-vac concentrator. A reconstituted residue in 200-400 μL Tetrahydrofuran (THF) was filtered through a 0.2 μ nylon filter before injection into high performance liquid chromatograph. At the mobile phase, Tetrahydrofuran (THF) (52:40:8) (v/v/v) configured at a rated flow of 2.0 mL min^{-1} and methanol were its constituents. Through a Photodiode array detector (PDA) configured at 450 nm, absorbance was recorded and the absorption spectra was comparatively analysed against the currently known benchmarks of β-carotene- Sigma Chemicals. Quantification of β-carotene was done by the use of peak areas defined in authentic standard. The vitamin B group was performed according to the standard method described by (AOAC, 2004).

3.12.9 Analysis of elemental composition

The AOAC method (1990) was used to determine the mineral content, whereby, two grams of the pulverized samples from the respective plants were placed in a clean, dry crucible and burned in a muffle furnace for 6 hours at 550 ^{o}C. Resultant ash was slowly heated in 10 ml of 5% HNO_3 for time of 20 minutes. Filtration was done using Whatman No 1 filter paper and the filtrates were used to determine mineral content. On the other hand, atomic absorption spectrophotometer (AAS) was employed in the process of

determining copper, calcium, magnesium, potassium, iron, zinc, manganese - and sodium content contained in the samples. Each standard was diluted according to its corresponding standard scheme. Then for each of the two types of Moringa, all tests were performed in triplicate.

3.13 Data analysis

The data were summarized using Microsoft Excel package then analysed using Minitab version 19.1. One-way analysis of variance (ANOVA) and student t-test were conducted appropriately at 0.05 Level of significance (95% level of confidence) to evaluate the effects of *M. oleifera* Lam and *M. stenopetala* Bac. leaves diet supplements on growth performance, blood indices values and gut microbiota. Where necessary, Fisher's Least Significant Difference post hoc test was done for pairwise separation and comparison of means.

3.14 Ethical considerations

The experiment was carried out in a controlled environment according to the (American Psychology Association's [APA], 2002), Ethics code. Four research Assistants were trained in care, maintenance and management of broiler chicken before commencement of the experiment. On ending the experiment – after the fifth week of the feed trials and all data collected, the remnants of the experimental chicks were humanely disposed. A registered Veterinarian supervised the euthanasia process carried out with minimal physiological and psychological disturbances. After euthanasia, the chicken carcasses were placed in plastic bags and sent for disposal, whereby, they buried deep underground, covered with disinfectant and lime.

Ethical clearance was obtained from Kenya Methodist University Scientific Ethics Review Committee (SERC), approval number KeMU/SERC/AGRI/73/2019 and a research license from the National Commission for Science, Technology and Innovation (NACOSTI), license number: NACOSTI/P/19/1697 (Appendix 16) before commencement of the study.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effects of *M. oleifera* and *M. stenopetala* extracts on growth performance

Like other farm animals, Poultry requires adequate supply of feed containing the necessary nutrients, to maintain their health and productivity. Previous studies have shown that successful production of quality broiler chicken depends on the nutritional quality of feed served, the ingredients used in feed formulation, procedures involved in feed, and manner in which the processed feed is given to chicken (Chehraghi et al., 2013; Hagan et al., 2016).

Furthermore, green plants have been recognized as one of the most abundant sources of protein, and health and growth promoting phytochemical constituents, which promote the health, performance in terms of growth, and quality of broilers (Azman & Yilmaz, 2005; Biesek et al., 2020; Chehraghi et al., 2013; Ololade & Iyayi, 2006).

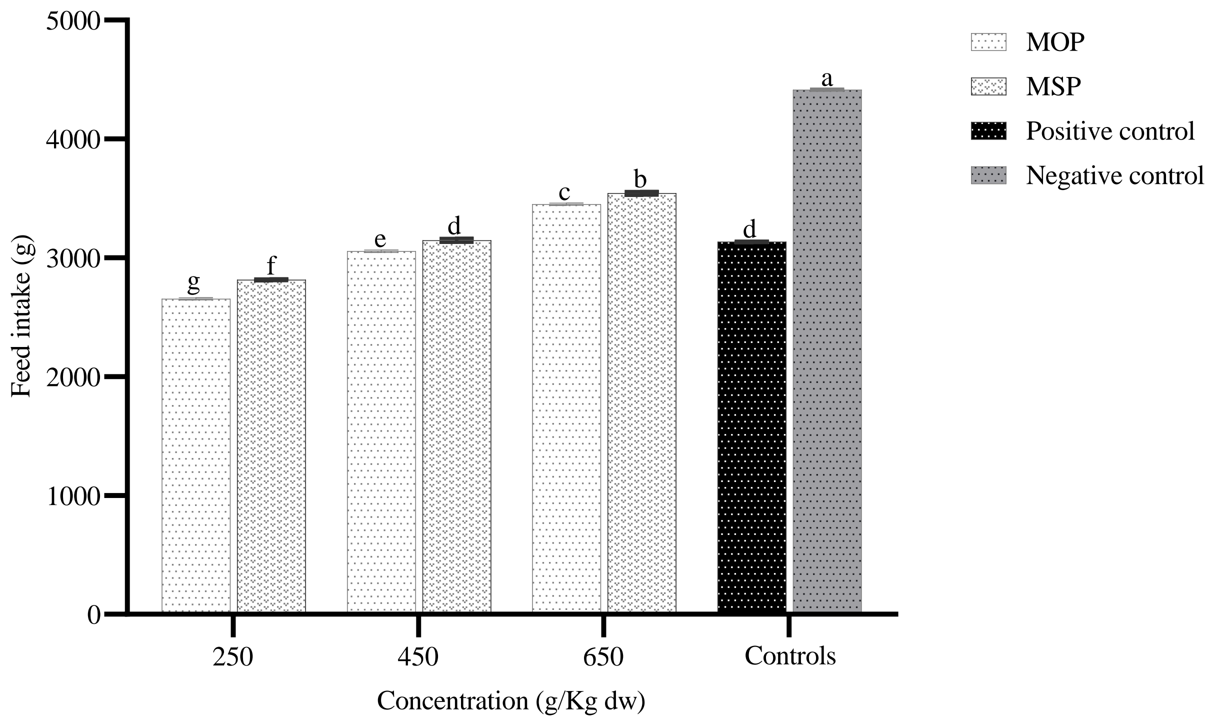
In this study, the implications of various concentrations of leaf powders and extracts (aqueous and ethanolic) contained in *Moringa oleifera* and *Moringa stenopetala* on growth performance of broiler chicken were evaluated. Gain of body weight, ratio at which feed is converted, and intake of feed were determined, then used as key indicators of growth performance of experimental chicken.

The results showed significant concentration-dependent increases in feed by broiler chicken that were fed on feed supplemented with graded powders of *M. oleifera* and *M. stenopetala* (Figure 4.1; $p<0.05$). Notably, broiler chicken that were fed on graded *M.*

stenopetala leaf powder-supplemented feed showed significantly higher feed intake compared with the feed intake by chicken fed on *M. oleifera* leaf powder-supplemented feed (Figure 4.1; p<0.05). Besides, the negative control chicken exhibited significantly higher feed intake in comparison with other chicken groups (Figure 4.1; p<0.05).

Figure 4.1

Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's feed intake

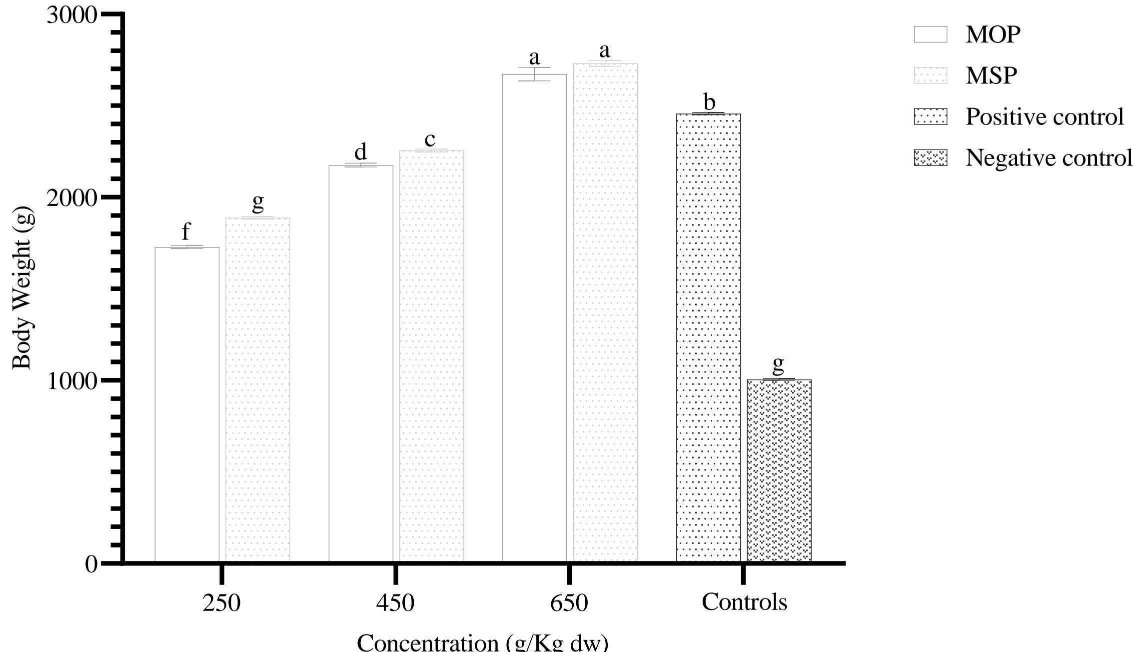


Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of (p<0.05) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOP: Leaf powder of *Moringa oleifera*; MSP: Leaf powder of *Moringa stenopetala;* Positive Control: Standard Commercial Broiler Feed; Negative Control: uncomplemented feed devoid of AGPs.

Besides, significant concentration-dependent increases in body weight of broiler chicken whose feeds were supplemented with graded powders of *M. oleifera* and *M. stenopetala* (Figure 4.2; $p<0.05$). Notably, body weights that were comparatively higher body were recorded in broiler chicken that were served graded *M. stenopetala* leaf powder-supplemented feed compared with the body weight of chicken fed on *M. oleifera* leaf powder-supplemented feed. The negative control chicken had a significantly lower body weight compared with the weights of chicken in all the other treatment groups.

Figure 4.2

Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's Body weight

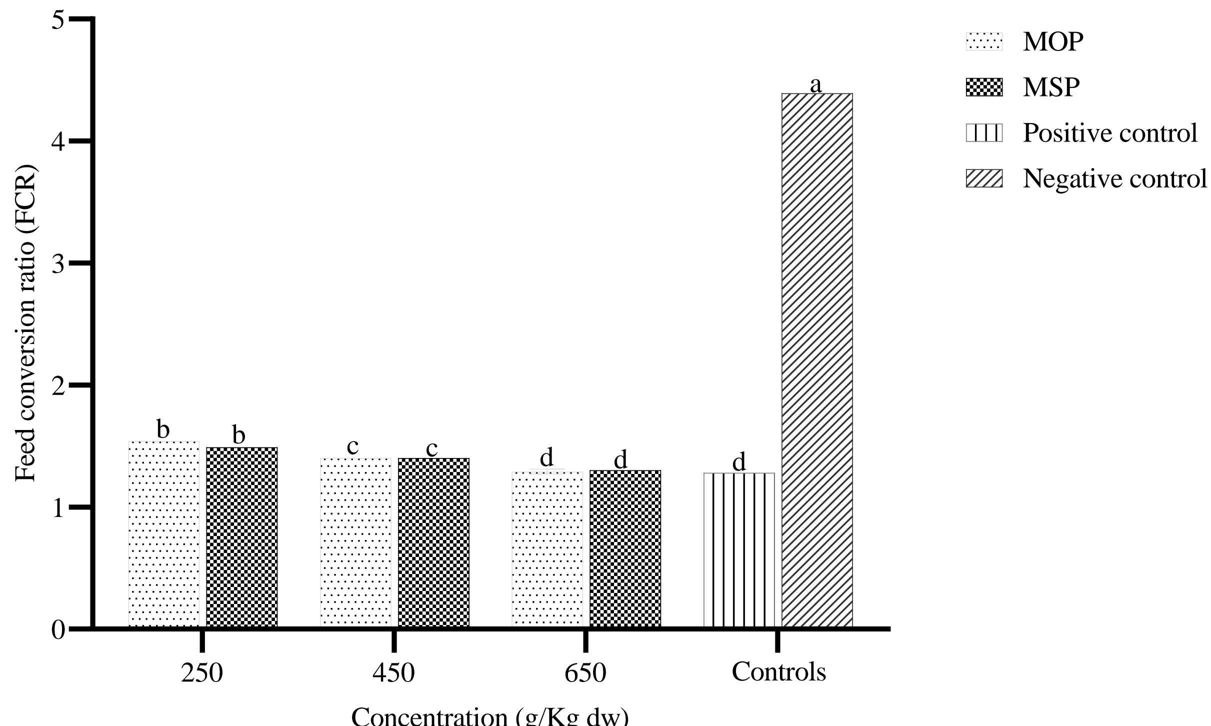


Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of ($p<0.05$) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOP: Leaf powder of *Moringa oleifera*; MSP: Leaf powder of *Moringa stenopetala*; Positive Control: Standard Commercial Broiler Feed; Negative Control: uncomplemented feed devoid of AGPs.

No significant difference in feed conversion ratios (FCRs) were observed among broiler chicken that were served with feed supplemented with 650 g/Kg dw containing powdered leaves from *M. stenopetala, M. oleifera*, and the positive control chicken (Figure 4.3; $p>0.05$). Similarly, the differences between feed conversion ratios recorded in chicken supplemented with 250 g/Kg dw, and 450 g/Kg dw, respectively, of leaf powders of the two studied plants were not significant (Figure 4.3; $p>0.05$).

Figure 4.3

Effects of the leaf powders of M. oleifera and M. stenopetala on broiler chicken's Feed conversion ratio (FCR)



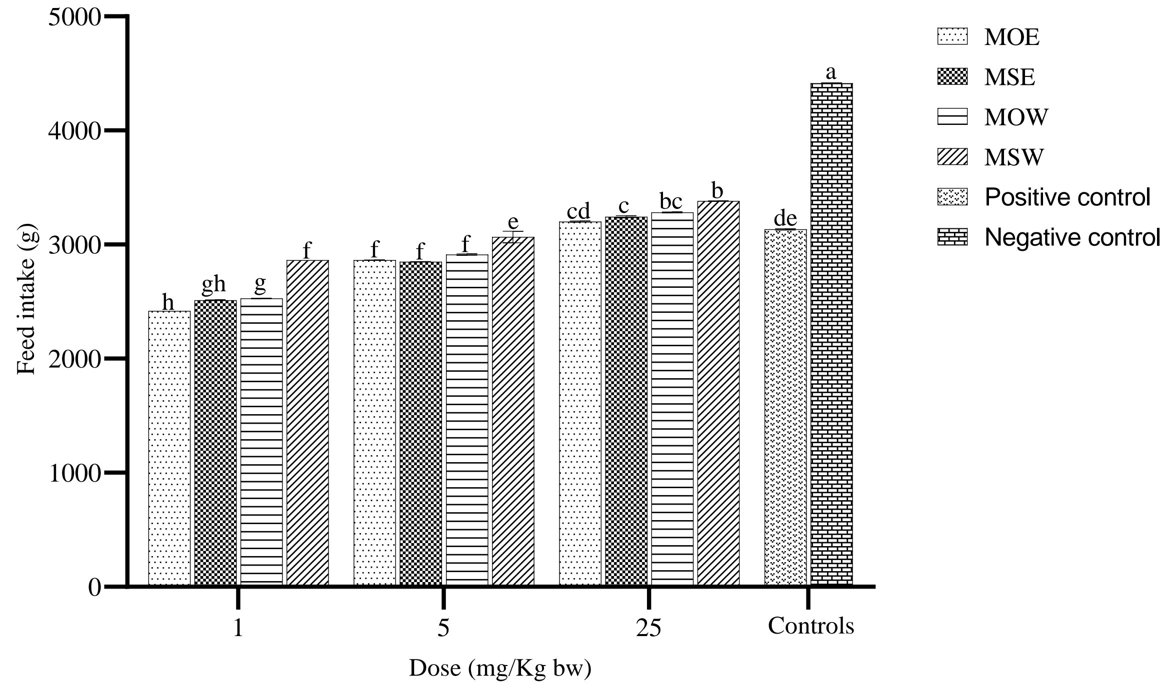
Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of ($p<0.05$) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOP: Leaf powder of *Moringa oleifera*; MSP: Leaf powder of *Moringa stenopetala;* Positive Control: Standard Commercial Broiler Feed; Negative Control: uncomplemented feed devoid of AGPs.

On the other hand, the experimental chicken that were orally administered with 1mg/Kg per body weight made from aqueous leaf extract of *M. stenopetala,* but those administered 5 mg/Kg of body weight of the ethanolic extracts from leaf of both plants, and the aqueous leaf extract of M. oleifera, respectively, showed no significant differences in feed intake (Figure 4.4; $p>0.05$). Nevertheless, positive dose-dependent

increases in feed intake by the experimental chicken were observed (Figure 4.4; p<0.05). Besides, the negative control chicken had a significantly higher feed intake compared with the feed intake recorded in all the other chicken in this study.

Figure 4.4

Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's feed intake



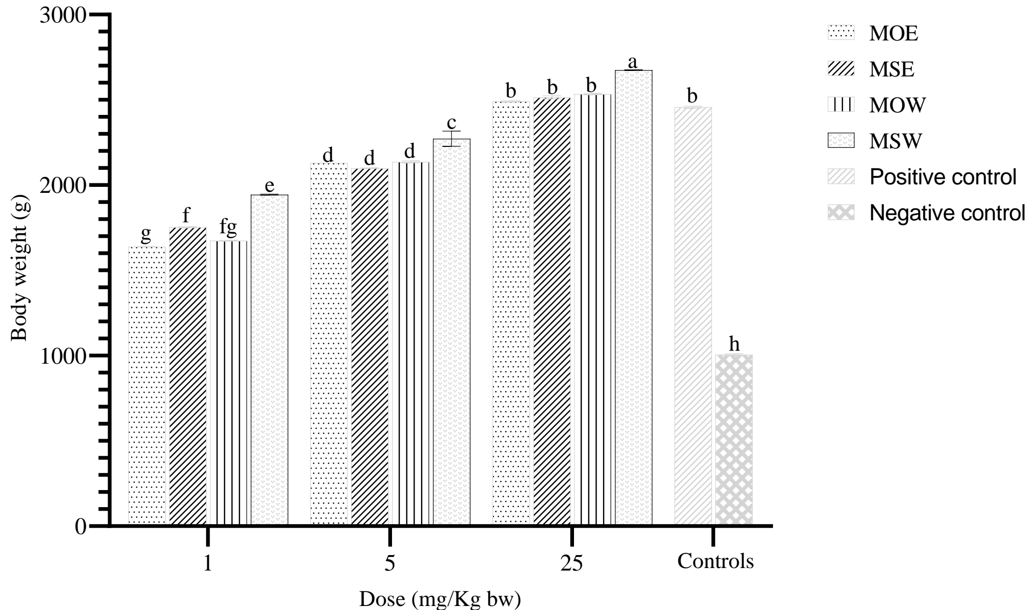
Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of (p<0.05) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOE: Ethanolic leaf extract of *Moringa oleifera*: MOW: Aqueous leaf extract of *Moringa oleifera;* MSE: Ethanolic leaf extract of *Moringa stenopetala;* MSW: Aqueous leaf extract of *Moringa stenopetala;* Positive Control: Standard Commercial Broiler Feed; Negative Control: placebo treated (10 ml/Kg bw of Normal saline).

The experimental broiler chicken that were treated with leaf extracts of ethanolic origin from *M. stenopetala* and *M. oleifera*, and the aqueous leaf extract of *M. oleifera*, at dosage levels at 5 mg/Kg b.w, and at 25 mg/Kg b.w, respectively, did not show

significant differences in body weights (Figure 4.5; p>0.05). on the contrary, the chicken that got 25 mg/kg bw containing aqueous leaf extract of *M. stenopetala* had a significantly more weight of body compared with the body weights recorded in all the other experimental chicken . The negative control chicken had the least body weight that was significant.

Figure 4.5

Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's Body weight



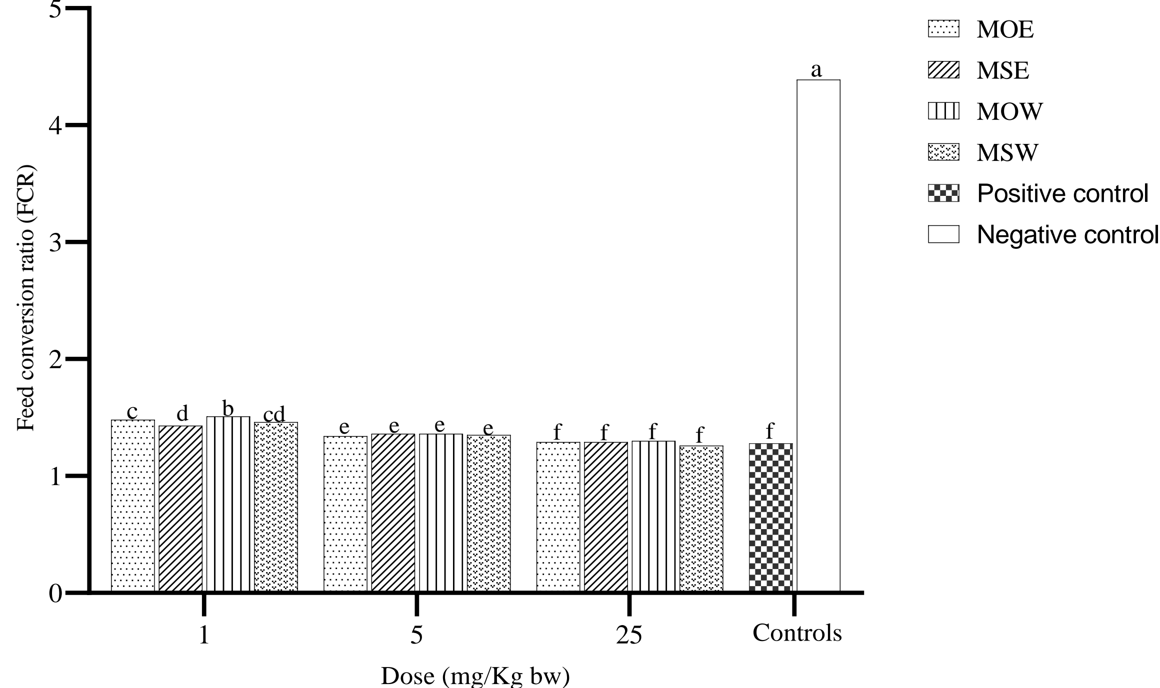
Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of (p<0.05) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOE: Ethanolic leaf extract of *Moringa oleifera*: MOW: Aqueous leaf extract of *Moringa oleifera*; MSE: Ethanolic leaf extract of *Moringa stenopetala;* MSW: Aqueous leaf extract of *Moringa stenopetala;* Positive Control: Standard Commercial Broiler Feed; Negative Control: placebo treated (10 ml/Kg bw of Normal saline).

The feed conversion ratios recorded in chicken that were treated with 5 mg per Kg of body weight of the ethanolic and aqueous leaf extracts of the two plants were not

significantly different (Figure 4.6; $p>0.05$). Similarly, conversion ratio of feed (FCR) of chicken that received 25 mg per Kg body weight of the aqueous and ethanolic leaf extracts of the studied plants, and the positive control were not significantly different (Figure 4.6; $p>0.05$). However, the negative control chicken had the highest feed conversion ratio while the ones that got treatment amount of 25 mg/Kg bw of plant extract under study had the least.

Figure 4.6

Effects of the ethanolic and aqueous leaf extracts of M. oleifera and M. stenopetala on broiler chicken's Feed conversion ratio (FCR)



Values are plotted as x̃±SEM; bars that are marked with different letters are indicative of significant difference of ($p<0.05$) by One-Way ANOVA done concurrently with Tukey's Post hoc test; MOE: Ethanolic leaf extract of *Moringa oleifera*: MOW: Aqueous leaf extract of *Moringa oleifera*; MSE: Ethanolic leaf extract of *Moringa stenopetala*; MSW: Aqueous leaf extract of *Moringa stenopetala;* Positive Control: Standard Commercial Broiler Feed; Negative Control: placebo treated (10 ml/Kg bw of Normal saline).

Research has indicated that feed intake in poultry is at times determined by elements like antinutritional phytocompounds in the feed (Awotedu and Ogunbamowo, 2019; Chinelo et al., 2014). For instance, tannins and saponins have been demonstrated to affect rate of feed intake and efficiency of feeding among broiler chicken adversely (Konietzny et al., 2006). Moreover, research has shown that tannins reduce dry matter of feed and impair

protein digestion and absorption in broiler chicken (Hagan et al., 2016). It is therefore suggestive that tannins, saponins, among other antinutritional factors contained in powdered leaves and extracts of *M. oleifera* and *M. stenopetala* could be in low concentrations, especially in *M. stenopetala,* to impede feed intake by the experimental chicken, and their weight gain. Findings of this have been determined to be consistent with those reported earlier by other scholars (Azman & Yilmaz, 2005; Biesek et al., 2020).

The effects of *Moringa oleifera* and *Moringa stenopetala* leaf powder supplements and extracts on growth performance of broiler chicken investigated in the current study can be attributed to the nutritional constituents present in the two studied plants (Rehman et al., 2018; Ambali & Furo, 2012; Voemesse et al., 2018). The increase in powder concentration, and extract dose resulted in increased chicken body weights, an indication of the presence of growth-promoting factors in the studied plant powders and extracts. The findings of this study corroborate well with a previous study conducted by (Zanu et al., 2012). Furthermore, significant differences in body weights of experimental chicks administered with extracts and those supplemented with powders of Moringa spp have been documented by (Ashong and Brown, 2011; John and Kenaleone, 2014; Alabi et al., 2017; Mahfuz and Piao 2019a) among other scholars, there by confirming the potential of the studied plants as alternative growth promoters in broiler chicken husbandry.

Besides, the study findings indicated that leaf extracts of ethanolic and aqueous origin possess growth promoting secondary metabolites, which effected a variety of body processes leading to appreciable weight gain, (Alabi et al., 2017). Ethanol and water,

among other polar solvents, extract antioxidant, among other health-promoting phytochemicals. Research has shown that polyphenolic compounds, vitamins, essential amino acids, and micronutrients in plants offer growth benefits to both animals and people (Rahman et al., 2013; Madende & Hayes, 2020)

The probable mechanism of growth performance of plant products could be through the reduction of pathogenic microbial infestation in the gastrointestinal tract, enhancing digestibility of the ingested feeds, and their metabolism, as well as enhancing immunity to infections (Nkukwana et al., 2014). The solvents used to extract the leaves of the two studied plants, possibly solubilised important amalgams which were concentrated and delivered to the experimental broiler chicken thereby conferring their beneficial effects (Nkukwana et al., 2014; Sarker et al., 2017).

Feed conversion ratio has been utilized to assess feed efficiency in animal husbandry. An enhanced feed conversion ratio (near 1) denotes value for capital and good returns to the farmer (Angela & Chizoba, 2019). Feed efficiency is influenced by several factors which include digestion, metabolism, and the general health of the animal (Salaheen et al., 2017). Therefore, the feed conversion ratios reported in this study were as a result of better feed intake, which effectively translated to higher weight gains in broiler chicken, indicating remarkable efficiency. However, the higher feed conversion ratio observed in the negative control group of chicken can be attributed to the low body weight gains resulting from insufficient or lack of the necessary nutritional factors in their feeds.

Moreover, feed conversion ratio (FCR), is used as an indicator of growth performance and as a predictor of capital value (Salim et al., 2018). The FCR results presented herein suggest that indeed the studied plant powders promoted growth of the broiler chicks comparatively with the positive control. This can in part be explained by the presence of bioactive phytoconstituents in the two studied *Moringa* species which act as antibiotics and immunomodulators (Mahfuz & Piao, 2019b;Alabi et al., 2017). These findings are supported by earlier studies by (Younis & Elbestawy, 2017; Karthivashan et al., 2015; Ebenebe et al., 2012; David et al., 2012). These FCRs are indicators of better economic returns and that the studied plants can be used as alternatives to raise broiler chicken.

Antibiotic growth promoters (AGPs) over the years have been used in the process of improving growth performance in broiler chicken with significant successes (Salim et al., 2018). However, serious safety concerns about expansive use of growth promoters in food animal husbandry have been raised prompting its ban in some regions including the European Union. This is because their benefits are marginal and are perceived to be outweighed by their adverse effects, especially when consumed by humans (Laxminarayan et al., 2015). As result, there is a research interest, particularly involving medicinal plants, in the quest for safer antibiotic alternatives in poultry farming aimed at promoting gut microflora, enhancing food digestibility, improving morphology, and as such foster meat quality (O 'neill, 2015;Sneeringer et al., 2015 ; Nkukwana et al., 2014).

The presence of probiotics, vitamins, mineral elements among other phytoactive molecules in feeds are thought to work towards promoting body weight gain in

conjunction with feed conversion ratio (Salaheen et al., 2017; Xie et al., 2014). Various studies have demonstrated that supplements of plant origin which improve feed conversion ratio are potential antibiotic growth promoter replacements (Gadde et al., 2017). In light of this, the studied plant powders and extracts have compounds which facilitate normal flora growth in the gastrointestinal tract while inhibiting pathogenic strains (Gadzirayi et al., 2012; Makkar et al., 2007; Salaheen et al., 2017). Therefore, nutrient competition is reduced and thereby undersupply is curtailed ensuring the chicks received optimum nutrients for optimal and healthy growth. Additionally, powdered leaf extracts of *M. oleifera* and *M. stenopetala* contain valuable phytocompounds, which may have enhanced digestibility of the served feeds, thereby promoting growth efficiently as evidenced in this study. Therefore, supplementation of feeds with the studied leaf powders or the administration of extracts may be an efficient and safer strategy to ensuring quality production and higher returns in broiler chicken husbandry.

4.2 *M. Oleifera* and *Stenopetala* Leaf Powder and Biochemical Parameters

Haematological and biochemical composition are important determining factor of health status in broiler chicken. Both haematology and biochemical parameters are observed as indicators of overall health of an animal.

4.2.1 Effects of *M. Oleifera* and *Stenopetala* Leaf Powder and Haematological Parameters of Broiler Chicken

Effects from plant leaf powders and extracts under study (aqueous and ethanolic) on the experimental chicks' haematological and biochemical parameters were investigated. Research has shown that haematologic parameters help to evaluate the physiological,

pathological, and health conditions of the body. Furthermore, haematological parameters are important markers for normal growth and thriving of humans and animals. Good haematologic parameter states are indicative of good health, thus better growth and performance of farm animals.

In this study, the haematological analysis revealed no significant difference in counts of red blood cells (RBC), levels of white blood cell counts (WBC), percentage lymphocytes, and packed cell volume (% PCV) among the experimental broiler chicks that received *M. stenopetala* and *M. oleifera* leaf powder-supplemented feeds ($p>0.05$; Table 4.1). However, the negative control chicks had significantly low WBC and RBC counts, and percentage PCV and lymphocytes compared with the chicks in the positive control group and those that received the studied plant powders.

Besides, notable variation in haemoglobin levels (Hb) was spotted in chicks that were served with a feed supplemented with *M. stenopetala* leaf powder at all the three levels ($P>0.05$; Table 4.1). Similarly, the Hb levels measured in chicks served with *M. oleifera* leaf powder-supplemented feed at the three studied concentrations were not significantly different ($P>0.05$). Notably, the Hb levels obtained for chicks that were served with *M. stenopetala* leaf powder supplemented feed were significantly higher than those of chicks that received M. oleifera leaf powder-supplemented feed at all concentrations ($P<0.05$; Table 4.1). Remarkably, the chicks that were served with feeds supplemented with the studied plant powders recorded significantly higher Hb levels than the positive control group chicks ($P<0.05$). The negative control chicks had significantly low Hb levels than all the other chicks.

Table 4.1

Effects of the leaf powders of M. oleifera sand M. stenopetala on haematologic parameters of experimental broiler chicks

Diet	Parameter/Trait				
	WBC (× 10^{3}/mm^3)	**Lymphocyte (%)**	**PCV (%)**	**RBC (×10^6/mm^3**	**Hb (g/dl)**
MOP 250	13.72±1.7^a	93.97±0.2^a	30.30±0.2^a	3.00±0.4^a	14.90±1.9^b
MOP 450	12.90±1.7^a	93.50±0.1^a	30.80±0.2^a	3.17±0.1^a	15.65±1.4^b
MOP 650	13.72±1.6^a	92.80±0.3 ^{a}	30.58±0.6^a	3.27±0.2^a	16.24±1.3^b
MSP 250	13.05±1.2 ^{a}	93.17±0.2 ^{a}	30.47±0.0^a	3.64±0.4^a	19.82±1.6^a
MSP 450	13.50±1.2 ^{a}	93.15±0.1^a	30.78±1.6^a	3.71±0.2^a	18.45±1.6^a
MSP 650	13.30±1.3 ^{a}	92.85±0.1 ^{a}	30.30±0.2^a	3.76±0.5^a	18.23±3.1^a
-ve Control	7.05±1.3^b	82.15±0.2^b	15.90±1.0^c	2.11±0.3^b	8.60±0.1^d
+ve Control	12.05±1.7^a	94.15±0.9^a	28.90±2.1^b	3.18±0.5^a	12.8±0.1^c

Values are presented as x̃±SEM; Means with similar superscript alphabets across the rows are not significantly different (One-Way ANOVA followed by Tukey's test)
MOP: *Moringa oleifera* powder; MSP: *Moringa stenopetala* powders

The study further evaluated effects of ethanolic and aqueous based leaf extracts of *M. oleifera* and *M. stenopetala* on haematologic parameters of experimental chicks were also evaluated. And the results are presented in Table 4.2. The WBC, RBC and Hb levels, and percentage PCV and lymphocytes measured in experimental chicks that received the studied plant extracts were not having any significant difference ($p>0.05$).

on the contrary, all the studied haematologic parameters were greatly lower in the negative control chicks compared with those in all the experimental and positive control chicks.

The percentage of lymphocyte counts was significantly higher in chickens in the positive control group than in all other chickens ($p<0.05$). On the contrary, the Hb content was significantly higher in the test chickens that received the examined plant extracts than in the positive control group, and negative control groups.

Table 4.2

Effects of the aqueous and ethanolic leaf extracts of M. oleifera and M. stenopetalaon haematologic parameters of experimental broiler chicks

Dose (mg/Kg bw)	Treatment	Parameter (trait)				
		WBC ($\times$ 10^{3}/mm^3)	**Lymphocyte (%)**	**PCV (%)**	**RBC ($\times 10^6$/mm^3**	**Hb (g/dl)**
1	MOE	11.70±0.86^a	87.92±0.0^b	26.18±1.0^a	3.51± 0.3^a	18.30±0.0^a
	MSE	11.90±0.32^a	87.75±0.1^b	27.63±1.0^a	3.50±0.1^a	18.10±1.0^a
	MOW	12.70±0.22^a	86.35±1.1^b	27.95±0.8^a	4.40±0.0^a	23.01±0.0^a
	MSW	12.60±0.11^a	88.75±0.2^b	26.45±1.4^a	3.40±0.3^a	17.80±0.0^a
5	MOE	12.30±0.34^a	88.22±0.7^b	26.25±1.1^a	3.60±0.0^b	19.60±1.0^a
	MSE	12.70±0.06^a	88.15±0.1^b	28.03±0.3^a	3.70±0.0^b	18.70±1.0^a
	MOW	11.30±0.84^a	88.10±0.1^b	27.55±1.0^a	3.80±0.3^a	20.70±1.0^a
	MSW	11.50±0.70^a	87.75±1.4^b	27.08±1.0^a	3.40±0.1^a	17.60±2.0^a
25	MOE	12.90±0.43^a	86.80±1.9^b	28.47±0.7^a	3.70±0.1^a	20.20±1.0^a
	MSE	12.20±0.03^a	87.40±2.1^b	26.13±1.6^a	3.50±0.0^a	21.90±1.0^a
	MOW	12.10±0.80^a	87.55±0.8^b	28.50±0.5^a	4.00±0.0^a	21.10±0.0^a
	MSW	12.70±0.91^a	87.92±1.0^b	28.18±1.0^a	3.83±0.1^a	18.30±0.0
-ve Control		7.05±1.30^b	82.15±0.2^c	15.90±1.0^b	2.11±0.3^b	8.60±0.1^c
+ve Control		12.05±1.7^a	94.15±0.9^a	28.90±2.1^a	3.18±0.5^a	12.8±0.1^b

Values are presented as x̃±SEM; Means with similar superscript alphabet within the same row are not significantly different (One-Way ANOVA followed by Tukey's test)
MOE: *Moringa oleifera* ethanolic leaf extract; MSE: *Moringa stenopetalaethanolic* leaf extract; MOW: *Moringa oleifera* aqueous leaf extract; MSW: *Moringa stenopetalaaqueous* leaf extract; +ve Control: positive control; -ve Control: negative control

Blood is an important tissue of the circulatory system of the body playing crucial functions of maintaining homeostasis and health (Abbas, et al., 2018; Makama et al., 2020). Analysis of haematologic constituents in animals reared in farms, to include red blood cells, haemoglobin levels, white blood cells, platelets, packed cell volume, among

others, helps to diagnose and monitor ill health, which are associated with feed toxicity or diseases(Doneley, 2010).During ill health, these parameters deviate from their normal levels/concentrations.

The leucocyte (white blood cells) majorly fights infections by phagocytosing foreign microbes and triggering an immunologic response to infections. High levels of white blood cells in the body are associated with better capability of antibody secretion, effective phagocytosis, and proper eradication of or resistance to diseases (Makama et al., 2020).

Leucocytes (white blood cells) are categorised into two groups based on the presence of granules in their cytoplasm as granulocytes (neutrophils, basophils, and eosinophils), and agranulocytes (monocytes and lymphocytes). Notably, the salient function of lymphocytes, especially T, B and natural killer cells (NK) is mounting immunologic responses to foreign epitopes. Low levels of lymphocyte counts are indicative of suppressed immunity to infections (Onunkwo et al., 2018).

In the present study, higher percentage lymphocyte counts were observed in experimental chicks that were treated with the aqueous and ethanolic leaf extracts of *M. oleifera* and *M. stenopetala* and those that were offered feed supplemented with leaf powders of the two studied plants. Notably, the obtained lymphocyte counts were higher than the normal reference ranges of 45-70 % and 54-73% (Onunkwo et al., 2018).

High white blood cell counts indicate the capacity to produce antibodies against injurious stimuli and higher resistance to diseases. However, overwhelmingly high white

blood cell counts are indicative of stress, toxicity, inflammatory response, among other pathologic conditions (Doneley, 2010; Oghenebrorhie & Oghenesuvwe, 2016). Besides, low white blood cell counts are indicative of suppressed immunity to infections, and reduced capability to thwart pathologic infections, which may lead to poor health, and even death (Doneley, 2010; Onunkwo et al., 2018).

The white blood cell counts of experimental chicks treated with the aqueous and ethanolic extracts and powders of *M. oleifera* and *M. stenopetala* obtained in this study were notably higher than the normal reference ranges of $3.05\times10^3/mm^3$ - $5.56\times10^3/mm^3$(Al-Nedawi, 2018b). These findings may be attributed to environmental stresses, sex, and genetic predisposition, which may have evoked heightened immunologic response by the birds(Fernandez et al., 1995; Livingston et al., 2020).

Due to the absence of adverse pathologic symptoms in experimental chicks, the high WBC counts could indicate better infection fighting abilities, which may have contributed to the chicks' good health and growth performance (Abbas et al., 2018). Further, it is suggestive that the incorporated powders and administered extracts contain active ingredients which either works to promote leucocyte production, health and immunologic functioning, prevent or wade off diseases when consumed by animals (Disetlhe et al., 2015). Additionally, this is an indication of recovery boosting effect of these powders in the chicken. These were evidenced by good health and survival observed during the entire treatment period.

Packed cell volume (PCV) and haemoglobin (Hb) are crucial parameters for examining the circulatory erythrocytes, the capacity of the bone marrow to produce healthy

erythrocytes, hence are important diagnostic markers for anaemia (Doneley, 2010). Higher levels of red blood cells, haemoglobin, and packed cell volume are indicative of higher oxygen-carrying capacity of blood, hence enhanced health.

Higher percentage packed cell volume values are clinically termed as polycythaemia and is associated with high population of red blood cells, or low volume of circulating plasma, which may result from physiologic adaptation to high altitudes, dehydration or pathologic retort to chronic diseases of the circulatory or respiratory system (Doneley, 2010; Oghenebrorhie & Oghenesuvwe, 2016). Additionally, polycythaemia can be an indication of rickets, iron storage disorder, hypoxia-induced, or non-hypoxic autonomous elevation of erythropoietin production (Onunkwo & George, 2015).

Mostly, PCV values of>56 % indicates pathologic polycythaemia in broiler chicken and in other poultry species (Onunkwo et al., 2018). Notably, the percentage PCV values obtained in experimental chicks fed on *M. oleifera* and *M. stenopetala* leaf powder supplements, and those administered with the aqueous and ethanolic leaf extracts of the two plants were below 35 %, and within the normal reference ranges of 22-35% and 25-49 %(Al-Nedawi, 2018b; Onunkwo et al., 2018). Therefore, the studied plant extracts, supplemented feeds did not adversely affect the PCV values in experimental chicks, indicating their safety and beneficial role in promoting health.

It is apparent that red blood cells are carriers of haemoglobin, the oxygen transporter in the body (Makama et al., 2020). Oxygen is a critical component of respiration, a biochemical process responsible for energy production in the body. The concentration of red blood cells is closely for energy production. Additionally, the red blood cells

facilitate the transportation of carbon dioxide from tissues to lungs for excretion. The red blood cell concentration is associated with haemoglobin levels, which in turn dictate the amount of oxygen/carbon dioxide carried (Makama et al., 2020). Because a higher concentration of RBCs is indicative of higher oxygen carrying capacity to tissues and carbon dioxide to lungs, lower levels can be attributable to ill health arising from insufficient oxygen for respiration, and inefficient excretion of carbon dioxide from the body.

In this study, haematological results obtained in experimental chicks treated with extracts containing ethanolic and aqueous extracts, and those whose feeds were supplemented with powdered leaves of *M. oleifera* and *M. stenopetala* were significantly higher, and corroborate those reported previously (Abbas et al., 2018). Research has demonstrated that high red blood cell count and haemoglobin content are due to high iron concentrations (Alabi et al., 2017). In the opinion of this, the heightened levels of haemoglobin reported in this study can be due to iron content in the leaf powders and extracts of the studied plants, which were supplied to the chicken's bodies.

Furthermore, research has shown the role of haemoglobin and red blood cells in the body ranging from oxygen and carbon (IV) oxide transport, which are key as far as body cellular respiration and health are concerned (Makama et al., 2020). Therefore, higher levels translate to enhanced health and performance of cells during growth and exercise (Ogunwole et al., 2016). As a result, it is conceivable that *M. oleifera* and *M. stenopetala* supplemented feeds and extracts conferred these benefits to the experimental broiler chicks, which contributed to their optimal health and growth performance.

Elsewhere, the high concentrations in haemoglobin and red blood cells have been linked to high protein content in *Moringa* meal (Ulfman et al., 2018; Mahfuz & Piao, 2019). Therefore, the nutritive value of the studied plant powder supplements and the studied extracts is indispensable in poultry and other animals' husbandry.

However, undesirable effects of *M. oleifera* at higher concentrations (>20 %) on hematologic parameters have been reported (Tijani et al., 2016). However, in this study, no adverse effects were observed when leaf powders and extracts of the two plant species were utilised in rearing broiler chicken. The differences can be attributed to the differences in concentrations and agro-ecological locations where these plants grew, which influence both nutritive and phytochemical composition (Aroche et al., 2018).

Generally, the findings showed that the studied leaf powders and extracts did not adversely alter the haematologic parameters and instead promoted their functioning and ultimate health. Based on these findings, the utilisation of these extracts and powders can be partly recommended to improve the quality of broiler chicken and to maximize returns.

4.2.2 Effects of *M. oleifera* and *M. stenopetala* leaf powder and extracts on biochemical parameters of broiler chicken

Biochemical parameters are invaluable indicators of the health state of both human beings and animals (Abd, 2014; Rezende et al., 2017). In this study, serum biochemical parameters in experimental chicks supplemented with plant powders that have been studies all through were articulated. The obtained results revealed that chicks that received 250 g/Kg dw of *M. oleifera* leaf powder had significantly higher serum glucose

levels than the glucose levels in all the chicks of other treatment groups ($p<0.05$; Table 4.3). Conversely, those that received a similar level of *M. stenopetala* leaf powder and 650 g/Kg dw of *M. oleifera* leaf powder had significantly lower glucose concentrations ($p<0.05$; Table 4.3). However, no distinguishable variation in levels of glucose were noted among experimental chicks which received *Moringa stenopetala* leaf powder at doses of 250 g/Kg dw and 450 g/Kg dw and those that were fed on 650 g /Kg bw of *M. oleifera* leaf powder ($p>0.05$; Table 4.9).

In terms of total protein concentration, the experimental chicks that were fed on 450 g/Kg dw of powdered *M. oleifera* leaf supplemented feed had a significantly higher protein concentration than those obtained for all the other chicks in respective treatments ($p<0.05$; Table 4.3). Contrastingly, the chicks that were given 650 g/Kg dw of *M. stenopetala* leaf powder had a significantly lower serum total protein concentration in comparison with the concentrations from other groups of chicks ($p<0.05$). Notably, at a dose of 250 g/Kg dw of *M. oleifera* leaf powder, no significant difference in chicks' protein concentration was noted when compared with the protein levels in chicks that were fed on a similar dose of the *M. stenopetala* leaf powder.

The serum albumin concentrations obtained in chicks fed on a meal supplemented with 250 g/Kg dw and 650 g/Kg dw of *M. oleifera* and *M. stenopetala* leaf powder and the control group chicks were not significantly different ($p>0.05$; Table 4.3). However, significantly higher serum albumin concentrations were obtained in chicks that fed on 450 g/Kg dw of M. oleifera leaf powder supplement compared with those obtained for all the other chicks in respective groups ($p<0.05$; Table 4.3). The chicks that were

supplemented with 450 g/Kg dw of *M. stenopetala* leaf powder had a significantly lower albumin concentration than those in other chicks.

Revelation from results is that no significant difference in calcium and creatinine concentrations in all the experimental chicks ($p>0.05$; Table 4.3). Similarly, there was no observable on serum urea concentrations obtained in chicks that were supplemented with 250 g/Kg dw and 450 g/Kg dw of both studied plant powders and 650 g/Kg dw of the *M. oleifera* leaf powder ($p>0.05$; Table 4.3). However, the chicks in the control group recorded significantly higher urea levels compared with those in all the other treatments of the experimental chicks.

Besides, the control group chicks had significantly higher total cholesterol concentration compared with the concentrations obtained in all the other chicks ($p<0.05$; Table 4.3). Conversely, the cholesterol concentrations measured in chicks supplemented with 450 g/Kg dw and 650 g/Kg dw of M. *Oleifera* leaf powder gave significantly lower total cholesterol levels than those in all the other experimental chicks.

Total triglyceride levels in chicks fed on 250 g/Kg dw supplement of *M. Oleifera* were notably high as compared to other chicks involved in the study ($p<0.05$; Table 4.3). Significantly lower serum triglyceride concentrations were observed in chicks that consumed 650 g/Kg dw of *M. oleifera* leaf powder inclusion in their feed compared with triglyceride concentrations in all the other experimental chicks. However, no notable difference in total triglyceride levels were noted in chicks served with 450 g/Kg dw of both *M. oleifera* and *M. stenopetala,* and 250 g/Kg dw of *M. Stenopetala* leaf powder supplemented feeds.

Table 4.3

Effects of the leaf powder of M. oleifera and M. stenopetala on serum biochemical parameters of experimental broiler chicks

Parameter (concentration)	Powder supplement						
	MOP			MSP			Control
	250 g/Kg dw	450 g/Kg dw	650 g/Kg dw	250 g/Kg dw	450 g/Kg dw	650 g/Kg dw	
Serum glucose (mmol/L)	142.10± 2.70[a]	123.9 0±1.20[b]	96.73 ±1.96[c]	87.00± 3.50[c]	111.20± 2.80[bc]	124.10± 1.10[b]	128.70± 2.60[b]
Total protein (g/L)	20.75 ±4.42[cd]	31.40± 2.82[a]	28.60± 3.00[ab]	19.92± 4.76[cd]	6.58± 0.10[e]	14.00± 0.86[d]	26.81± 2.69[bc]
Albumin (g/dl)	3.73± 0.35[ab]	5.60± 0.37[a]	4.52±0.52[ab]	3.72± 0.74[ab]	1.74± 0.45[b]	3.51 ±0.50[ab]	3.83 ±0.76[ab]
Creatinine (mg/dl)	1.91 ±0.73[a]	2.80 ±1.05[a]	2.99 ±0.93[a]	3.71 ±1.25[a]	3.59± 1.39[a]	2.30 ±1.35[a]	2.16 ±1.48[a]
Blood Urea (mg/dl)	11.10± 1.02[bc]	11.89 ±1.20[bc]	11.60± 1.20[bc]	10.52 ±1.44[bc]	11.24 ±0.73[bc]	13.20± 1.10[b]	18.30 ±1.90[a]
Total Cholesterol (mg/dl)	126.60 ±1.90[d]	82.90± 2.20[f]	82.08± 5.73[f]	152.30± 2.90[b]	145.80 ±4.80[c]	102.70 ±1.40[e]	168.80 ±3.40[a]
Triglyceride (mg/dl)	125.60 ±1.20[a]	49.56± 9.74[d]	82.08± 5.73[c]	40.03± 5.55[d]	50.92 ±7.84[d]	88.30 ±1.05[c]	93.30± 1.70[b]
Calcium (mmol/L)	11.03± 2.40[a]	8.34 ±1.30[a]	13.40± 0.26[a]	10.04± 2.01[a]	10.45 ±2.63[a]	11.10 ±0.61[a]	9.90± 0.98[a]

Values are presented as x̃±SEM; Means with similar superscript alphabet within the same row are not significantly different (One-Way ANOVA followed by Tukey's test)

MOP: *Moringa oleifera* powder; MSP: *Moringa stenopetala* powder

The results for the effects observed from the extracts of the *Moringa oleifera* and *Moringa stenopetala* on the biochemical parameters in blood of the broiler chicks are presented in Table 4.4. The results revealed that the glucose levels in the chicks that received leaf extracts of the studied plants at all dose levels and those in the control group indicated no notable difference.

The total protein levels in the blood serum of the chicks that received the sum total of every plant extract and those in the control groups were significantly lower than those that received aqueous extracts from *M. oleifera* at 25 mg/kg bw ($p<0.05$). However, the total protein levels in the blood serum of the chicks that got the aqueous extracts of M. oleifera at 1mg/kg bw and 5 mg/kg be dose levels, *M. stenopetala* at 1 mg/Kg bw and the ethanolic extract of *M. oleifera* at 5 mg/Kg bw were not meaningfully different ($p>0.05$; Table 4.4).

The albumin content in the blood serum of the chicks that received the aqueous extracts of *M. stenopetala* dosage levels computed at 25 mg/kg bw was meaningfully lower when compared to those of the other chicken that received the rest of the studied plants leaf extracts and those in the controlled group of experimental animals.

In contrast, the serum albumin content of chickens in the control group and chickens that received plant leaf extracts was tested at a dose of 1 mg/kg body weight and 25 mg/kg bw, except for the water-receiving group. The dose of *M. stenopetala* extract was 25 mg/kg bw, and there was no significant difference between them. Similarly, the serum albumin content of chickens treated with plant leaf extracts at a dose of 5 mg/kg bw did not demonstrate significant difference.

Furthermore, the blood serum creatinine concentrations in the chicks in all the treatment groups were not significantly different. However, the blood creatinine in the blood serum of the chicks that received the studied plants leaf extracts at 1 mg/ml and 5 mg/Kg bw were similar ($p>0.05$;Table 4.4). Additionally, at all doses, the blood urea levels in the control chickens were significantly higher than chickens treated with the leaf extracts of the test plants. Moreover, the total cholesterol levels and calcium content in the blood serum of the chicks in all the study groups did not demonstrate any significant difference. However, the total triglyceride levels in the blood serum of the chicks that received the aqueous extracts of *M. stenopetala* at dosages of 5 mg per Kg body weight and 25 mg/Kg bw were significantly higher than the levels obtained for the rest of chicks under research.

In addition, the total triglyceride levels in the blood serum of the chicks that received the ethanolic extracts of the *M. stenopetala* at 1 mg/kg of body weight and 25 mg/kg of body weight dose levels, the aqueous and ethanolic extracts of *M. oleifera* at 1 mg/Kg bw and the control group were not significantly different. Also, the total triglyceride levels obtained in the treatment groups that received the ethanolic extracts of *M. oleifera* and *M. stenopetala,* and the aqueous extracts of *M. oleifera* at dosages of 5 mg per Kg of rated body weight which did not have any difference that was noteworthy.

Table 4.4

Effects of the leaf aqueous and ethanolic extracts of M. oleifera and M. stenopetala on serum biochemical parameters of experimental broiler chicken

Dose (mg/Kg bw)	Extract	Serum glucose (mmol/L)	Total protein (g/L)	Albumin (g/dl)	Creatinine (mg/dl)	Blood Urea (mg/dl	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Calcium (mmol/L)
1	MOE	71.66±4.23[a]	14.14±4.44[bcde]	5.18±1.16[ab]	2.18±0.61[a]	42.50±15.60[ab]	193.70±38.40[a]	97.06±0.80[ab]	10.22±4.15[a]
	MOW	119.60±18.50[a]	24.89±2.04[abc]	3.08±0.87[ab]	2.11±1.12[a]	47.80±10.20[ab]	100.36±5.88[a]	69.35 ±9.62[ab]	8.49± 1.11[a]
	MSE	88.36±6.03[a]	4.03±0.64[e]	2.78±0.98[ab]	0.44±0.35[a]	45.70±13.80[ab]	162.80±29.00[a]	92.30±26.50[ab]	8.51±1.95[a]
	MSW	92.22±6.58[a]	2.90 ±0.42[abc]	2.36±0.23[ab]	3.46±0.71[a]	46.09±5.36[ab]	135.30±38.90[a]	50.80±10.20[b]	11.12±0.86[a]
5	MOE	103.00±18.70[a]	18.12±3.07[abc]	4.07±0.96[a]	1.62±0.76[a]	48.40±15.90[ab]	148.00±23.90[a]	106.70±9.20[abc]	10.72±2.53[a]
	MOW	102.70±26.90[a]	21.10±4.54[abc]	3.05±0.92[a]	1.57±0.94[a]	52.65±8.79[ab]	127.40 ±25.30[a]	83.42±7.30[abc]	9.55±1.80[a]
	MSE	136.9±22.3[a]	26.38±3.84[ab]	5.01±1.48[a]	2.27±1.17[a]	53.00 ±11.80[ab]	91.40±15.70[a]	81.20±24.80[abc]	9.09±1.00[a]
	MSW	115.41±7.17[a]	9.49±1.45[c]	3.94 ±1.10[a]	2.09±0.45[a]	52.40±16.00[ab]	167.90 ±20.80[a]	157.10±28.60[a]	8.34±1.25[a]
25	MOE	106.2±15.4[a]	4.833±0.80[de]	2.89±0.24[ab]	5.43±0.71[a]	20.55±0.73[bc]	172.00±40.50[a]	50.60±13.00[bc]	11.95±.64[a]
	MOW	124.4±23.4[a]	29.85±1.15[a]	3.05±0.92[ab]	2.22±1.49[a]	42.60±12.70[abc]	131.40± 44.30[a]	97.80±21.70[bc]	9.94±1.27[a]
	MSE	76.65±7.65[a]	20.17±5.70[abcd]	3.86±1.09[ab]	4.47±0.80[a]	35.13±9.74[abc]	124.90±26.80[a]	116.70±18.70[ab]	12.59±3.09[a]
	MSW	108.20±18.4[a]	4.03±0.64[e]	1.93±0.25[b]	4.44± 0.52[a]	24.78±1.99[bc]	120.6±19.00[a]	175.2±12.70[a]	10.26±1.17[a]
Control group		128.70±28.6[a]	26.81±2.69[abc]	3.83±0.76[ab]	2.16±1.48[a]	73.30±10.90[a]	108.80±37.40[a]	93.30±7.70 [ab]	9.90± 0.98 [a]

Values are presented as x̃±SEM; Means with similar superscript alphabet within the same column are not significantly different (One-Way ANOVA followed by Tukey's test)

MOE: *M. oleifera* ethanolic extract; MSE: *M. stenopetala* ethanolic extract; MOW: *M. oleifera* aqueous extract; MSW: *M. stenopetala* aqueous extract

Changes in body metabolism, organ morphology and feeding behaviour, especially when supplemented or alternative feeds are served to birds are expected (Rezende et al., 2017). These can be detected in serum biochemical parameters and the information used for mitigation and improvement purposes. Serum glucose levels are good markers for the feeding state of the animal. High levels partly denote replete state whereas low levels indicate fasting state. In view of this, in almost all the groups of chicks that were fed on the two studied plant powders, the glucose levels did not have any differentiable levels in comparison with those in the control group. This denotes better feeding and replete state which in turn translates to normal health and growth in animals (Smith et al., 2018).

The total protein concentrations in the experimental chicks were within the reported normal reference ranges of 20-45 g/L except for the group that received 650 g/Kg dw of *M. stenopetala* leaf powder supplemented feed (Hussein & Jassim, 2019; Rezende et al., 2017). Proteins are synthesized by liver and play various roles in the body by acting as mediators of metabolism (enzymes), growth (structural proteins), regulators of signal transduction and metabolism (hormones), defence against diseases (antibodies), osmoregulation, buffers among other functions (Criscitiello et al., 2020; Café et al., 2012; Rezende et al., 2017). Therefore, the protein content can be a good indicator of the state of these functions in the animal. In this study, the studied plants leaf powder supplements did not adversely influence protein concentrators in the plasma of the experimental chicks.

In this study, higher serum albumin levels were obtained in experimental chicks whose feed was supplemented with the studied plants leaf powders. These results were higher than those reported at an earlier date by (Rezende et al., 2017). The

rationale is based on high protein consumption offered by the *Moringa* feed supplements. Albumin is the major transport of drugs among other molecules in blood. Additionally, it facilitates maintenance of blood osmotic pressure, therefore ensuring steady health in animals (Rezende et al., 2017; Tijani et al., 2016). Therefore, these benefits were suggestively conferred by the supplemented feeds which were served to experimental broiler chicks.

Blood urea and creatinine concentrations are influenced by protein content in the animal. Furthermore, creatinine is an indicator of muscle activity, therefore, high levels depict increased muscle metabolism (Ghanavi et al., 2017;Café et al., 2012). Researches indicate that older broiler chicken have low urea and creatinine levels due to their reduced turnovers (Orlowski et al., 2017;Zaefarian et al., 2019). In this study, inclusion of *Moringa oleifera* and *Moringa stenopetala* in the feeds did not significantly affect creatinine levels in broiler chicken. This partly suggest that these feeds in one way or another helped maintained the muscle turnover thereby wading away wastage. This was evidenced in the health thriving and growth of these experimental chicken.

The total cholesterol concentrations were greatly reduced in experimental broiler chicks that were supplemented with the studied plant powders. High total cholesterol has been implicated in cardiovascular syndromes among other pathologies (Kothari et al., 2019;Bahmani et al., 2015). Therefore, *M. oleifera* and *M. stenopetala* leaf powders contain phytosterols, which when consumed, reduce the bad cholesterol in the body. These phytosterols have been shown to contain high density lipoproteins (HDL) which has been demonstrated to confer many health benefits to humans (Stohs & Hartman, 2015).

Moreover, in this study, the total triglycerides were greatly reduced in almost all groups of experimental chicks. Previous studies have shown that triglycerides are synthesized in the intestinal mucosa as well as in the liver tissue following digestion of dietary components (Abbas et al., 2018; Mahfuz & Piao, 2019a; Rezende et al., 2017). The triglyceride levels are influenced by sex, age and hormonal status of the animal (Phelps et al., 2019). In this study, the high triglyceride levels reported in the experimental group that received 250 g/Kg dw of *M. oleifera* supplemented feed could be due to the sex influence.

It was noted that the calcium content was not influenced by *Moringa* spp leaf inclusion in the feeds. Calcium plays important roles in bone formation and health (Vannucci et al., 2018;Rezende et al., 2017). Therefore, the studied plant powders may have maintained astrocytic homeostasis thereby ensuring normal health.

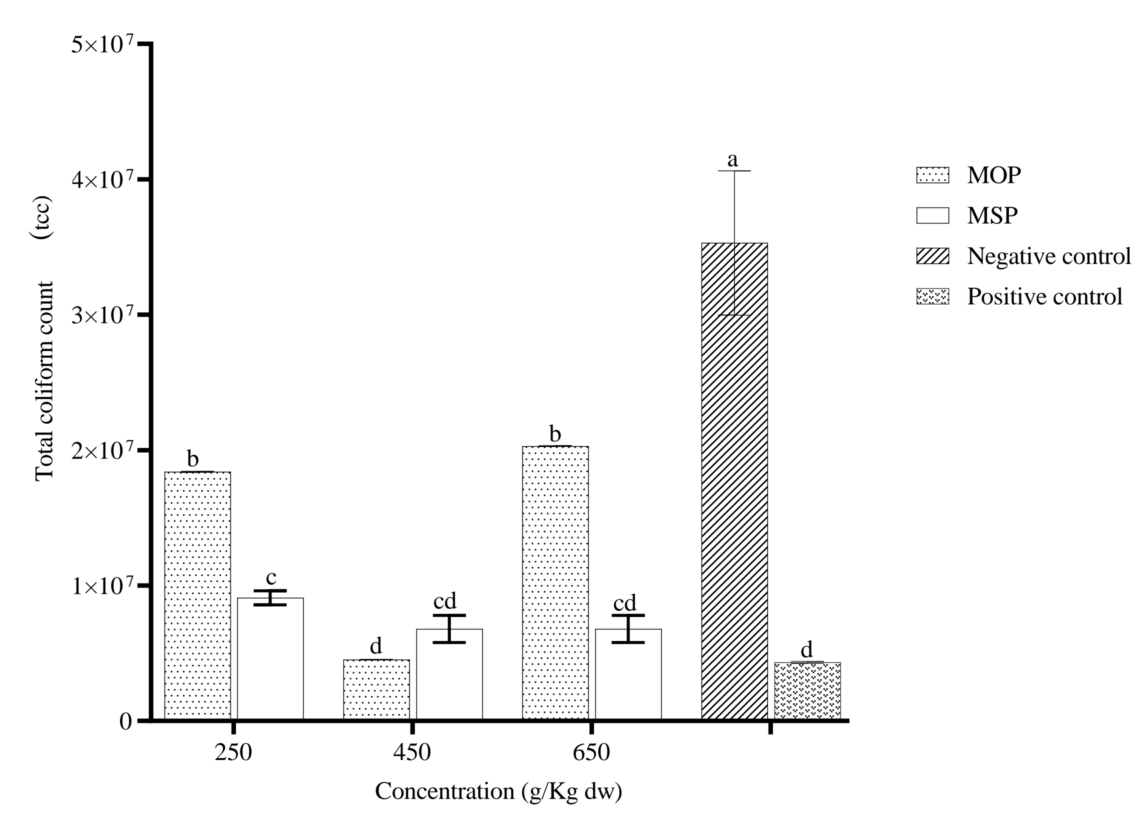
4.3 Effects of *M. stenopetala* and *oleifera* extracts on the gut microbiota

The results for the effect of the *M. oleifera* and *M. stenopetala* leaf powder on the levels of the gut microbiota in the broiler chicks are represented in Figure 4.7. The results showed that the negative control recorded significantly higher levels of the gut microbiota as in comparison with other groups treated ($p<0.05$; Figure 4.7).

Levels of gut microbiota in the chicks that fed on the feed supplemented with *M. oleifera* leaf powder at dose levels of 250 g/Kg dw and 650 g/Kg dw showed no level of significance ($p>0.05$; Figure 4.7). Similarly, the levels of the gut microbiota in broiler chicks that fed on the feed that was supplemented with the leaf powder of *M. stenopetala* at all dose levels and *M. oleifera* at dose of 450 g/Kg dw and those in the positive control were did not have any notable deviation between groups.

Figure 4.7

Effects of the M. leaf powder supplements on total coliform count of gut microbiota in broilers



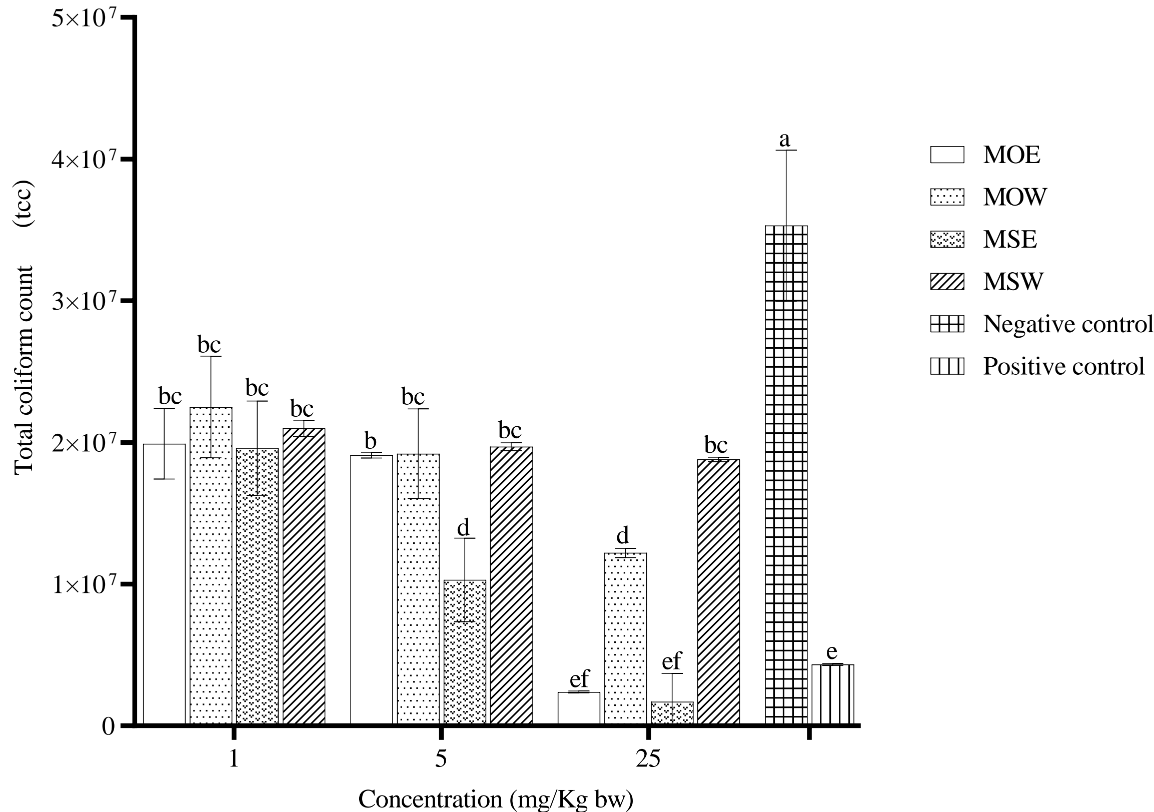
Values are plotted as x̃±SEM, Bars with same superscript letter within respective dose levels not having any notable differences (one-way ANOVA done sequentially with Fisher's LSD; $p>0.05$); MOP: *Moringa oleifera* powder; MSP: *Moringa stenopetala* powder; 250,450 and 450 represent the concentration in g/Kg dw; Positive control: Standard commercial feed; Negative control: uncomplimented feed devoid of AGPs.

The results for the effects of the aqueous and ethanolic leaf extracts of the M. oleifera and *M. stenopetala* on levels of gut microbiota are represented in Figure 4.8. The results indicated that the negative control group registered significantly higher levels of the gut microbiota as compared to the rest of the treatment groups. For broiler chickens that received the ethanolic and aqueous related extracts from M. oleifera tree at dose level of 5 mg/kg body weight and 25 mg/kg body weight, as well

as the ones receiving *M. stenopetala* extract at dosage of 1 mg/Kg; and, *M. stenopetala* administered in all levels indicated no significant difference between the experimental groups. Compared with all other treatment groups, the positive control group had the lowest intestinal flora value.

Figure 4.8

Effects of the aqueous and ethanolic leaf extracts of M. oleifera and M. stenopetala on the total coliform count of gut microbiota



The values expressed as x̃ ±SEM, indicating that there is no significant difference in the superscript letters corresponding to the same parameters (between rows) (one-way ANOVA followed by Fisher's LSD; p>0.05);MOE: ethanolic leaf extract of *Moringa oleifera*: MOW: Aqueous leaf extract of *Moringa oleifera*; MSE: Ethanolic leaf extract of *Moringa stenopetala*; MSW: aqueous leaf extract of *Moringa stenopetala;*1,5 and 25 represent the concentration in mg/Kg bw.; Positive control-Standard commercial feed; Negative control-uncomplimented feed devoid of AGPs

Dietary supplementation of plant-derived materials(powders/extracts/ concoctions/ tinctures) has been shown to performance of chicken growth in broilers (Aroche et al., 2018;Kothari et al., 2019). However, the specific mechanism through which this is achieved has not been elucidated. Research data has revealed the significance of

antibiotic growth promoters (AGPs) in regard to the modulation of gut microbiota and growth performance of broiler chicken (Salaheen et al., 2017).

Earlier studies have implicated antimicrobial-mediated growth improvement in broiler chicken, facilitated by a reduction in pathogenic bacteria in the gut to reduce nutrient competition (Pham et al., 2020;Fajardo et al., 2016). Furthermore, reduction in microbial population also reduces microbial metabolites which impede proper growth of chicken (Gadde et al., 2017).

It has also been speculated that antibiotics interact with immune cells, reduce inflammatory retort (Tarradas et al., 2020). Moreover, the animals' appetite is supressed as muscle catabolism is triggered, thereby reducing energy wastage which is then directed to production (Wicks et al., 2019;Wicks et al., 2019). Therefore, inclusion of antibiotics in the feeds ultimately ensures balanced microbiota and in a cascade of various mechanisms increase energy return from the consumed feed leading to improved growth performance (Maastricht & Version, 2020;Salaheen et al., 2017; Walter et al., 2011).

The antimicrobial effects of *Moringa* species has been demonstrated (Maastricht & Version, 2020;Raghavendra et al., 2016). Perhaps, the gut microbiota homeostasis observed in *Moringa* species fed chicken can be attributed to the antibacterial activity of these plants. Research has shown that prolonged use and inappropriate concentrations of synthetic antibiotics has undesirable health effects (Laws et al., 2019). Moreover, even though they promote growth performance, their accumulation in the body due to inefficient metabolism and excretion has been identified as

contributor to antibiotic resistance (Manyi-Loh et al., 2018;Cheng et al., 2019;Steczny et al., 2017).

It is beyond reasonable doubt that the need for safer growth promoters is warranted. Based on the obtained results, from powdered leaves, and extracts of *M. oleifera* and *M. stenopetala* significantly improved beneficial gut microbiota which was reflected in enhanced growth and health of experimental chicks. These findings were consistent with earlier studies which found that inclusion of *Moringa* spp. leaf in broiler chicken significantly improved gut microbiota and growth performance (Alabi et al., 2017; Edu et al., 2019; Nkukwana et al., 2014; Sarker et al., 2017).

4.4 Phytochemical and nutrient composition of *M. oleifera* and *stenopetala* leaf

As per objectives of this study, qualitative and relative abundance of phytochemical compounds of the studied plant powders were evaluated. Moreover, crude protein, carbohydrate, crude lipid, amino acid, and vitamin concentration in the samples collected from *M. oleifera* and *M. stenopetala* were determined. Additionally, mineral element content in the studied plant samples were determined.

4.4.1 Qualitative and quantitative phytochemical composition of *M. oleifera* and *M. stenopetala* leaf powders

Research has shown that the *Moringa* species possess a wide range of phytochemical compounds which contribute to various pharmacologic activities (Rani et al., 2018). Due to differences in agro-climatic differences in various regions of the world, it is important to carry out phytochemical screening of local plants to determine their phytochemical profile. Moreover, intrinsic, and extrinsic factors have shown to play

major roles in phytochemical composition and quantities of secondary metabolites (Altemimi et al., 2017).

In this study, the qualitative screening of phytochemicals showed that there are multiple groups of pharmacologically active compounds in *M. oleifera* and *M. stenopetala* leaf powder, including, flavonoids, cardiac glycosides, phenolic compounds, saponins, phytosterols, and coumarin and terpenoids. Also, groups of functional compounds with nutritional value were discovered, including carbohydrates, vitamins, lipids, and proteins (Table 4.5).

Table 4.5

Qualitative phytochemical composition of leaf powders of Moringa

Phytochemical	Test/treatment	*M. oleifera*	*M. stenopetala*
Carbohydrates	Benedict's test	+	+
	Fehling's Test	+	+
Proteins	Biurettest	+	+
	Ninhydrin Test	+	+
Lipids	Sudan III test	+	+
Vitamin C	Dichlorophenolindophenol (DCPIP) Test	+	+
Alkaloids	Dragendorff's test	+	+
	Mayer's Test	+	+
Tannins	$FeCl_3$ test	+	+
	Lead sub acetate test	+	+
Saponins	Frothing test	+	+
Flavonoids	Ammonia test	+	+
	Hcl acid test	+	+
	Na_2Co_3 (Sodium carbonate) test	+	+
	NaOH	+	+
Phenols	$FeCl_3$ test	+	+
Glycosides	Keller-Killiani test (Cardiac glycosides)	+	+
Coumarins	$FeCl_3$ test	+	+
	Alcoholic $FeCl_3$ test	+	+
Steroids	Salkowski test	+	+
Terpenoids	Salkowski test	+	+

+: Present

Furthermore, qualitative phytochemical composition of the aqueous and ethanolic leaf extracts of *M. oleifera* and *M. stenopetala* was determined in this study. The results revealed the presence of alkaloids, phenols, tannins, flavonoids, saponins, coumarins, terpenoids, steroids, and glycosides in the aqueous and ethanolic leaf extracts of the two studied plants. Additionally, carbohydrates, lipids, vitamin C, and proteins were detected. Table 4.6 presents the results.

Table 4.6

Qualitative phytochemical composition of the aqueous and ethanolic leaf extracts of M. oleifera and M. stenopetala

		M. oleifera		***M. stenopetala***	
Phytochemical	**Test/treatment**	**Aqueous**	**Ethanolic**	**Aqueous**	**Ethanolic**
Carbohydrates	Benedict's test	+	+	+	+
	Fehling's Test	+	+	+	+
Proteins	Biurettest	+	+	+	+
	Ninhydrin Test	+	+	+	+
Lipids	Sudan III test	+	+	+	+
Vitamin C	Dichlorophenolindophenol (DCPIP) Test	+	+	+	+
Alkaloids	Dragendorff's test	+	+	+	+
	Mayer's Test	+	+	+	+
Tannins	$FeCl_3$ test	+	+	+	+
	Lead sub acetate test	+	+	+	+
Saponins	Frothing test	+	+	+	+
Flavonoids	Ammonia test	+	+	+	+
	Hcl acid test	+	+	+	+
	Na_2Co_3 (Sodium carbonate) test	+	+	+	+
	NaOH	+	+	+	+
Phenols	$FeCl_3$ test	+	+	+	+
Glycosides	Keller-Killiani test (Cardiac glycosides)	+	+	+	+
Coumarins	$FeCl_3$ test	+	+	+	+
	Alcoholic $FeCl_3$ test	+	+	+	+
Steroids	Salkowski test	+	+	+	+
Terpenoids	Salkowski test	+	+	+	+

+: Present

Besides, quantitative phytochemical analysis of different degrees of phytochemical clusters *M. stenopetala, M. oleifera* and leaf samples was performed in this study and the findings are presented in Table 4.7. Moreover, a comparison between the concentrations of the selected phytochemicals in *M. oleifera* and *M. stenopetala* was done. The resultant findings were that the relative abundancies of flavonoids, alkaloids and saponins in the leaf powder of *M. stenopetala* were significantly higher than those in the leaf powder of *M. oleifera*. However, the percentage concentration of phenols was significantly higher in the leaf powder of *M. oleifera* than in the leaf powder of *M. stenopetala*.

Table 4.7

Relative abundancies of selected phytochemicals in Moringa leaf powder

Phytochemical	**Abundance (%) Mean±SEM**	
	Moringa oleifera	***Moringa stenopetala***
Flavonoids (%)	$1.12{\pm}0.20^{b}$	$5.64{\pm}1.93^{a}$
Alkaloids (%)	$0.06{\pm}0.00^{b}$	$22.93{\pm}0.55^{a}$
Phenols (%)	$46.81{\pm}3.29^{a}$	$4.20{\pm}1.52^{b}$
Saponins (%)	$14.00{\pm}0.51^{b}$	$35.75{\pm}2.10^{a}$

Values are presented as Mean±SEM; Means with dissimilar superscript letter across the rows are significantly different (un-paired student t-test; $p<0.05$)

Previous studies have shown that *M. oleifera* and *M. stenopetala* have high antioxidant activities due to their high flavonoid content. It has been determined that flavanol and glycoside compounds falling in this category confer antioxidant activity. According to Wang et al. (2017), rutin, quercetin, kaempferol, myricetin, rhamnetin and apigenin are the prominent flavonoid compounds with profound pharmacologic activity.

Elsewhere, the leaves from the plant named *M. oleifera* contain gallic acid as their main phenolic compound. Other phenolic compounds like ellagic acid, caffeic acid, chlorogenic acid, ferulic acid, o-coumaric acid among others have been identified in *M. oleifera* extracts (Otieno et al., 2016). Apart from their remarkable antioxidant properties, plant-derived phenolics exhibit antidiabetic, anti-inflammatory, anticancer, among other bioactivities (Rani et al., 2018; Seifu, 2015).

Therefore, it is suggestive that consumption of diets containing *Moringa* powder can confer these beneficial effects. Antioxidant properties of plant phytoconstituents exhibit hepatoprotective efficacies against chemical and drug induced toxicity in animals. Furthermore, antioxidant phytocompounds ameliorate oxidative stress either solely or synergistically thereby promoting health.

In addition, studies have shown the importance of the concentration of total phenols, total flavonoids and ascorbic acid as parameters for the antioxidant capacity of medicinal plants (Aryal et al., 2019;Subedi et al., 2014; Rumit et al., 2010; Brahmi et al., 2012; Raghavendra, et al., 2013). Saponins are known to have hypocholesterolaemia, anti-inflammatory, haemolytic (Leon & Johanna, 2018).

Alkaloids on the other hand possess analgesic, antispasmodic, and bactericidal properties (Umaru et al., 2019; Tlili et al., 2019; Batiha et al., 2020and Naser et al., 2013). The presence and abundance of these phytocompounds is beneficial and possibly the reason behind the extensive use of these plants both as food and as medicines and thus health promoters. As a result, inclusion of Moringa leave powders in poultry feeds is expected to offer these benefits and hence promote healthy growth.

The solvent of extraction of plant materials determines the concentration and type of active principles extracted (Altemimi et al., 2017; Dhanani et al., 2017). In this study, water and ethanol were used due to their extensive application and ability to extract and concentrate pharmacologically valuable compounds. In this study, all the phytochemical compounds that were present in powders made from M. oleifera together *M. stenopetala* were also present in the extracts. This indicated that the polarities of water and ethanol were suitable and corresponded to the phytochemicals present in the two plant materials, and successfully extracted them (Dhanani et al., 2017). This suggests that administration of these extracts, in therapeutic forms, could potentially offer the bioactive principles just like the powders, and may be a good replacement.

4.4.2 Proximate composition of leaf powders of *M. oleifera* and *M. stenopetala*

The obtained results revealed that the leaf powders of *M. stenopetala* had significantly higher percentages of crude fibre and carbohydrate content than those in the leaf powder of *M. oleifera* ($p<0.05$; Table 4.8). However, no notable deviations in the percentage crude lipid and crude protein concentrations were observed between the leaf powders of the two plants ($p>0.05$; Table 4.8)

Table 4.8

Proximate composition of selected parameters in leaf powders of Moringa

Parameter	Leaf powder	
	M. oleifera	***M. stenopetala***
Crude fibre (%)	1.58 ± 0.45^{b}	5.09 ± 2.47^{a}
Crude Lipid (%)	6.06 ± 1.63^{a}	5.03 ± 0.05^{a}
Crude Protein (%)	23.05 ± 0.62^{a}	19.42 ± 3.70^{a}
Carbohydrate (%)	41.97 ± 0.72^{b}	46.01 ± 0.04^{a}

Values are presented as Mean±SEM; Means with dissimilar superscript letter across the rows are significantly different (un-paired student t-test; $p<0.05$)

Previous studies have shown that there are inconsistencies in percentage proximate composition in samples from *Moringa* species (Stadtlander & Becker, 2017). Partly, this is attributable to the agroecological zone where the plants are grown, the developmental stage at which the parts are harvested, as well as specific growth conditions (Lamidi et al., 2017). Considering these factors, the results reported in the current study varied from those of other scholars such as (Bamishaiye et al., 2011; Valdez-Solana et al., 2015; Abdulkadir et al., 2016) among others. It is however notable that crude fibre helps to promote digestion, by increasing food bulk and reducing transit time along the gastrointestinal tract.

Lipids, carbohydrates and proteins are important nutritional components of any mean, which when consumed play both structural and physiological roles in the body. At optimal levels, these biologically important molecules promote health, growth, and defend against or alleviate diseases. Therefore, it is conceivable that their quantities in the two *Moringa* species may have contributed significantly to the good health and growth performance in broiler chicken, used in this study.

4.4.3 Nutrient composition of leaf powders of *M. oleifera* and *M. stenopetala*

It is undisputable that proteins are essential components of all animal tissues and therefore, significantly influences growth and performance of broiler chicken (Gadzirayi et al., 2012; Sarker et al., 2017). Consequently, it is imperative to understand nutritional requirements of essential amino acids as this helps to determine the appropriate feeding formula to achieve optimal results (Slavin, 2012).

Furthermore, optimal amino acid concentration in feed helps broiler chicken to gain weight and breast meat thereby maximizing profits. In view of these, this study sought to determine the concentration of some essential amino acids in the studied plant powders. The results showed lack of substantial differences in the composition of the selected essential amino acids in the leaf powders of *M. stenopetala* altogether with M. oleifera ($p>0.05$; Table 4.9).

Table 4.9

Proximate concentration of essential amino acids in the leaf powders

Amino acid	Concentration of amino acids in mg/100 g of DW	
	M. oleifera	***M. stenopetala***
Phenylalanine	146.6 ±1.01[a]	143.7 ± 0.74 [a]
Valine	183.4 ± 0.63[a]	187.8 ± 0.253 [a]
Threonine	264.4 ± 0.001[a]	263.7 ± 0.23 [a]
Tryptophan	300 ± 2.52 [a]	300 ± 1.021 [a]
Methionine	300 ± 0.17 [a]	300 ± 0.84 [a]
Leucine	264.4 ± 0.17 [a]	263.7 ± 0.58 [a]
Isoleucine	292.8 ± 0.23 [a]	292.7 ± 0.18 [a]
Lysine	292.8 ± 0.02 [a]	292.7 ± 0.003 [a]
Histidine	231.1 ± 0.0 [a]	217.9 ± 0.10 [a]
Cysteine	142.7 ± 0.391[a]	136.5 ± 1.06 [a]
Tyrosine	298.6±1.092[a]	294.4 ± 0.98 [a]

Values are expressed as Mean±SEM; Means that do not share a superscript across the rows are significantly different at p≤0.05.

Studies have shown that sulphur containing amino acids like methionine and cysteine are critical in many ways in the body including promotion of proper enzymatic activity. Furthermore, methionine is a precursor of cysteine, and together play vital roles in protein synthesis in the body (Leeson, 2008; Mehdi et al., 2018).

Just like methionine and cysteine, lysine is also a limiting amino acid often used as a reference for rationing essential amino acids in an appropriate formula (Shumo et al., 2019; Ravindran, 2014). Hence, it is important to accurately determine the ratio of methionine, lysine and cysteine required in chicken feed to offer optimal growth results to gain commercial advantage.

The National Research Council guidelines have been adopted by many poultry nutritionists to formulate feeds (Academy, 1994). However, research has shown that the amino acid estimates in these guidelines are way too low for optimal broiler chicken growth performance (Jha et al., 2019). As a result, it is important to have methionine and lysine concentrations above the National Research Council recommended levels as in the case of the current study so that optimal benefits are achieved.

Additionally, the significance of proteins in immunity cannot be overemphasized (Farkhoy et al., 2012; Gadzirayi et al., 2012; Rezende et al., 2017). Various studies have shown that amino acids, in appropriate concentrations play great roles in improving growth and immunity performance in animals. It is therefore anticipated that the presence and abundance of these essential amino acids in the studied pant powders potentially improved the growth and performance of the immune system of the studied broiler chicken thereby thwarting infections and maintaining optimal health.

4.4.4 Composition of selected vitamins in leaf powders of *M. oleifera* and *M. stenopetala*

Vitamins are important in health as they play important roles as catalytic coenzymes, cofactors and as precursors for synthesis of key molecules in the body (Yoshii et al., 2019;Abidemi, 2013; Shanmugavel et al., 2018; Yoo et al., 2016). In this regard, selected essential vitamin composition in the two studied Moringa species was determined in this study.

Upon determination of proximate vitamin concentration, it was observed that thiamine levels in both studied plant powders were not considerably varied ($p>0.05$; Table 4.10). However, the concentrations of pyridoxine, niacin, folic acid, and ascorbic acid were significantly higher in *M. stenopetala* leaf powder than in the leaf powder of *M. oleifera* ($p<0.05$; Table 4.10).

Table 4.10

Concentration of selected vitamins in leaf powders of M. oleifera and M. stenopetala

Vitamin	**Concentration of vitamins in mg/100 g of DW**	
	Moringa oleifera	***Moringa stenopetala***
Thiamine (Vit. B1)	0.85 ± 0.001^{a}	0.70 ± 0.004^{a}
Pyridoxine (Vit. B6)	0.25 ± 0.003^{a}	0.16 ± 0.003^{b}
Niacin (Vit. B3)	0.45 ± 0.002^{a}	0.33 ± 0.004^{b}
Folic acid (Vit. B9)	0.27 ± 0.003^{a}	0.24 ± 0.002^{b}
B-carotene (Vit. A)	0.27 ± 0.003^{a}	0.24 ± 0.002^{b}
Ascorbic acid (Vit. C)	8.63 ± 0.003^{a}	51.04 ± 0.007^{b}

Values are presented as Mean±SEM; Means that do not share a superscript across the rows are significantly different at α=0.05 (un-paired student t-test).

Research has shown that vitamin A and C supplementation in meals improves immunity and growth in broiler chicken. Notably, various studies have established that the National Research Council recommended vitamin levels in poultry are way too low for optimal growth and performance especially in broiler chicken (Dalólio et al., 2015).

For instance, ascorbic acid (vitamin C) requirements for optimal immunocompetence in broiler chicken are much higher, up to 10-fold, than those stipulated in the National Research Council (Carr & Vissers, 2018). The amounts of vitamin A obtained in this study for both *Moringa* species were higher with *M. stenopetal* are cording significantly higher levels. Certainly, the beneficial effects associated with this vitamin were conferred to the experimental chicks ensuring their health, growth, and performance.

Abundant evidence has demonstrated the consequences of vitamin deficiency in animals (Abidemi, 2013;Tuohimaa, 2012;Diab & Krebs, 2018). In broiler chicken, inadequate supply of vitamin C has been associated with impaired immunity and vulnerability to infections. However, it should be noted that very high concentrations of vitamin A leads to hypervitaminosis, which can increase morbidity as a results of malabsorption syndrome or other adverse events (Diab and Krebs, 2018).

Vitamin C helps maintain the integrity and health of mucous membranes of the eyes, gastrointestinal and respiratory tracts thereby optimizing native immune defence against pathogenic insults (Bouba et al., 2012; Raghavendra et al., 2016; Valdez-Solana et al., 2015). Besides, other studies have shown that vitamin C in appropriate concentrations enhance utilization of stress-associated corticosteroids and facilitate stress alleviation thereby improving survival rate in broiler chicken.

On the other hand, β-carotene (Vitamin A) is important in vision and as an intracellular free radical scavenger (antioxidant) (Edu et al., 2019; Ravindran, 2014; Seifu, 2014). Free radicals have been recognized as key triggers and driving factors in many diseases (Pallio et al., 2017). Oxidative stress emanating from excessively

generated free radicals damages cell membranes and other biologically important biomolecules leading to impaired functioning and ultimately death. As a remedy, antioxidant vitamins like A, C, D, E and K work to quench free radicals thereby averting oxidative stress by restoring redox homeostasis in the body (Tan et al., 2018; Mitchel Otieno et al., 2016; Xu and Howard, 2012).

In the body, Thiamine (vitamin B1) function as coenzyme cocarboxylase (thiamine pyrophosphate, TPP) which has significant role in the tricarboxylic acid cycle (TCA) (Pavlović, 2019). The TCA cycle integrates the metabolism of carbohydrates, lipids as well as proteins geared towards energy synthesis. Vitamin B1 is the major coenzyme in all enzymatic decarboxylation reactions of α-keto acids.

Vitamin B9 (folic acid) takes the form of 5, 6, 7, 8-tetrahydrofolic acid (THF) and is important in processes involving transferring singular carbon atoms in a diverse array of biochemical reactions. Most importantly, the biosynthetic relationship in THF medicated carbon unit transfer exist in reactions of amino acid interconversions in the body (Abidemi, 2013; Bouba et al., 2012). For instance, synthesis of homocysteine, mono and polyglutamate as well as methionine. Additionally, folic acid is important in the synthesis of purines and pyrimidines which are critical in the genome. Folic acid also helps in the formation of haemoglobin which carry oxygen in the body. Its deficiency is associated with various anomalies including anaemia (Pavlović, 2019).

Vitamin B3 (Niacin) is a part of nicotinamide containing coenzymes; nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD). All these coenzymes are crucial links in the reactions involved in

carbohydrate, protein and lipid metabolism which furnish the animal with essential energy for various functions (Gasperi et al., 2019;Cantó et al., 2015). Moreover, nicotinamide containing enzymes comprise of a group of hydrogen transfer agents in the body from oxidizable substrates to oxygen, in detoxification reactions and in maintaining redox homeostatic states, ensuring normal body functioning and health (Pallio et al., 2017).

On the other hand, vitamin B6 (Pyridoxine) is converted to pyridoxal-5-phosphate which in turn takes part in the transamination reactions of amino acids. Transamination reactions are important in the interconversion of amino acids in the liver; therefore, deficiency is associated with detrimental results (Parra et al., 2018;Detox, 2018) The abundance of these essential vitamins in the studied plant leaves is an indicator of nutritional value and adequacy in supply, therefore ensuring proper growth and performance of broiler chicken.

4.4.5 Composition of selected mineral elements in leaf powders of *M. oleifera* and *M. stenopetala*

Mineral elements are micronutrients required in small concentrations in the body for various metabolic functions (Raghavendra et al., 2016). In the daily diet, both minor and major micronutrients are required in appropriate amounts in the food/feed so as to meet the body requirements (Bouba et al., 2012; Rahman et al., 2013). Mineral deficiency is associated with various diseases and disorders that affect both animals and humans (Raghavendra et al., 2016; Valdez-Solana et al., 2015).

In this study, the results indicated significantly higher calcium levels in *Moringa oleifera* leaf powder than in the leaf powder of *Moringa stenopetala* ($p<0.05$; Table

4.11). Similarly, the leaf powder of *Moringa stenopetala* had a significantly higher concentration of sodium compared with the concentration of this mineral in the *Moringa oleifera* leaf ($p<0.05$; Table 4.11). However, no significantly differences were observed in all the other mineral concentrations between the studied plant powders ($p>0.05$; Table 4.11). These results collaborate with those earlier reported in literature (Raghavendra et al., 2016; Valdez-Solana et al., 2015).

Table 4.11

Mineral element concentration in leaf powders of M. oleifera and M. stenopetala

Element	Concentration of elements in mg/100 g of DW	
	M. oleifera	***M. stenopetala***
Cu	0.08 ± 0.07^{a}	0.15 ± 0.08^{a}
Mn	0.84 ± 04^{a}	0.46 ± 0.04^{a}
Fe	0.21 ± 0.13^{a}	0.39 ± 0.02^{a}
Zn	6.65 ± 1.51^{a}	22.26 ± 0.50^{a}
Ca	1652.30 ± 4.02^{a}	1042.30 ± 34.30^{b}
Mg	37.069 ± 0.11^{a}	294.70 ± 41.10^{a}
Na	163.90 ± 8.09^{b}	33.38 ± 1.59^{a}
K	$304.50 \pm 57.1\ ^{a}$	$443.50\pm19.50\ ^{a}$

Values are presented as Mean±SEM; Means that do not share a superscript across the rows are significantly different by unpaired student t-test ($p<0.05$).

Research has shown that manganese, copper and magnesium, among others, play important roles as coenzymes and cofactors for various enzymes in the body. Their deficiency has been associated with various diseases attributable to either defunct or

improper enzymatic activity. Besides, iron is an important mineral involved in haemoglobin synthesis. Haemoglobin is an important carrier molecule for oxygen to tissues and carbon dioxide from tissues to lungs for excretion. Iron deficiency has been associated with anaemia among other diseases in the body (Raghavendra et al., 2016a; Valdez-Solana et al., 2015).

Zinc promotes digestion of food in the gastrointestinal tract, enhances proper growth and reproduction in humans and animals. Its deficiency has been associated with impaired growth, indigestion and sterility/impotence. Besides, it promotes optimal enzymatic activity in the body, thereby ensuring proper body physiology. Calcium is critical muscular physiology, motor activity, and bone synthesis, among others, in the body, and its deficiency may cause deleterious effects (Valdez-Solana et al., 2015).

As such, helpful gains from the studied minerals were potentially conferred to the experimental chicks via the supplemented feeds in this study (Bouba et al., 2012; Mbailao et al., 2014; Raghavendra et al., 2016; Valdez-Solana et al., 2015). Perhaps, the improved growth and performance of broiler chicks witnessed in this study, may have been sourced from the presence of these minerals in adequate levels, which consequently met the daily dietary requirements.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter provides a summarisation of key findings and presents the conclusions that were drawn from the study based on the obtained results of each research objective. Furthermore, various recommendations made based on the study finding, and research prospects are also highlighted.

5.2 Summary of the study

Based on the objectives of this study, the summary was that: the present study investigated the effects of *M. oleifera* and *M. stenopetala* leaf powder-supplemented feeds of growth performance of experimental broiler chicken. The obtained findings revealed that the studied graded powders and extracts at the three dose levels, significantly enhanced growth performance. This was observed in growth performance indicators, including improved feed intake, higher body weight gains, and lower feed conversion ratios in the experimental chicken. The low feed conversion rations recorded in the experimental chicken indicated feed utilization efficiency; hence, utilization of the studied plant powders and extracts could maximize financial returns. Besides, the effects of the leaf powders and extracts (aqueous and ethanolic) of the studied plants on haematological and biochemical parameters were examined in the study. The results showed that the feeds supplemented with graded and powdered leaves of *M. oleifera* and *M. stenopetala,* and the orally administered aqueous and ethanolic extracts of these plants, did not cause haematoxicity; instead, they enhanced the haemoglobin and white blood cell levels in experimental chicken's blood. Based on the study findings, it was

concluded that the leaf powders and the studied extracts of *M. oleifera* and *M. stenopetala* do not cause haematoxicity and contain active principles, which promote health, and their haemaprotective effects were probably conferred into experimental chicken during the treatment period.

The results further showed no alteration of the selected biochemical parameters in experimental chicken which received powdered and graded *M. oleifera* and *M. stenopetala* leaf extracts.

Moreover, the effects of the studied leaf powders and extracts of *M. oleifera* and *M. stenopetala* in experimental chicken's gut microbiota were evaluated in this study. The results revealed low total coliform counts in chicken fed on the studied powders and those administered with the aqueous and ethanolic leaf extracts. It was concluded that these finding could be due to existence of phytocompounds with antimicrobial elements, in the studied powders and extracts which may have inhibited the growth or killed the pathogenic bacteria in the gut of experimental chicken. Eradication of pathogenic bacteria enhances the growth of beneficial normal flora strains of bacteria, which promote food digestibility, absorption as well as synthesis of important vitamins like vitamin K, which are beneficial to the body.

This study evaluated the proximate, phytochemical, and nutrient composition found inside leaf powders and extracts of *M. oleifera* and *M. stenopetala*. Phytochemical experimental investigations demonstrate presence of various health-promoting phytochemical compounds, which may have fostered health and growth of experimental chicken.

In terms of proximate composition of the studied plant powders, it obtained results shown that *M. stenopetala* has more crude fibre than *M. oleifera.* Conversely, it was concluded that *M. oleifera* leaf powder is a suitable source of calcium as it had higher abundance than *M. stenopetala*. However, both leaf powders have similar mineral element profile and as such, either of the powders can be used as sources of feed supplements.

Furthermore, the study findings showed that the leaf powders of the two studied plants are endowed with a variety of important nutrients known to promote health in animals and humans.

5.3 Conclusions Drawn from this Study

The leaf powders and the studied extracts of the two plants are potent enhancers of growth performance in broiler chicken, and probably contain active principles, which are responsible for these effects.

The determined biochemical parameters are indicators of normal tissue metabolism and normal physiology of body organs. Therefore, based on the results obtained, it can be inferred that the studied powders and extracts do not cause biochemical toxicity in broiler chicken. Moreover, it was concluded that the leaf powders and the tested extracts of *M. oleifera* and *M. stenopetala* probably contain concentrated bioactive principles which either prevent damage to cellular components, maintaining their normal physiology and homeostasis or by averting stress. Therefore, the enhanced growth performance evidenced in chicken treated with the studied plant extracts, and those fed on the studied plants' leaf powder-supplemented

feeds, are thought to be due to the presence of health -promoting bioactive phytocompounds.

It was also concluded from the obtained results, that the studied plant extracts and powders do not affect normal flora strains and the enhanced growth performance witnessed in the treated chicks could be partly due to the presence of phytochemicals which deter proliferation of pathogenic strains while promoting gut health.

Furthermore, it was concluded that the two plant powders and extracts possess pharmacologically important phytochemicals which are associated with proper growth, amelioration of oxidative stress, antimicrobial activity, immunomodulation, and maintenance of health. Quantitative phytochemical analysis showed that M. oleifera leaf powder had significantly higher phenolic content than that of *M. stenopetala*, depicting higher antioxidant potency. Nevertheless, the studied plant powders have appreciable concentration of bioactive compounds known to promote health and growth performance.

It was also concluded that the leaf powders of the studied plants have essential amino acids in optimal levels. The amino acid concentrations in the leaf powders of the studied plants are not significantly different, hence, either can supply optimal concentrations required for optimal health and growth. Furthermore, the conclusion was that the leaf powder of *Moringa oleifera* has significantly higher concentration of the assayed vitamins than those in the leaf powder of *Moringa stenopetala.* Generally, the present study concluded that the leaf powders and extracts (aqueous and ethanolic) of *M. oleifera* and *M. stenopetala* are promoters of broiler chicken's growth performance, they deter proliferation of pathogenic microbes while

promoting normal flora and gut health, are nontoxic to blood and biochemical parameters, and contain phytochemicals and nutrient and non-nutrient components, associated with growth performance. Leaves of the studied *Moringa* species are good sources of nutrients.

5.3 Recommendations

In this section, recommendations are made based on research findings from the study as well as recommendations for further studies.

5.3.1. Recommendations from the Study

This study recommends the use of *M. oleifera* and *M. stenopetala* powdered leaves as alternative and safer broiler chicken feed supplements, due to the remarkable results witnessed herein. Also, the use of the studied plant extracts as growth promoters is recommended as they are of portable, and therapeutic quantities, and less laborious, yet with remarkable outcomes. This could alleviate harvesting of huge volumes of these plants, thereby causing a biodiversity crisis.

Since the studied plant powders and extracts showed remarkable results in terms of growth performance, their utilization could maximize economic returns compared to the use of commercially synthesized antibiotic growth promoters, which have been associated with undesirable health effects. Moreover, the studied powders derived from leaves of *M. oleifera* and *M. stenopetala* can be used as sources of essential vitamins, mineral elements and essential amino acids in broiler chicken husbandry. Owing to their antimicrobial effects against gut coliforms, the aqueous and ethanolic leaf extracts, and leaf powders of the studied plants can be used to enhance gastric

health and digestion in broiler chicken. As a result, this will improve feed utilization, boost growth, thwart infections, and better outcome.

5.3.2. Recommendations for Further Studies

Further studies geared towards the validation and authentication of *M. oleifera* and *M. stenopetala* usage in rearing other poultry species, and farm animals are encouraged. Moreover, dosage optimisation of the studied plant extracts to ensure maximum productivity in broiler chicken should be determined. Moreover, the inclusion formula for the leaf powders in the feeds to produce optimal performance should be investigated further.

Furthermore, the specific mechanisms through which the studied plant powders and extracts promote health and proper growth in broiler chicken should be established. Because the two studied *Moringa* species present a promising, safer, cost effective and viable strategy to enhancing growth performance in broiler chicken, and perhaps all the other farm animals, their proper cultivation and protection-through policy formulations and sensitization, should be done to avert extinction.

REFERENCES

Abbas, A. K., Litchman, A. H., & Pillai, S. (2020). *Basic Immunology: Functions and Disorders of the Immune System*, (6th ed.). Elsavier Inc. https://books.google.co.ke/books?hl=en&lr=&id=bSuFDwAAQBAJ&oi=fnd&pg=PP1&ots=PgGVfLdS5G&sig=A5f1ryHKOj_c92QVzrGv3R4sSP4&redir_esc=y#v=onepage&q=inflammation&f=false

Abbas, R., Abdul-Lateef, A. N., Mehmood, A, A., & Jameel, Y. J. (2018). Haematological and Biochemical Indices of Broiler Chicks Fed At Different Levels of Moringa oleifera Leaf Meal. *Biochemical Cell Architecture*, *18*(2), 1931–1936.https://uoqasim.edu.iq/e_learning/pub_folder/Biochem.%20Cell.%20Arch..pdf

Abd El-Hack, M. E., Alagawany, M., Elrys, A. S., Desoky, E. S. M., Tolba, H. M. N., Elnahal, A. S. M., Elnesr, S. S., & Swelum, A. A. (2018). Effect of forage Moringa oleifera l. (moringa) on animal health and nutrition and its beneficial applications in soil, plants and water purification. *Agriculture (Switzerland), 8*(9) 1-10. https://doi.org/10.3390/agriculture8090145

Abd, S. K. (2014). Effect of effective microorganisms on some biochemical parameters in broiler chicks. *Iraqi Journal of Veterinary Sciences, 28*(1), 1–4. https://doi.org/10.33899/ijvs.2014.89463

Abdel-Wareth, A. A. A., Hammad, S., Khalaphallah, R., Salem, W. M., & Lohakare, J. (2019). Synbiotic as eco-friendly feed additive in diets of chickens under hot climatic conditions. *Poultry Science, 98*(10), 4575–4583. https://doi.org/10.3382/ps/pez115

Abdulkadir, A. R., Zawawi, D. D., & Jahan, M. S. (2016). Proximate and phytochemical screening of different parts of Moringa oleifera. *Russian Agricultural Sciences, 42*(1), 34–36. https://doi.org/10.3103/s106836741601002x

Abdulla, J. M., Rose, S. P., Mackenzie, A. M., & Pirgozliev, V. R. (2017). Feeding value of field beans (Vicia faba L. var. minor) with and without enzyme containing tannase, pectinase and xylanase activities for broilers. *Archives of Animal Nutrition, 71*(2), 150–164. https://doi.org/10.1080/1745039X.2017.1283823

Abidemi, O. O. (2013). Proximate Composition and Vitamin Levels of Seven Medicinal Plants. *International Journal of Engineering Science Invention, 2*(5),

47–50. http://www.ijesi.org/papers/Vol%202(5)/version-4/F254750.pdf

Abrar, T., Azmach, N., & Campus, A. (2017). The miraculous moringa trees : From nutritional and medicinal point of views in tropical regions. *Medicinal Plants Studies, 5*(4), 151-162. https://www.plantsjournal.com/archives/?year=2017&vol=5&issue=4&part=C&ArticleId=666

Abu Hafsa, S. H., Ibrahim, S. A., Eid, Y. Z., & Hassan, A. A. (2020). Effect of dietary Moringa oleifera leaves on the performance, ileal microbiota and antioxidative status of broiler chickens. *Journal of Animal Physiology and Animal Nutrition, 104*(2), 529–538. https://doi.org/10.1111/jpn.13281

Abuye, C., Urga, K., Knapp, H., Selmar, D., Omwega, A. ., Imungi, J., & Winterhalter, P. (2003). A composotitional Study of Moringa Stenopetala leaves. *East African Medical Journal, 80*(5), 247–252. https://pubmed.ncbi.nlm.nih.gov/16167740/

Academy, N. P. (1994). Nutrient Requirements of Poultry. *In Nutrient Requirements of Poultry,* (9th ed.). The National Academies Press. https://doi.org/10.17226/2114

Achilonu, M., Shale, K., Arthur, G., Naidoo, K., & Mbatha, M. (2018). Phytochemical Benefits of Agroresidues as Alternative Nutritive Dietary Resource for Pig and Poultry Farming. *Journal of Chemistry, 1*(1), 1-15. https://doi.org/10.1155/2018/1035071

Adamu, M., & Boonkaewwan, C. (2014). Protective Effects of Moringa Stenopetala Leaf Supplemented Diets on Eimeria tenella Infected Broiler Chickens. Agricultural and natural science, 47(3), 398-406. https://li01.tci-thaijo.org/index.php/anres/article/view/243067/165876

Adedapo, A. A., Falayi, O. O., & Oyagbemi, A. A. (2015). Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. *Journal of Basic and Clinical Physiology and Pharmacology, 26*(5), 491–499. https://doi.org/10.1515/jbcpp-2014-0105

Advisory, E., & Down, C. (2019). *Poultry sector study.* http://www.bdsknowledge.org/dyn/bds/docs/960/DFID%20Poultry%20Sector%20Study%20180419.pdf

Aftab, U., Bedford, M. R., & Creswell, D. (2018). Prospects of improving efficiency

of feed utilisation in broiler. *World's Poultry Science Journal, 74*(3), 427–441. https://doi.org/10.1017/S0043933918000466

Al-Nedawi, A. M. (2018). Reference hematology for commercial Ross 308 broilers. *Online Jounrnal of Veterinary Research, 22*(7), 566–570. https://doi.org/10.13140/RG.2.2.34589.97768

Alabi, O. J., Malik, A. D., Ng'Ambi, J. W., Obaje, P., & Ojo, B. K. (2017). Effect of aqueous Moringa oleifera (Lam) leaf extracts on growth performance and carcass characteristics of hubbard broiler chicken. *Revista Brasileira de Ciencia Avicola, 19*(2), 273–280. https://doi.org/10.1590/1806-9061-2016-0373

Alagawany, M., Elnesr, S. S., & Farag, M. R. (2018). The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. *Iranian Journal of Veterinary Research, 19*(3), 157–164. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6184034/

Alayande, K. A., Aiyegoro, O. A., & Ateba, C. N. (2020). Probiotics in animal husbandry: Applicability and associated risk factors. *Sustainability (Switzerland), 12*(3), 1–12. https://doi.org/10.3390/su12031087

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants, 6*(4). https://doi.org/10.3390/plants6040042

Andrade, E. L., Bento, A. F., Cavalli, J., Oliveira, S. K., Schwanke, R. C., Siqueira, J. M., Freitas, C. S., Marcon, R., & Calixto, J. B. (2016). Non-clinical studies in the process of new drug development - Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas, 49*(12), 5646. https://doi.org/10.1590/1414-431X20165646

Angela, N. U., & Chizoba, N. O. (2019). Effects of <i>Moringa oleifera</i> Leaves and Lysine on Growth Performance of Broiler Chicks. *Advances in Applied Physiology, 4*(1), 1-10. https://doi.org/10.11648/j.aap.20190401.11

Anjum, M. S., & Chaudhry, A. S. (2010). Using enzymes and organic acids in broiler diets. *Journal of Poultry Science, 47*(2), 97–105. https://doi.org/10.2141/jpsa.009082

Apajalahti, J., & Vienola, K. (2016). Interaction between chicken intestinal microbiota and protein digestion. *Animal Feed Science and Technology, 221*(1), 323–330. https://doi.org/10.1016/j.anifeedsci.2016.05.004

Aroche, R., Martínez, Y., Ruan, Z., Guan, G., Waititu, S., Nyachoti, C. M., Más, D., & Lan, S. (2018). Dietary Inclusion of a Mixed Powder of Medicinal Plant Leaves Enhances the Feed Efficiency and Immune Function in Broiler Chickens. *Journal of Chemistry, 1*(1), 1-7. https://doi.org/10.1155/2018/4073068

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants, 8*(4), 96. https://doi.org/10.3390/plants8040096

Atta, A. H., Nasr, S. M., Almaweri, A. H., Sedky, D., Mohamed, A. M., Desouky, H. M., & Shalaby, M. A. (2018). Phytochemical, antioxidant and hepatoprotective effects of different fractions of Moringa oleifera leaves methanol extract against liver injury in animal model. *Asian Pacific Journal of Tropical Medicine, 11*(7), 423–429. https://doi.org/10.4103/1995-7645.237186

Awotedu, O. L., & Ogunbamowo, P. O. (2019). Nutritional , Anti-nutritional and Phytochemical Profile of the Leaves and Fruits of Synsepalum dulcificum (Schumach. & Thonn.) Daniell. *American Journal of Biological Chemistry, 7*(3), 53–59. https://miraburst.com/wp-content/uploads/2020/01/Nutritionals-Anti-Nutritional-and-Phytochemical-Profile-of-the-Leaves-and-Fruit-of-the-Miracle-Fruit.pdf

Azman, M. A., & Yilmaz, M. (2005). The growth performance of broiler chicks fed with diets containing cottonseed meal supplemented with lysine. *Revue de Medecine Veterinaire, 156*(2), 104–106. https://www.revmedvet.com/2005/RMV156_104_106.pdf

Bahmani, M., Mirhoseini, M., Shirzad, H., Sedighi, M., Shahinfard, N., & Rafieian-Kopaei, M. (2015). A Review on Promising Natural Agents Effective on Hyperlipidemia. *Journal of Evidence-Based Complementary and Alternative Medicine, 20*(3), 228–238. https://doi.org/10.1177/2156587214568457

Bain, M. M., N.Y., & Dunn, I. C. (2016). Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges. *British Poultry Science, 57*(3), 330–338. https://doi.org/10.1080/00071668.2016.1161727

Bamishaiye, E. I., Olayemi, F. F., Awagu, E. F., & Bamshaiye, O. M. (2011).

Proximate and phytochemical composition of Moringa oleifera leaves at three stages of maturation. *Advance Journal of Food Science and Technology, 3*(4), 233–237. https://agris.fao.org/agris-search/search.do?recordID=DJ2012066731

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules, 10*(2), 202. https://doi.org/10.3390/biom10020202

Batool, R., Khan, M. R., Sajid, M., Ali, S., & Zahra, Z. (2019). Estimation of phytochemical constituents and in vitro antioxidant potencies of Brachychiton populneus (Schott & Endl.) *R.Br. BMC Chemistry, 13*(1), 1–15. https://doi.org/10.1186/s13065-019-0549-z

Bayu, F., Afework, M., Geleta, B., Ergete, W., & Makonnen, E. (2020). Effect of Chronic Administration of Aqueous Leaves Extract of Moringa Stenopetala on Blood Parameters and Histology of Liver and Kidney in Rats. *Ethiopian Journal of Health Sciences, 30*(2), 259–268. https://doi.org/10.4314/ejhs.v30i2.14

Beski, S. S. M., Swick, R. A., & Iji, P. A. (2015). Specialized protein products in broiler chicken nutrition: A review. *Animal Nutrition, 1*(2), 47–53. https://doi.org/10.1016/j.aninu.2015.05.005

Bettencourt, E. M. V., Tilman, M., Narciso, V., da Silva Carvalho, M. L., & de Sousa Henriques, P. D. (2015). The livestock roles in the wellbeing of rural communities of Timor-Leste. *Revista de Economia e Sociologia Rural, 53*(1), 63–80. https://doi.org/10.1590/1234-56781806-94790053s01005

Bhatnagar-Panwar, M., Bhatnagar-Mathur, P., Bhaaskarla, V. V. A., Dumbala, S. R., & Sharma, K. K. (2013). Rapid, accurate and routine HPLC method for large-scale screening of pro-vitamin A carotenoids in oilseeds. *Journal of Plant Biochemistry and Biotechnology, 24*(1), 84–92. https://doi.org/10.1007/s13562-013-0239-1

Bibi, Y., Nisa, S., Zia, M., Waheed, A., & Ahmed, S. (2012). In vitro cytotoxic activity of Aesculus indica against breast adenocarcinoma cell line. *(MCF-7) and phytochemical analysis.* 25(1), 183–187.https://pubmed.ncbi.nlm.nih.gov/22186328/

Biesek, J., Kuźniacka, J., Banaszak, M., Kaczmarek, S., Adamski, M., Rutkowski, A., Zmudzińska, A., Perz, K., & Hejdysz, M. (2020). Growth performance and carcass quality in broiler chickens fed on legume seeds and rapeseed meal.

Animals, 10(5), 1–16. https://doi.org/10.3390/ani10050846

Biovision Foundation. (2020). *Moringa / Moringa oleifera*. https://www.infonet-biovision.org/EnvironmentalHealth/Trees/Moringa-Moringa-oleifera

Bor, T., Aljaloud, S. O., Gyawali, R., & Ibrahim, S. A. (2016). *Antimicrobials from herbs, spices, and plants. In Fruits, Vegetables, and Herbs: Bioactive Foods in Health Promotion*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802972-5.00026-3

Borda-Molina, D., Zuber, T., Siegert, W., Camarinha-Silva, A., Feuerstein, D., & Rodehutscord, M. (2019). Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. *Poultry Science, 98*(7), 2906–2918. https://doi.org/10.3382/ps/pez038

Boschloo, R. (2019). *Analysis poultry sector Ghana 2019* . https://www.rvo.nl/sites/default/files/2019/12/Update-poultry-report-ghana-2019.pdf

Bouba, A. A., Njintang, N. Y., Foyet, H. S., Scher, J., Montet, D., & Mbofung, C. M. F. (2012). Proximate Composition, Mineral and Vitamin Content of Some Wild Plants Used as Spices in Cameroon. *Food and Nutrition Sciences, 3*(4), 423–432. https://doi.org/10.4236/fns.2012.34061

Boukandoul, S., Casal, S., & Zaidi, F. (2018). The potential of some moringa species for seed oil production†. *Agriculture, 8*(10), 1–13. https://doi.org/10.3390/agriculture8100150

Bounous, D. I., Wyatt, R. D., Gibbs, P. S., Kilburn, J. V., & Quist, C. F. (2000). Normal hematologic and serum biochemical reference intervals for juvenile wild turkeys. *Journal of Wildlife Diseases, 36*(2), 393–396. https://doi.org/10.7589/0090-3558-36.2.393

Cantó, C., Menzies, K. J., & Auwerx, J. (2015). NAD+ Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. *Cell Metabolism, 22*(1), 31–53. https://doi.org/10.1016/j.cmet.2015.05.023

Cardoso, L., & Almeida, A. (2013). *Seasonal weight loss - an assessment of losses and implications on animal welfare and production in the tropics: Southern Africa and Western Australia as case studies. Food and Agricultural Oranizations of the United Nations*. https://www.cabdirect.org/cabdirect/abstract/20133118162

Carr, A. C., & Vissers, M. M. C. (2018). *Vitamin C and Human Health. In Vitamin C and Human Health.* https://doi.org/10.3390/books978-3-906980-62-1

Carrasco, J. M. D., Casanova, N. A., & Miyakawa, M. E. F. (2019). Microbiota, gut health and chicken productivity: What is the connection. *Microorganisms, 7*(10), 1–15. https://doi.org/10.3390/microorganisms7100374

Carré, B. (2015). *A model for calculation of growth and feed intake in broiler chickens on the basis of feed composition and genetic features of broilers*. https://doi.org/10.13140/RG.2.1.1447.4962

Carron, M., Alarcon, P., Karani, M., Muinde, P., Akoko, J., Onono, J., Fèvre, E. M., Häsler, B., & Rushton, J. (2017a). The broiler meat system in Nairobi, Kenya: Using a value chain framework to understand animal and product flows, governance and sanitary risks. *Preventive Veterinary Medicine, 147*(8), 90–99. https://doi.org/10.1016/j.prevetmed.2017.08.013

Carron, M., Alarcon, P., Karani, M., Muinde, P., Akoko, J., Onono, J., Fèvre, E. M., Häsler, B., & Rushton, J. (2017b). The broiler meat system in Nairobi, Kenya: Using a value chain framework to understand animal and product flows, governance and sanitary risks. *Preventive Veterinary Medicine, 147*(2), 90–99. https://doi.org/10.1016/j.prevetmed.2017.08.013

Carvalho, I. T., & Santos, L. (2016). Antibiotics in the aquatic environments: A review of the European scenario. *In Environment International, 94*(1), 736–757). https://doi.org/10.1016/j.envint.2016.06.025

Cerrate, S., & Corzo, A. (2019). Lysine and Energy Trends in Feeding Modern Commercial Broilers. *International Journal of Poultry Science, 18*(1), 28–38. https://doi.org/10.3923/ijps.2019.28.38

Cervantes, H. M. (2015). Antibiotic-free poultry production: Is it sustainable. *Journal of Applied Poultry Research, 24*(1), 91-97. https://doi.org/10.3382/japr/pfv006

Chadd, S. (2007). Future trends and developments in poultry nutrition. *Proceedings of the International Conference Poultry in the 21st Century, 1*(1), 1–29. http://www.fao.org/ag/againfo/home/events/bangkok2007/docs/part1/1_7.pdf

Chaiban, C., Robinson, T. ., Fèvre, E. M., Ogola, J., & Vanwambeke, S. (2018). *Characterization of small-scale commercial chicken production in a distant rural area of a lower-middle-income country.* https://dial.uclouvain.be/pr/boreal/object/boreal:214205

Chehraghi, M., Zakeri, A., & Taghinejad-Roudbaneh, M. (2013). Effects of different feed forms on performance in broiler chickens. *European Journal of Experimental Biology, 3*(4), 66–70. https://www.imedpub.com/articles/effects-of-different-feed-forms-on-performance-in-broiler-chickens.php?aid=14706

Cheng, G., Ning, J., Ahmed, S., Huang, J., Ullah, R., An, B., Hao, H., Dai, M., Huang, L., Wang, X., & Yuan, Z. (2019). Selection and dissemination of antimicrobial resistance in Agri-food production. *Antimicrobial Resistance and Infection Control, 8*(1), 1–13. https://doi.org/10.1186/s13756-019-0623-2

Chinelo C., N., Obi, U, N., & Florence N, E. (2014). Phytochemical, Antinutrient and Amino Acid Composition of Synsepalum dulcificum Pulp. *IOSR Journal of Pharmacy and Biological Sciences, 9*(2), 25–29. https://doi.org/10.9790/3008-09252529

Clavijo, V., & Flórez, M. J. V. (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poultry Science, 97*(3), 1006–1021. https://doi.org/10.3382/ps/pex359

Cox, C. (2014). Security Procedures. *An Introduction to LTE, 1*(1), 203–213. https://doi.org/10.1002/9781118818046.ch12

Criscitiello, M. F., Kraev, I., & Lange, S. (2020). *Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID- 19.* https://stacks.cdc.gov/view/cdc/87050/cdc_87050_DS1.pdf

D'Odorico, P., Davis, K. F., Rosa, L., Carr, J. A., Chiarelli, D., Dell'Angelo, J., Gephart, J., MacDonald, G. K., Seekell, D. A., Suweis, S., & Rulli, M. C. (2018). The Global Food-Energy-Water Nexus. Reviews of Geophysics, 56(3), 456–531. https://doi.org/10.1029/2017RG000591

Daba, M. (2016). Miracle Tree: A Review on Multi-purposes of Moringa oleifera and Its Implication for Climate Change Mitigation. *Journal of Earth Science & Climatic Change, 7*(8), 1-5. https://doi.org/10.4172/2157-7617.1000366

Dabbou, S., Gasco, L., Rotolo, L., Pozzo, L., Tong, J. M., Dong, X. F., Rubiolo, P., Schiavone, A., & Gai, F. (2018). Effects of dietary alfalfa flavonoids on the performance, meat quality and lipid oxidation of growing rabbits. *Asian-Australasian Journal of Animal Sciences,* 31(2), 270–277. https://doi.org/10.5713/ajas.17.0284

Dalólio, F. S., Albino, L. F. T., Lima, H. J. D., da Silva, J. N., & Moreira, J. (2015).

Estresse térmico por calor e vitamina E em rações para frangos de corte como medida atenuante. *Acta Scientiarum - Animal Sciences, 37*(4), 419–427. https://doi.org/10.4025/actascianimsci.v37i4.27456

Dankowiakowska, A., Bogucka, J., Sobolewska, A., Tavaniello, S., Maiorano, G., & Bednarczyk, M. (2018). *Effects of in ovo injection of prebiotics and synbiotics on the productive performance and microstructural features of the superficial pectoral muscle in broiler chickens*. https://doi.org/10.3382/ps/pez202

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods, 8*(3), 1–27. https://doi.org/10.3390/foods8030092

David, L. S., Vidanarachchi, J. K., Samarasinghe, K., Cyril, H. W., & Dematawewa, C. M. B. (2015). Effects of Moringa based Feed Additives on the Growth Performance and Carcass Quality of Broiler Chicken. *Tropical Agricultural Research, 24*(1), 12-20. https://doi.org/10.4038/tar.v24i1.7985

De Bruyn, J., Wong, J., Bagnol, B., Pengelly, B., & Alders, R. (2015). Family poultry and food and nutrition security. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 10*(13), 1-9. https://doi.org/10.1079/PAVSNNR201510013

De Oliveira, W. D. C., Silva, T. P. D. E., De Araújo, M. J., Edvan, R. L., Oliveira, R. L., & Bezerra, L. R. (2019). Changes in hematological biomarkers of Nellore cows at different reproductive stages. *Acta Scientiarum - Animal Sciences, 41*(1), 1–7. https://doi.org/10.4025/actascianimsci.v41i1.45725

Detox, W. H. (2018). *Love your liver and get more out of life*. https://www.theholistichealthapproach.com/blog/love-your-liver

Diribi, K., Scott, D. Y., David, W, O., Edward, A., & Matin, R. B. (2017). Variation in the mineral element concentration of Moringa oleifera Lam. and M. Stenopetala (Bak. f.) Cuf.: Role in human nutrition. *PloS One, 12*(4), 1–26. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0175503

Dhama, K., Tiwari, R., Khan, R. U., Chakraborty, S., Gopi, M., Karthik, K., Saminathan, M., Desingu, P. A., Sunkara, L. T., & Perumal. (2014). Growth Promoters and Novel Additives Improving Poultry Production and Health, Bioactive and Beneficial Applications: The tends and Advances-A Review. *International Journal of Pharmacology, 10*(3), 129–159. https://scialert.net/abstract/?doi=ijp.2014.129.159

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry, 10*(1), 1193-1199. https://doi.org/10.1016/j.arabjc.2013.02.015

Diab, L., & Krebs, N. F. (2018). Vitamin excess and deficiency. *Pediatrics in Review, 39*(4), 161–179. https://doi.org/10.1542/pir.2016-0068

Dudonné, S., Vitrac, X., Coutiére, P., Woillez, M., & Mérillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry, 57*(5), 1768-1774. https://doi.org/10.1021/jf803011r

Edu, N. E., Ekpo, P. B., Umoyen, A. J., Thomas, T. L., Akpan, M. O., Leo, E. A., & Job, I. E. (2019). Evaluating the Effect of Moringa (Moringa oleifera) Leaf Supplemented Feed on the Growth and Carcass Quality of Broilers in Calabar. *Asian Journal of Advanced Research and Reports, 7*(1), 1–7. https://doi.org/10.9734/ajarr/2019/v7i130162

EFSA. (2019). *Technical Report on the notification of leaf powder of Moringa Stenopetala as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283. EFSA Supporting Publications.* https://doi.org/10.2903/sp.efsa.2019.en-1672

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology, 4*(1), 1–10. https://doi.org/10.3389/fphar.2013.00177

El-katcha, M. I., Soltan, M. A., Sharaf, M. M., & Hasen, A. (2016). Growth Performance , Immune Response , Blood serum parameters , Nutrient Digestibility and Carcass Traits of Broiler Chicken as Affected by Dietary Supplementation of Garlic Extract (Allicin). *Biblio medical, 49*(2), 50–64. https://doi.org/10.5455/ajvs.219261

El-Menawey, M., Attallah, A., Abdel-Aziz, Y., M.Yossif, M., & Gharib, H. (2019). Characterization and Constrains of Semi-Intensive Broiler Production System in Rural Sector of Al-Sharkia and El-Qaliobia Governorates, Egypt. Egyptian. *Poultry Science Journal, 39*(2), 311–326. https://doi.org/10.21608/epsj.2019.34356

Elangovan, M., Mathi, P., & Bhogireddy, N. (2014). Analysis of Phytochemicals, Antibacterial and Antioxidant activities of Moringa oleifera Lam. Leaf extract-

an in vitro study. *International Journal of Drug Development & Research, 6*(4), 173. https://doi.org/10.21608/epsj.2019.34356

Etim, N. N. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science, 2*(1), 37–47. https://doi.org/10.12735/as.v2i1p37

Etim, N. N., Williams, M. E., Akpabio, U., & Offiong, E. E. A. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science, 2*(1), 37–47. https://doi.org/10.12735/as.v2i1p37

Fajardo, S., García-Galvan, R., F., Barranco, V., Galvan, J. C., & Batlle, S. F. (2016). *We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %.* https://doi.org/http://dx.doi.org/10.5772/57353

FAO. (2002). *Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Gidelines for the evaluation of probiotics in food. In world Health Organization*. FAO

FAO. (2013). *Poultry Development Review. In Food and Agriculture Organization of the United Nations*. FAO

FAO. (2014). *Family poultry development − Issues, opportunities and constraints. Animal Production and Health. In Animal Production and Health.* http://www.fao.org/docrep/019/i3595e/i3595e.pdf

FAO. (2018). *Livestock production systems spotlight cattle and poultry sectors in Kenya.* https://doi.org/10.1016/s0301-6226(97)89729-1

FAO, F. H. (2012). *Impact of animal nutrition on animal welfare*. FAO

Farkhoy, M., Modirsanei, M., Ghavidel, O., Sadegh, M., & Jafarnejad, S. (2012). Evaluation of protein concentration and limiting amino acids including lysine and Met + Cys in prestarter diet on performance of broilers. *Veterinary Medicine International, 2*(2) 189-394. https://doi.org/10.1155/2012/394189

Fej, J., Kron, I., Pellizzeri, V., Eliašov, A., Campone, L., Gervasi, T., Bartolomeo, G., Cicero, N., & Babejov, A. (2019). *Antioxidant Properties of Moringa oleifera Lam . from Caribbean Island of Saint Lucia.* https://www.google.com/search?q=Antioxidant+Properties+of+Moringa+oleifera+lam+.+from+caribbean+island+of+saint+lucia&spell=1&sa=x&ved=2ahukewicoo_vqdrxahwh3eakhbixb_cqkeeckab6bagbec8

Ferdous, M. F., Arefin, M. S., Rahman, M. M., Ripon, M. M. R., Rashid, M. H., Sultana, M. R., Hossain, M. T., Ahammad, M. U., & Rafiq, K. (2019). Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. *Journal of Advanced Veterinary and Animal Research, 6*(3), 409–415. https://doi.org/10.5455/javar.2019.f361

Fikremariam, H. D., Zewdu, Y. A., & Abeje, E. W. (2019). Effect of tree age on nutritional, anti-nutritional and proximate composition of Moringa Stenopetala leaves in South west Ethiopia. *African Journal of Food Science, 13*(7), 129–134. https://doi.org/10.5897/ajfs2018.1745

Fitsum, M. (2014). Poultry Production System and Role of Poultry Production in Tigray Region , Northern Ethiopia : A Review. *Journal of Biology, Agriculture and Healthcare, 4*(27), 154–160.https://core.ac.uk/download/pdf/234660477.pdf

Food and Agricultural Organization of the United Nations. (2018). *Meat Market Review - Key trends in 2018*. http://www.fao.org/3/CA2129EN/ca2129en.pdf

Frutos, P., Hervás, G., Giráldez, F. J., & Mantecón, A. R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research*, 2(2), 191. https://doi.org/10.5424/sjar/2004022-73

Fuentes, M. K., Tristán, T. Q., Guzmán Maldonado, S. H., Valdivia-Flores, A. G., & Ortíz-Martínez, R. (2019). Effect of Moringa oleifera intake on productive and toxicological parameters in broiler chickens. *Revista Mexicana De Ciencias Pecuarias,* 10(4), 1013–1026. https://doi.org/10.22319/rmcp.v10i4.4575

Gadde, U., Kim, W. H., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Animal Health Research Reviews, 18*(1), 26–45. https://doi.org/10.1017/S1466252316000207

Gaddet, U., Kimt, W., & S.Lillehoj, H. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry : a review. *Animal Health Reseach Reviews,1*(1), 1–20. https://doi.org/10.1017/S1466252316000207

Gadzirayi, C. T., Masamha, B., Mupangwa, J. F., & Washaya, S. (2012). Performance of broiler chickens fed on mature Moringa oleifera leaf meal as a protein supplement to soyabean meal. *International Journal of Poultry Science, 11*(1), 5–10. https://doi.org/10.3923/ijps.2012.5.10

Gagliardi, A., Totino, V., Cacciotti, F., Iebba, V., Neroni, B., Bonfiglio, G., Trancassini, M., Id, C. P., Pantanella, F., & Schippa, S. (2018). *Rebuilding the Gut Microbiota Ecosystem*. https://doi.org/10.3390/ijerph15081679

Gakuya, D. W., Mbugua, P. N., Mwaniki, S. M., Njuguna, A., Muchemi, G. M., & Njuguna, A. (2014). Effect of supplementation of Moringa oleifera (LAM) leaf meal in layer chicken feed. *International Journal of Poultry Science, 13*(7), 379–384. https://doi.org/10.3923/ijps.2014.379.384.

Galvan, R., F., Barranco, V., Galvan, J. C., Batlle, Sebastian FeliuFajardo, S., & García. (2016). *We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %.* https://doi.org/http://dx.doi.org/10.5772/57353

Gasperi, V., Sibilano, M., Savini, I., & Catani, M. V. (2019). Niacin in the central nervous system: An update of biological aspects and clinical applications. *International Journal of Molecular Sciences, 20*(4), 1–26. https://doi.org/10.3390/ijms20040974

Gaucher, M., Quessy, S., Letellier, A., Arsenault, J., & Boulianne, M. (2015). Impact of a drug-free program on broiler chicken growth performances , gut health , Clostridium perfringens and Campylobacter jejuni occurrences. *Journal Name Poultry Science, 94*(8), 1791–1801. https://doi.org/10.3382/ps/pev142

Gay, M., & Kai, M. (2019). *African moringa tree.* https://en.wikipedia.org/wiki/Moringa_stenopetala

Geleta, Makonnen, & Debella. (2015). Toxicological Evaluations of the Crude Extracts and Fractions of Moringa Stenopetala Leaves in Liver and Kidney of Rats. *Journal of Cytology & Histology, 7*(01), 1–6. https://doi.org/10.4172/2157-7099.1000383

Ghanavi, J., Farnia, P., & Ghomi, H. (2017). The Effectiveness of Cold Atmospheric Plasma by Inhaling Anesthetic Mask or through Bronchoscopy against COVID-19. *Biomedical and Biotechnology Research Journal (BBRJ), 50*(8), 553–558. https://doi.org/10.4103/bbrj.bbrj

Gigante, A., & Atterbury, R. J. (2019). Veterinary use of bacteriophage therapy in intensively-reared livestock. *Virology Journal, 16*(1), 155. https://doi.org/10.1186/s12985-019-1260-3

Gizaw, S., & Tegegne, A. (2015). *Classification of small ruminant production sub-systems in Ethiopia : Implications for designing development interventions.*

https://hdl.handle.net/10568/69412

Glasgow, L., Forde, M., Brow, D., Mahoney, C., Fletcher, S., & Rodrigo, S. (2019). Antibiotic Use in Poultry Production in Grenada. *Veterinary Medicine International, 1*(1), 1-7. https://doi.org/10.1155/2019/6785195

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness, 5*(2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001

Gore, M. M. (2018). Extraction and Physicochemical Characterization of Oil from Maringa Stenopetala Seeds. *International Journal of Applied Engineering Research, 11*(6), 1–7. https://doi.org/10.9790/5736-1106010107

Hagan, M. A. S., Donkoh, A., & Awunyo-Vitor, D. (2016). Growth performance and economic evaluation of broiler Chicken fed with rain tree (Samanea saman) seed meal. *Cogent Food & Agriculture, 2*(1), 1-10. https://doi.org/10.1080/23311932.2016.1277445

Hagan, O. C. K., Nsiah, P., Obiri-yeboah, D., Yirdong, F., Annan, I., Eliason, S., Nuvor, S. V, & College, C. (2018). *Vaccination against hepatitis B in Ghana : A pilot study*. https://doi.org/10.4081/jphia.2018

Hagos, Z., Yirga Brhane, M., Zenebe Teka, M., Krishna Chaithanya, K., & Gopalakrishnan, V. K. (2018). Proximate analysis of the methanolic and aqueous leaves extracts of Moringa Stenopetala. *Drug Invention Today, 10*(12), 2577–2582.https://jprsolutions.info/files/final-file-5baf83ee219672.70824383.pdf

Hamasalim, H. J. (2016). Synbiotic as Feed Additives Relating to Animal Health and Performance. *Advances in Microbiology, 6*(4), 288–302. https://doi.org/10.4236/aim.2016.64028

Hamid, H. A., Ramli, A. N. M., & Yusoff, M. M. (2017). Indole alkaloids from plants as potential leads for antidepressant drugs: A mini review. *Frontiers in Pharmacology, 8*(26), 1-7. https://doi.org/10.3389/fphar.2017.00096

Hamuel, J. (2012). Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. *Phytochemicals, 1*(1), 1-34. https://doi.org/10.5772/26052

Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H. I., Huang, L., Dai, M., Wang, Y., Liu, Z., & Yuan, Z. (2014). Benefits and risks of antimicrobial use in food-

producing animals. *Frontiers in Microbiology, 5*(6), 1–11. https://doi.org/10.3389/fmicb.2014.00288

Hassan, H. M. A., El-Moniary, M. M., Hamouda, Y., El-Daly, E. F., Youssef, A. W., & Abd El-Azeem, N. A. (2016). Effect of different levels of Moringa oleifera leaves meal on productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. *Asian Journal of Animal and Veterinary Advances, 11*(1), 60–66. https://doi.org/10.3923/ajava.2016.60.66

Hassan, H. M. A., Samy, A., Youssef, A. W., & Mohamed, M. A. (2018). Using Different Feed Additives as Alternative to Antibiotic Growth Promoter to Improve Growth Performance and Carcass Traits of Broilers. *International Journal of Poultry Science, 17*(6), 255–261. https://doi.org/10.3923/ijps.2018.255.261

Hassan, S. K. U., Khalique, A., Pasha, T. N., & Sahota, A. W. (2018). Effect of dried Moringa oleifera leaves on growth performance and immune response of broilers. *Journal of Animal and Plant Sciences, 28*(6), 1579–1583.https://www.thefreelibrary.com/effect+of+dried+moringa+oleifera+leaves+on+growth+performance+anD...-a0567631848

HLPE. (2016). *Sustainable agricultural development for food security and nutrition: what roles for livestock? A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security (Issue July).* http://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/HLPE_Reports/HLPE-Report-10_EN.pdf

Holland, N. D. (2013). *Digestive system. Developments in Aquaculture and Fisheries Science.* https://doi.org/10.1016/B978-0-12-396491-5.00008-3

Hussein, H. H., & Jassim, J. M. (2019). The Influence of Moringa oleifera leaf meal and their acqueous and ethanolic leaf extractions on growth performance and Blood parameters of broiler chicken. Plant Archives. 19(2), 1841–1848. http://www.plantarchives.org/SPL%20ISSUE%20SUPP%202,2019/321%20(1841-1848).pdf

Idris, M., Jami, M., Hammed, A., & Jamal, P. (2016). Moringa oleifera Seed Extract: A Review on Its Environmental Applications. *International Journal of Applied Environmental Sciences,* 11(6), 1469–1486. http://www.plantarchives.org/SPL%20ISSUE%20SUPP%202,2019/321%20(1841-1848).pdf

Idris, O. A., Wintola, O. A., & Afolayan, A. J. (2019). Comparison of the proximate composition, Vitamins (Ascorbic acid, α-Tocopherol and retinol), anti-nutrients (phytate and oxalate) and the GC-MS analysis of the essential oil of the root and leaf of Rumex Crispus l. *Plants, 8*(3) 51. https://doi.org/10.3390/plants8030051

Imran, M., Nazar, M., Saif, M., Khan, M. A., Sanaullah, D., Vardan, M., & Javed, O. (2016). Role of Enzymes in Animal Nutrition: A Review. *PSM Veterinary Research, 1*(2), 38–45. http://journals.psmpublishers.org/index.php/vetres/article/view/84

International Committee for Standardization in Haematology. (1978). Protocol for type testing equipment and apparatus used for haematological analysis. C*linical Pathology, 3*(1), 275–279.https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwja_YXLttrxAhUZEWMBHcfTCIIQFjABegQIAhAD&url=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fpmc%2Farticles%2FPMC1145243%2F&usg=AOvVaw3G-MIAUCIvbIySpliW8zpd

Iyaya, E.A., & Alalade, O.A. (2006). Alalade_lyayi_Chemical Composition and the feeding calue of Azolla Meal for egg-type chicks_2006.pdf. *Asian Network for Scientific Information, 5*(2), 137–141. https://agris.fao.org/agris-search/search.do?recordID=DJ2012051659

Jacob, J., & Pescatore, T. (2013). Avian Digestive System. University of Kentucky - Cooperative Extension Service. *College of Agriculture, 11*(1), 1–4. https://poultry.extension.org/articles/poultry-anatomy/avian-digestive-system/

Jacques, A. S., Arnaud, S. S. S., Fréjus, O. O. H., & T.Jacques, D. (2020). Review on biological and immunomodulatory properties of Moringa oleifera in animal and human nutrition. *Journal of Pharmacognosy and Phytotherapy, 12*(1), 1–9. https://doi.org/10.5897/jpp2019.0555

Jadhav, K., Sharma, K. S., Katoch, S., Sharma, V., & Mane, B. G. (2015). Probiotics in Broiler Poultry Feeds: A Review. *Journal of Animal Nutrition and Physiology, 1*(15), 4–16.https://www.researchgate.net/publication/298865794_Probiotics_in_Broiler_Poultry_Feeds_A_Review

Jamal, M., shareef, M., & Sajid, S. (2017). Lincomycin and tetracycline resistance in poultry. Review. *Matrix Science Pharma, 1*(1), 33–38. https://doi.org/10.26480/msp.01.2017.33.38

Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of HerbMed Pharmacology, 7*(1), 1–7. https://doi.org/10.15171/jhp.2018.01

Jha, R., Singh, A. K., Yadav, S., Berrocoso, J. F. D., & Mishra, B. (2019). Early Nutrition Programming (in ovo and Post-hatch Feeding) as a Strategy to Modulate Gut Health of Poultry. *Frontiers in Veterinary Science, 6*(3), 1–10. https://doi.org/10.3389/fvets.2019.00082

Ji,E, K., Hyun,S. L., Yeong,H, H., Geun, B. K., Sung, H. L., EP, L., & DM, B. (2015). Dietary Capsicum and Curcuma longa oleoresins increase intestinal microbiome and necrotic enteritis in three commercial broiler breeds. *Res Vet Sci, 102*(1), 150–158. https://pubmed.ncbi.nlm.nih.gov/26412535/

Jin, L. Z., Ho, Y. W., Abdullah, N., & Jalaludin, S. (1997). Probiotics in poultry: modes of action. *World's Poultry Science Journal, 53*(4), 351–368. https://doi.org/10.1079/wps19970028

Kamboh, A. A., Khan, M. A., Kaka, U., Awad, E. A., Memon, A. M., Saeed, M., Korejo, N. A., Bakhetgul, M., & Kumar, C. (2018). Effect of dietary supplementation of phytochemicals on immunity and haematology of growing broiler chickens. *Italian Journal of Animal Science, 17*(4), 1038–1043. https://doi.org/10.1080/1828051X.2018.1438854

Kanakri, K., Carragher, J., Hughes, R., Muhlhausler, B., & Gibson, R. (2018). The Effect of Different Dietary Fats on the Fatty Acid Composition of Several Tissues in Broiler Chickens. *European Journal of Lipid Science and Technology, 120*(1), 170-237. https://doi.org/10.1002/ejlt.201700237

Karthivashan, G., Arulselvan, P., Alimon, A. R., Safinar Ismail, I., & Fakurazi, S. (2015). Competing role of bioactive constituents in Moringa oleifera extract and conventional nutrition feed on the performance of cobb 500 broilers. *BioMed Research International, 1*(1), 1-10. https://doi.org/10.1155/2015/970398

Kekudaa, T. R. P., Raghavendrab, H. L., Solomonc, T., & Duress, D. (2016). Antifungal and antiradical potential of Moringa Stenopetala (Baker. *Journal of Bioscience and Agriculture Research, 11*(1), 906–922. https://doi.org/10.18801/jbar.110116.111

Kheravii, S. K., Morgan, N. K., Swick, R. A., Choct, M., & Wu, S.-B. (2018). Roles of dietary fibre and ingredient particle size in broiler nutrition. *World's Poultry Science Journal, 74*(2), 301–316. https://doi.org/10.1017/S0043933918000259

Khor, K. Z., Lim, V., Moses, E. J., & Abdul Samad, N. (2018). *The in Vitro and in Vivo Anticancer Properties of Moringa oleifera. Evidence-Based Complementary and Alternative.* Medicine.https://doi.org/10.1155/2018/1071243

Klasing, K. C. (2015). *Nutritional Requirements of Poultry - Poultry - Veterinary Manual.* https://www.msdvetmanual.com/poultry/nutrition-and-management-poultry/nutritional-requirements-of-poultry

KNBS. (2019). *2019 Kenya Population and Housing Census.Kenya National Bureau of Statistics*

Koche, D., Shirsat, R., & Kawale, M. (2016). An Overerview of Major Classes of Phytochemicals : Their Types and Role in Disease Prevention. *Hislopia Journal, 1*(2), 1–11.https://mafiadoc.com/download/an-overerview-of-major-classes-of-phytochemicals_5c63bbad097c47f54d8b462d.html

Kogut, M. H. (2019). The effect of microbiome modulation on the intestinal health of poultry. *Animal Feed Science and Technology, 250*(8), 32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.008

Kong, C., & Adeola, O. (2014). Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. *Asian-Australasian Journal of Animal Sciences, 27*(7), 917–925. https://doi.org/10.5713/ajas.2014.r.02

Konietzny, U., Jany, K. D., & Greiner, R. (2006). Phytate - An undesirable constituent of plant-based foods. *Journal Fur Ernahrungsmedizin, 8*(3), 18–28. https://www.kup.at/kup/pdf/6239.pdf

Kothari, D., Lee, W. D., Niu, K. M., & Kim, S. K. (2019). The genus Allium as poultry feed additive: *A review. Animals, 9*(12), 9–11. https://doi.org/10.3390/ani9121032

Kuli Kuli Foods. (2021). *What are the parts of a moringa tree and their benefits.* https://blog.kulikulifoods.com/2018/04/11/what-are-the-parts-of-a-moringa-tree-and-their-benefits/

Kumar, M., Kumar, V., Roy, D., Kushwaha, R., & Vaswani, S. (2014). Application of Herbal Feed Additives in Animal Nutrition - A Review. *International Journal of Livestock Research, 4*(9), 1. https://doi.org/10.5455/ijlr.20141205105218

Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with

promising therapeutic applications. *Biotechnology Reports, 24*(1), 3-7. https://doi.org/10.1016/j.btre.2019.e00370

Kumar, S., Chen, C., Indugu, N., Werlang, G. O., Singh, M., Kim, W. K., & Thippareddi, H. (2018). Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics, immunity, growth performance and prevalence of foodborne pathogens. *PLoS ONE, 13*(2), 1–23. https://doi.org/10.1371/journal.pone.0192450

Kumssa, D. B., Joy, E. J. M., Young, S. D., Odee, D. W., Ander, E. L., Magare, C., Gitu, J., & Broadley, M. R. (2017). Challenges and opportunities for Moringa growers in southern Ethiopia and Kenya. *PLoS ONE, 12*(11), 1-7. https://doi.org/10.1371/journal.pone.0187651

Laganá, C., Ribeiro, A. M. L., Kessler, A. M., Kratz, L. R., & Pinheiro, C. C. (2007). Effect of the supplementation of vitamins and organic minerals on the performance of broilers under heat stress. *Revista Brasileira de Ciencia Avicola, 9*(1), 39–43. https://doi.org/10.1590/S1516-635X2007000100006

Lai, W., Cao, A., Li, J., Zhang, W., & Zhang, L. (2018). Effect of high dose of bile acids supplementation in broiler feed on growth performance, clinical blood metabolites, and organ development. *Journal of Applied Poultry Research, 27*(4), 532–539. https://doi.org/10.3382/japr/pfy040

Lamidi, W. A., Murtadha, M. A., & Ojo, D. O. (2017). Effects of Planting Locations on the Proximate Compositions of Moringa oleifera leaves. *Journal of Applied Sciences and Environmental Management, 21*(2), 331. https://doi.org/10.4314/jasem.v21i2.14

Laws, M., Shaaban, A., & Rahman, K. M. (2019). Antibiotic resistance breakers: Current approaches and future directions. *FEMS Microbiology Reviews, 43*(5), 490–516. https://doi.org/10.1093/femsre/fuz014

Laxminarayan, R., Van Boeckel, T., & Teillant, A. (2015). *The economic costs of withdrawing antimicrobial growth promoters from the livestock sector 78*. OECD Publishing. https://doi.org/10.1787/18156797

Leeson, S. (2008). Future developments in poultry nutrition. Carolina Feed Industry Association 35th. *Poultry Nutrition Conference, 35*(1), 4–8.http://www.fao.org/ag/againfo/home/events/bangkok2007/docs/part1/1_7.pdf

Leon, M., & Johanna, M. (2018). The synopsis of Biological and Pharmacological Events in Saponins. *International Journal of Latest Engineering Science,*

1(2),1–5. https://www.ijlesjournal.org/2018/volume-1%20issue-2/ijles-v1i2p101.pdf

Leone, A., Fiorillo, G., Criscuoli, F., Ravasenghi, S., Santagostini, L., Fico, G., Spadafranca, A., Battezzati, A., Schiraldi, A., Pozzi, F., Di Lello, S., Filippini, S., & Bertoli, S. (2015). Nutritional characterization and phenolic profiling of Moringa oleifera leaves grown in chad, sahrawi refugee camps, and Haiti. *International Journal of Molecular Sciences, 16*(1), 18923–18937. https://doi.org/10.3390/ijms160818923

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *International Journal of Molecular Sciences, 16*(2), 12791–12835. https://doi.org/10.3390/ijms160612791

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2016). Moringa oleifera seeds and oil: Characteristics and uses for human health. *International Journal of Molecular Sciences, 17*(12), 1–14. https://doi.org/10.3390/ijms17122141

Lilburn, M. S., & Loeffler, S. (2015). Review Early intestinal growth and development in poultry. *Journal Name Poultry Science, 94*(7), 1569–1576. https://doi.org/10.3382/ps/pev104

Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M. E., Chi, F., Cravens, R. L., Oh, S., & Gay, C. G. (2018). Phytochemicals as antibiotic alternatives to promote growth and enhance host health. *Veterinary Research, 49*(1), 1–18. https://doi.org/10.1186/s13567-018-0562-6

Liverpool-Tasie, L. S. O., Sanou, A., & Tambo, J. A. (2019). Climate change adaptation among poultry farmers: evidence from Nigeria. *Climatic Change, 157*(3–4), 527–544. https://doi.org/10.1007/s10584-019-02574-8

Maas Pavlović, D. (2019). Thiamine Deficiency and Benfotiamine Therapy in Brain Diseases. *American Journal of Biomedical Science & Research, 3*(1), 1–5. https://doi.org/10.34297/ajbsr.2019.03.000621

Maastricht, P., & Version, D. (2020). *Regulation of skeletal muscle recover. ProefschriftMaken Maastricht*. https://doi.org/10.26481/dis.20181221ak

Madende, M., & Hayes, M. (2020). Fish by-product use as biostimulants: An overview of the current state of the art, including relevant legislation and regulations within the EU and USA. *Molecules, 25*(5), 1-17.

https://doi.org/10.3390/molecules25051122

Mahfuz, S., & Piao, X. S. (2019a). Application of moringa(Moringa oleifera) as natural feed supplement in poultry diets. *Animals, 9*(431), 1–19. https://doi.org/10.3390/ani9070431

Mahfuz, S., & Piao, X. S. (2019b). Application of Moringa (Moringa oleifera). *Natural. Animals, 9*(7), 431. https://dx.doi.org/10.3390%2Fani9070431

Makama, R.S., Duru, S., Onimisi, P.A., Moses, O. (2020). Growth performance and hematological parameters of broiler chicken fed raw and boiled senna obtusifolia seed meal. *Nigerian Journal of Animal Science, 22*(3), 271–278.https://www.ajol.info/index.php/tjas/article/view/202146

Makeri, H. K., Ayo, J. O., Aluwong, T., & Minka, N. S. (2017). Daily rhythms of blood parameters in broiler chickens reared under tropical climate conditions. *Journal of Circadian Rhythms, 15*(1), 1-14. https://doi.org/10.5334/jcr.151

Makkar, H. P. S., Francis, G., & Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal, 1*(9), 1371–1391. https://doi.org/10.1017/S1751731107000298

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *In Molecules, 23*(4). 1-13. https://doi.org/10.3390/molecules23040795

Maroccolo, S. (2013). *Inclusion of a species-specific probiotic or calcium diformate in young calves diets: effects on gut microbial balance, health status and growth performance*.https://core.ac.uk/download/pdf/187905089.pdf

Mbailao, M., Mianpereum, T., & Albert, N. (2014). Proximal and Elemental Composition of Moringa oleifera (Lam) Leaves from Three Regions of Chad. *Journal of Food Resource Science, 3*(1), 12–20. https://doi.org/10.3923/jfrs.2014.12.20

Mbuza, F., Manishimwe, R., Mahoro, J., Simbankabo, T., & Nishimwe, K. (2017). Characterization of broiler poultry production system in Rwanda. *Tropical Animal Health and Production, 49*(1), 71–77. https://doi.org/10.1007/s11250-016-1160-0

Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M. Lou, Chorfi, Y., Suresh, G., Rouissi, T., Brar, S. K., Côté, C., Ramirez, A. A., & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition, 4*(2), 170–178. https://doi.org/10.1016/j.aninu.2018.03.002

Mekonnen, Y., & Dräger, B. (2003). Glucosinolates in Moringa Stenopetala. *Planta Medica., 69*(4), 380-382.https://pubmed.ncbi.nlm.nih.gov/12709911/

Melesse, A. (2014). Significance of scavenging chicken production in the rural community of Africa for enhanced food security. *World's Poultry Science Journal, 70*(3), 593–606. https://doi.org/10.1017/S0043933914000646

Melesse, A., Tiruneh, W., & Negesse, T. (2011). Effects of feeding Moringa Stenopetala leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. *Tropical and Subtropical Agroecosystems, 14*(2), 485–492. https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/876

Melesse, Aberra, Getye, Y., Berihun, K., & Banerjee, S. (2013). Effect of feeding graded levels of Moringa Stenopetala leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens. *Livestock Science, 157*(2–3), 498–505. https://doi.org/10.1016/j.livsci.2013.08.012

Meresa, A., Fekadu, N., Degu, S., Tadele, A., & Geleta, B. (2017). An Ethno Botanical Review on Medicinal Plants Used for the Management of Hypertension. *Clinical and Experimental Pharmacology, 7*(2), 1-16. https://doi.org/10.4172/2161-1459.1000228

Mikore, D., & Mulugeta, E. (2017). Determination of proximate and mineral compositions of Moringa oleifera and Moringa Stenopetala leaves cultivated in Arbaminch Zuria and Konso, Ethiopia. *African Journal of Biotechnology, 16*(15), 808–818. https://doi.org/10.5897/ajb2017.15919

Ministry of Agriculture. (2019). *Draft National Stock Policy. In Republic of Kenya.*

Mitchel Otieno, O., James Mucunu, M., Laetitia Wakonyu, K., Daniel Waweru, G., Stephen Gitahi, K., Francis Okumu, O., & Okumu Mitchel Otieno, C. (2016). Phytochemical profile and antioxidant capacity of leaves of Moringa oleifera (Lam) extracted using different solvent systems. *Journal of Pharmacognosy and Phytochemistry JPP, 5*(4), 302–308. http://hdl.handle.net/11295/100535

Mohamed., A. M., Osman,A., S., & Hadey, A. (2017). Performance, immunology and biochemical parameters of Moringa oleifera and/or Cichorium intybus

addition to broiler chicken ration. *Journal of Veterinary Medicine and Animal Health, 9*(10), 255–263. https://doi.org/10.5897/jvmah2017.0611

Mohammadagheri, N., Najafi, R., & Najafi, G. (2016). Effects of dietary supplementation of organic acids and phytase on performance and intestinal histomorphology of broilers. *Veterinary Research Forum : An International Quarterly Journal, 7*(3), 189–195. http://www.ncbi.nlm.nih.gov/pubmed/27872714%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5094164

Mohammadi Gheisar, M., & Kim, I. H. (2018). Phytobiotics in poultry and swine nutrition–a review. *Italian Journal of Animal Science, 17*(1), 92–99. https://doi.org/10.1080/1828051X.2017.1350120

Moodley, I. (2017). Acute toxicity of Moringa oleifera leaf powder in rats. *Journal of Medicinal Plants Studies, 5*(5), 180–185. https://www.plantsjournal.com/archives/2017/vol5issue5/PartC/5-4-24-284.pdf

Moreno-Mendoza, Y., López-Villarreal, K. D., Hernández-Martínez, C. A., Rodríguez-Tovar, L. E., Hernández-Coronado, A. C., Soto-Domínguez, A., Hume, M. E., & Méndez-Zamora, G. (2021). Effect of moringa leaf powder and agave inulin on performance, intestinal morphology, and meat yield of broiler chickens. *Poultry Science, 100*(2), 738–745. https://doi.org/10.1016/j.psj.2020.11.058

Morgan, N. (2017). Managing gut health without reliance on antimicrobials in poultry. *Animal Production Science., 57*(11), 2270–2279. https://www.publish.csiro.au/AN/pdf/AN17288

N.Savithramma, M. L. R., & B. P. (2011). Phytochemical Studies of Dysophylla myosuroides(Roth.) Benth.In. Wall and Talinum cuneifolium (Vahl.) *Willd.Res. East,* 3(2), 163–169. https://doi.org/10.3923/rjphyto.2010.234.241

Napagoda, M. T., Sundarapperuma, T., Fonseka, D., Amarasiri, S., & Gunaratna, P. (2018). An Ethnobotanical Study of the Medicinal Plants Used as Anti-Inflammatory Remedies in Gampaha District, Western Province, Sri Lanka. *Scientifica, 1*(1), 1-9. https://doi.org/10.1155/2018/9395052

Ndung'u, J., Anino, E., Njuguna, D., Mwangangi, R., Jepkorir, M., Mbugua, R., Chepng'etich, J., Ngule, C., & Mwitari, P. (2018). Phytochemical Screening and Synergistic Anti-proliferative Activity against Selected Cancer Cell Lines of Moringa oleifera and Indigofera arrecta Leaf Extracts. *European Journal of Medicinal Plants, 23*(2), 1–11. https://doi.org/10.9734/ejmp/2018/41021

Neves, D., Banhazi, T., & Nääs, I. (2014). On the Biomechanical Characteristics. *Brazilian Journal of Poultry Science, 16*(2), 1–16. https://doi.org/10.1080/17461391.2018.1554707

Njuguna, C. (2018). Impact of improved poultry production technologies among smallholder indigenous chicken farmers in Kakamega and Makueni– Kenya. *Journal of Chemical Information and Modeling, 53*(9), 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Nkukwana, T. T., Muchenje, V., Pieterse, E., Masika, P. J., Mabusela, T. P., Hoffman, L. C., & Dzama, K. (2014). Effect of Moringa oleifera leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Livestock Science, 161*(1), 139–146. https://doi.org/10.1016/j.livsci.2014.01.001

Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E., & Domínguez-Perles, R. (2016). Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of Moringa oleifera Lam. *Industrial Crops and Products, 83*(1), 166–176. https://doi.org/10.1016/j.indcrop.2015.12.032

O 'neill, J. (2015). *Antimicrobials in Agriculture and the Environment: Reducing Unnecessary Use and Waste the Review on Antimicrobial Resistance.* http://www.biomerieuxconnection.com/antimicrobials-in-agriculture-and-the-environment-reducing-unnecessary-use-and-waste/

Ogbe, A. ., & Affiku, J. (2019). Proximate Study, Mineral and Anti-Nutrient Composition of Moringa oleifera Leaves Harvested From Lafia, Nigeria: Potential Benefits in Poultry Nutrition and Health. *Journal of Microbiology, Biotechnology and Food Sciences, 9*(1), 296–308. https://agris.fao.org/agris-search/search.do?recordID=SK2012100062

Ogbuewu, I. P., Emenalom, O. O., & Okoli, I. C. (2017). Alternative feedstuffs and their effects on blood chemistry and haematology of rabbits and chickens: a review. *Comparative Clinical Pathology, 26*(2), 277–286. https://doi.org/10.1007/s00580-015-2210-0

Ogunwole, O. A., Lawal, H. O., Idowu, A. I., Oladimeji, S. O., Abayomi, F. D., & Tewe, O. O. (2016). *National Animal Production*. Ahmadu Research Institute

OIE. (2019). Animal welfare and broiler chicken production systems. *Terrestrial Animal Health Code, 1*(1), 1–8.https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapit

re_aw_broiler_chicken.pdf

Okoronkwo, N. E., Mba, K. C., & Nnorom, I. C. (2017). Estimation of Protein Content and Amino Acid Compositions in Selected Plant Samples Using UV-Vis Spectrophotometeric Method. *American Journal of Food Science and Health,* *3*(3), 41–46. https://www.researchgate.net/publication/319057476_Estimation_of_Protein_Content_and_Amino_Acid_Compositions_in_Selected_Plant_Samples_Using_UV-Vis_Spectrophotometeric_Method

Okumu, M., Mbaria, J., Kanja, L., Gakuya, D., Kiama, S., Ochola, F., & Okumu, P. (2016). Acute toxicity of the aqueous-methanolic Moringa oleifera (Lam) leaf extract on female Wistar albino rats. *International Journal of Basic and Clinical Pharmacology,* *5*(5), 1856–1861. https://doi.org/10.18203/2319-2003.ijbcp20163153

Oleforuh-Okoleh, V. U., Ndofor-Foleng, H. M., Olorunleke, S. O., & Uguru, J. O. (2015). Evaluation of Growth Performance, Haematological and Serum Biochemical Response of Broiler Chickens to Aqueous Extract of Ginger and Garlic. *Journal of Agricultural Science,* *7*(4), 1-6. https://doi.org/10.5539/jas.v7n4p167

Onunkwo, D., & George, O. . (2015). Effects of Moringa oleifera Leaf Meal on the Growth Performance and Carcass Characteristics of Broiler Birds. *Agriculture and Veterinary Science (IOSR-JAVS),* *8*(3), 63–66. https://doi.org/10.9790/2380-08326366

Orlowski, S., Flees, J., Anthony, N., & Dridi, S. (2017). Differential expression of water channel- and noncoding RNA biogenesis-related genes in three lines of chickens under a short-term water restriction. *Poultry Science, 96*(12), 4172–4181. https://doi.org/10.3382/ps/pex263

Oumbortime, N., Tété-bénissan, A., Onagbesan, O. M., & Tona, K. (2020). Use of Moringa oleifera leaves in broiler production chain . 1 - Effect on Sasso breeder hens performances , internal quality of hatching eggs and serum lipids. *Veterinary Medicine and Science, 1*(1), 1–7. https://doi.org/10.1002/vms3.235

Paikra, B., Dhongade, H., & Gidwani, B. (2017). Phytochemistry and Pharmacology of Moringa oleifera Lam. *Pharmacopuncture,* *20*(3), 194–200.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5633671/

Palial, R., Sharma, V., & Pracheta. (2011). A review on Horse Radish Tree (Moringa oleifera): A multipurpose Tree with High Economic and Commercial

Importance. *Asian Journal of Biotechnology, 3*(4), 317–328.https://scialert.net/abstract/?doi=ajbkr.2011.317.328

Pallio, G., Irrera, N., Pizzino, G., Bitto, A., Cucinotta, M., Altavilla, D., Squadrito, F., Mannino, F., & Arcoraci, V. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity, 2*(17), 1–13. https://doi.org/10.1155/2017/8416763

Parra, M., Stahl, S., & Hellmann, H. (2018). Vitamin B6 and Its Role in Cell Metabolism and Physiology. *Cells, 7(*7), 84. https://doi.org/10.3390/cells7070084

Parrotta, J. A. (2005). *Moringa oleifera Lam*. Enzyklopädie der Holzgewächse

Pham, V. H., Kan, L., Huang, J., Geng, Y., Zhen, W., Guo, Y., Abbas, W., & Wang, Z. (2020). Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. *Journal of Animal Science and Biotechnology, 11*(1), 1–18. https://doi.org/10.1186/s40104-019-0421-y

Phelps, T., Snyder, E., Rodriguez, E., Child, H., & Harvey, P. (2019). The influence of biological sex and sex hormones on bile acid synthesis and cholesterol homeostasis. *Biology of Sex Differences, 1*0(1), 1–12. https://doi.org/10.1186/s13293-019-0265-3

Pirgozliev, V., Rose, S. P., & Ivanova, S. (2019). Feed additives in poultry nutrition. *Bulgarian Journal of Agricultural Science, 25*(1), 8–11.https://www.google.com/aclk?sa=l&ai=dchcsewju5oy-x9rxahxqgqyahvgedasyabadggj3cw&ae=2&sig=aod64_13grnr4a1etbufizkv41zp5sagmq&q&nis=1&adurl&ved=2ahukewibz-w-x9rxahwjcmmbhslmdkoq0qx6bageeae

Poultry Hub. (n.d.). *Feed Ingredients*. http://www.poultryhub.org/nutrition/feed-ingredients/

Poultry Hub. (2021). *Feed additives - Poultry Hub.* http://www.poultryhub.org/nutrition/feed-ingredients/feed-additives/

Qaisrani, S. N., Van Krimpen, M. M., Kwakkel, R. P., Verstegen, M. W. A., & Hendriks, W. H. (2015a). Diet structure, butyric acid, and fermentable carbohydrates influence growth performance, gut morphology, and cecal fermentation characteristics in broilers. *Poultry Science, 94*(9), 2152–2164. https://doi.org/10.3382/ps/pev003

Qaisrani, S. N., Van Krimpen, M. M., Kwakkel, R. P., Verstegen, M. W. A., & Hendriks, W. H. (2015b). Dietary factors affecting hindgut protein fermentation in broilers: A review. *World's Poultry Science Journal, 71*(1), 139–160. https://doi.org/10.1017/S0043933915000124

Raghavendra, H., Kekuda, T., Vijayananda, B., Duressa, D., & Solomon, T. (2016). Nutritive Composition and Antimicrobial Activity of Moringa Stenopetala (Baker f.) Cufod. *Journal of Advances in Medical and Pharmaceutical Sciences, 10*(3), 1–9. https://doi.org/10.9734/jamps/2016/29987

Rahman, M., Parvin, M., Sarker, R., & Islam, M. (2013). Effects of growth promoter and multivitamin-mineral premix supplementation on body weight gain in broiler chickens. *Journal of the Bangladesh Agricultural University, 10*(2), 245–248. https://doi.org/10.3329/jbau.v10i2.14914

Ramachandran, C., Peter, K. V, & Gopalakrishnan, P. K. (1980). Drumstick (Moringa oleifera): A Multipurpose Indian Vegetable. *Economic Botany, 34*(3), 276–283.https://agris.fao.org/agrissearch/search.do?recordID=US19810696208

Rani, N. Z. A., Husain, K., & Kumolosasi, E. (2018). Moringa genus: A review of phytochemistry and pharmacology. *Frontiers in Pharmacology, 9*(2), 1–26. https://doi.org/10.3389/fphar.2018.00108

Ravindran, V. (2013). Poultry Feed Availability and Nutrition in Developing Countries: Main Ingredients used in Poultry Feed Formulation. *Poultry Development Review, 2*(10), 694–695. http://www.fao.org/3/al706e/al706e.pdf

Ravindran, V. (2014). *Poultry. Elsevier Ltd.* https://doi.org/10.1016/B978-0-12-384731-7.00024-6

Ravindran, V. (2012). *Poultry feed availability and nutritionin developing countries : Feed supplements and additives.* http://www.fao.org.

Réhault-Godbert, S., Guyot, N., & Nys, Y. (2019). The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. *Nutrients, 11*(3), 1–26. https://doi.org/10.3390/nu11030684

Rehman, H. F., Zaneb, H., Masood, S., Yousaf, M. S., Ashraf, S., Khan, I., Shah, M., Khilji, M. S., & Rehman, H. (2018). Effect of Moringa oleifera leaf powder supplementation on pectoral muscle quality and morphometric characteristics of tibia bone in broiler chickens. *Revista Brasileira de Ciencia Avicola, 20*(4), 817–824. https://doi.org/10.1590/1806-9061-2017-0609

Rehman, H., Mahmood, S., Ahmad, F., Aslam, M. M., Abbas, G., Mahmood, A., & Sajid, M. (2018). Comparative Effect of Replacement of Canola Meal with Moringa oleifera Leaf Meal (MOLM) on Hemato-chemical Profile in Broilers. *Advances in Zoology and Botany, 6*(1), 19–25. https://doi.org/10.13189/azb.2018.060102

Rezende, M. S., Mundim, A. V., Fonseca, B. B., Miranda, R. L., Oliveira, W., & Lellis, C. G. (2017). Profile of serum metabolites and proteins of broiler breeders in rearing age. *Revista Brasileira de Ciencia Avicola, 19*(4), 583–586. https://doi.org/10.1590/1806-9061-2016-0338

Rodrigo, R., Guichard, C., & Charles, R. (2007). Clinical pharmacology and therapeutic use of antioxidant vitamins. *Fundamental and Clinical Pharmacology, 21*(2), 111–127. https://doi.org/10.1111/j.1472-8206.2006.00466.x

Rodrigues, I., & Choct, M. (2018). The foregut and its manipulation via feeding practices in the chicken. *Poultry Science, 97*(9), 3188–3206. https://doi.org/10.3382/ps/pey191

Russell, W. R., Baka, A., Björck, I., Delzenne, N., Gao, D., Griffiths, H. R., Hadjilucas, E., Juvonen, K., Lahtinen, S., Lansink, M., Loon, L. Van, Mykkänen, H., östman, E., Riccardi, G., Vinoy, S., & Weickert, M. O. (2016). Impact of Diet Composition on Blood Glucose Regulation. Critical Reviews in *Food Science and Nutrition, 56*(4), 541–590. https://doi.org/10.1080/10408398.2013.792772

Rust, J. M. (2019). The impact of climate change on extensive and intensive livestock production systems. *Animal Frontiers, 9*(1), 20–25. https://doi.org/10.1093/af/vfy028

Saini, R. K., Sivanesan, I., & Keum, Y. S. (2016). Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. *3 Biotech, 6*(2), 1–14. https://doi.org/10.1007/s13205-016-0526-3

Salaheen, S., Kim, S. W., Haley, B. J., Van Kessel, J. A. S., & Biswas, D. (2017). Alternative growth promoters Modulate broiler gut microbiome and enhance body weight gain. *Frontiers in Microbiology, 8*(10), 1–11. https://doi.org/10.3389/fmicb.2017.02088

Salajegheh, A., Salarmoini, M., Afsharmanesh, M., & Salajegheh, M. H. (2018). Growth performance , intestinal microflora , and meat quality of broiler chickens fed lavender (Lavandula angustifolia) powder. *Animal, 6*(1), 31–38.

https://doi.org/10.22103/jlst.2018.10340.1195

Salim, H. M., Huque, K. S., Kamaruddin, K. M., & Beg, M. A. H. (2018). Global restriction of using antibiotic growth promoters and alternative strategies in poultry production. *Science Progress, 101*(1), 52–75. https://doi.org/10.3184/003685018X15173975498947

Salois, M. J., Cady, R. A., & Heskett, E. A. (2016). The Environmental and Economic Impact of Withdrawing Antibiotics from US Broiler Production. *Journal of Food Distribution Research, 47*(1), 79–80.https://ageconsearch.umn.edu/record/232315/files/14_Salois.pdf

Samboko, P. C., Zulu-Mbata, O., & Chapoto, A. (2018). Analysis of the animal feed to poultry value chain in Zambia. *Development Southern Africa, 35*(3), 351–368. https://doi.org/10.1080/0376835X.2018.1480932

Samour, J. (2005). Diagnostic Value of Haematology. *Clinical Avian Medicine, 1*(1), 1–24. https://www.ivis.org/library/clinical-avian-medicine/diagnostic-value-of-hematology

Sarker, M. S. K., Rana, M. M., Khatun, H., Faruque, S., Sarker, N. R., Sharmin, F., & Islam, M. N. (2017). Moringa leaf meal as natural feed additives on the growth performance and meat quality of commercial broiler chicken. *Asian Journal of Medical and Biological Research, 3*(2), 240–244. https://doi.org/10.3329/ajmbr.v3i2.33576

Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of Medicinal Plants. *Pharmacognosy and Phytochemistry, 1*(6), 168–183. https://doi.org/10.1007/978-1-4614-3912-7_4

Schar, D., Sommanustweechai, A., Laxminarayan, R., & Tangcharoensathien, V. (2018). Surveillance of antimicrobial consumption in animal production sectors of low- and middle-income countries: Optimizing use and addressing antimicrobial resistance. *PLoS Medicine, 15*(3), 1–9. https://doi.org/10.1371/journal.pmed.1002521

Schiavone, A., Cullere, M., De Marco, M., Meneguz, M., Biasato, I., Bergagna, S., Dezzutto, D., Gai, F., Dabbou, S., Gasco, L., & Zotte, A. D. (2017). Partial or total replacement of soybean oil by black soldier fly larvae (Hermetia illucens L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Italian Journal of Animal Science, 16*(1), 93–100. https://doi.org/10.1080/1828051X.2016.1249968

Sebola, N. A., & Mokoboki, H. K. (2019). Influence of Dietary Moringa oleifera Leaf Meal on Haematological Parameters, Serum Biochemical Indices and Weight of Internal Organs of Chickens. *SciFed Food & Dairy Technology Journal,* *7*(12), 1042-1048. https://doi.org/http://dx.doi.org/10.17582/journal.aavs/2019/7.12.1042.1048

Seifu, E. (2014). Actual and Potential Applications of Moringa Stenopetala , Underutilized Indigenous Vegetable of Southern Ethiopia : A Review. *Agruculture and food research,* *3*(4), 8–19.https://www.sciencetarget.com/Journal/index.php/IJAFR/article/view/381

Selaledi, L. A., Hassan, Z. M., Manyelo, T. G., & Mabelebele, M. (2020). The current status of the alternative use to antibiotics in poultry production: An African perspective. *Antibiotics,* *9*(9), 1–18. https://doi.org/10.3390/antibiotics9090594

Shanmugavel, G., Prabakaran, K., & George, B. (2018). Evaluation of Phytochemical Constituents of Moringa oleifera (Lam.) Leaves Collected From Puducherry Region, South India. *International Journal of Zoology and Applied Biosciences, 3*(1), 1–8. http://www.ijzab.com

Shefaa A.M., El-Mandrawy, & Ismail., S. A. A. (2017). Selective Hematological, Biochemical and Pathological Alterations of Newcastle Virus in Naturally Infected and Vaccinated Broilers in Damietta Governorate of Egypt. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. *Veterinary Medicine, 74*(2), 139. https://doi.org/10.15835/buasvmcn-vm:0002

Shousha, W. G., Aboulthana, W. M., Salama, A. H., Saleh, M. H., & Essawy, E. A. (2019). Evaluation of the biological activity of Moringa oleifera leaves extract after incorporating silver nanoparticles, in vitro study. *Bulletin of the National Research Centre, 43*(1) 1-8. https://doi.org/10.1186/s42269-019-0221-8

Shroha, A., Bidhan, D. S., Sihag, S. S., & Yadav, D. C. (2019). Body Weight, Feed Consumption and FCR of Broiler Chicken upon Dietary Supplementation of Ajwain (Trachyspermum ammi). *International Journal of Current Microbiology and Applied Sciences,* *8*(2), 2189–2198. https://doi.org/10.20546/ijcmas.2019.802.253

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K. M., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Scientific Reports,* *9*(1), 1–13. https://doi.org/10.1038/s41598-019-46603-z

Singh, L., ti, J., & Singh, J. (2019). Medicinal and Nutritional Values of Drumstick Tree (Moringa oleifera) - A Review. *International Journal of Current Microbiology and Applied Sciences, 8*(5), 1965–1974. https://doi.org/10.20546/ijcmas.2019.805.228

Slavin, J. (2012). Dietary guidelines: Are we on the right path. *Nutrition Today, 47*(5), 245–251. https://doi.org/10.1097/NT.0b013e31826c50af

Slawinska, A., Dunislawska, A., Plowiec, A., Radomska, M., Lachmanska, J., Siwek, M., Tavaniello, S., & Maiorano, G. (2019). Modulation of microbial communities and mucosal gene expression in chicken intestines after galactooligosaccharides delivery In Ovo. *PLoS ONE, 14(*2), 1–23. https://doi.org/10.1371/journal.pone.0212318

Śliżewska, K., Markowiak, P., Żbikowski, A., & Szeleszczuk, P. (2019). Effects of synbiotics on the gut microbiota, blood and rearing parameters of chickens. *FEMS Microbiology Letters, 366*(11) 1-11. https://doi.org/10.1093/femsle/fnz116

Slow food Foundation. (2020). *Moringa Stenopetala.* https://www.fondazioneslowfood.com/en/ark-of-taste-slow-food/moringa-stenopetala-2/

Smith, R. L., Soeters, M. R., Wüst, R. C. I., & Houtkooper, R. H. (2018). Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocrine Reviews, 39*(4), 489–517. https://doi.org/10.1210/er.2017-00211

Sneeringer, S., MacDonald, J., Key, N., McBride, W., & Mathews, K. (2015). *Economics of Antibiotic Use in U.S. Livestock Production*. https://www.ers.usda.gov/publications/pub-details/?pubid=45488

Solis-cruz, B., Hernandez-patlan, D., Hargis, B. M., & Tellez, G. (2018). *Use of Prebiotics as an Alternative to Antibiotic Growth Promoters in the Poultry Industry*. IntechOpen. http://dx.doi.org/10.5772/intechopen.89053

Souza, C. S., Vieites, F. M., Justino, L. R., Lima, M. F. de, Chaves, A. S., Cardoso, V. da S., Sousa, F. D. de R., Costa, T. F., Minafra, C. S., & Lima, C. A. R. de. (2020). Importance of intestinal health in broilers. *Research, Society and Development,* 9(3), 1–18. https://doi.org/10.33448/rsd-v9i3.2475

Stadtlander, T., & Becker, K. (2017). Proximate Composition, Amino and Fatty Acid Profiles and Element Compositions of Four Different Moringa Species. *Journal*

of Agricultural Science, 9(7), 46. https://doi.org/10.5539/jas.v9n7p46

Steczny, K., Kokoszynski, D., Bernacki, Z., Wasilewski, R., & Saleh, M. (2017). Growth performance, body measurements, carcass composition and some internal organ characteristics in young pekin ducks. *South African Journal of Animal Sciences, 47*(3), 399–406. https://doi.org/10.4314/sajas.v47i3.16

Stohs, S. J., & Hartman, M. J. (2015). Review of the safety and efficacy of Moringa oleifera. *Phytotherapy Research, 29*(6), 796–804. https://doi.org/10.1002/ptr.5325

Su, B., & Chen, X. (2020). *Current Status and Potential of Moringa oleifera Leaf as an Alternative Protein Source for Animal Feeds. Frontiers Media S.A*. https://doi.org/10.3389/fvets.2020.00053

Subedi, L., Timalsena, S., Duwadi, P., Thapa, R., Paudel, A., & Parajuli, K. (2014). Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. *Journal of Traditional Chinese Medicine, 34*(5), 584–590. https://doi.org/10.1016/s0254-6272(15)30067-4

Surai F, P. (2016). Antioxidant Systems in Poultry Biology: Superoxide Dismutase. *Journal of Animal Research and Nutrition, 1*(1), 1–8. https://doi.org/10.21767/2572-5459.100008

Suresh, G., Das, R. K., Kaur Brar, S., Rouissi, T., Avalos Ramirez, A., Chorfi, Y., & Godbout, S. (2018). Alternatives to antibiotics in poultry feed: molecular perspectives. *Critical Reviews in Microbiology, 44*(3), 318–335. https://doi.org/10.1080/1040841X.2017.1373062

Svihus, B., & Itani, K. (2019). Intestinal Passage and Its Relation to Digestive Processes. *Journal of Applied Poultry Research, 28*(3), 546–555. https://doi.org/10.3382/japr/pfy027

Tallentire, C. W., Leinonen, I., & Kyriazakis, I. (2018). Artificial selection for improved energy efficiency is reaching its limits in broiler chickens. *Scientific Reports, 8*(1), 1–10. https://doi.org/10.1038/s41598-018-19231-2

Tamilselvi, N. A., & Arumugam, T. (2019). Health Benefits, Therapeutic and Pharmacological Properties of Moringa- A Review. *Journal of Pharmaceutical Research International, 25*(1), 1–11. https://doi.org/10.9734/jpri/2018/37179

Tamiru, M., Ashagrie, S., Alkhtib, A., Getachew, M., Demeke, S., Hassen, W., Worku, Z., & Burton, E. (2020). Performance of broilers and layers

supplemented with Moringa Stenopetala leaf meal under hot humid tropical conditions. *Animal Production Science, 60*(17), 1987–1994. https://doi.org/10.1071/AN19671

Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Rahman, H. S. (2018). Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Frontiers in Pharmacology, 9*(10), 1–28. https://doi.org/10.3389/fphar.2018.01162

Tarradas, J., Tous, N., Esteve-garcia, E., & Brufau, J. (2020). The control of intestinal inflammation: A major objective in the research of probiotic strains as alternatives to antibiotic growth promoters in poultry. *Microorganisms, 8*(2), 1-18. https://doi.org/10.3390/microorganisms8020148

Teng, P., & Kim, W. K. (2018). Review : Roles of Prebiotics in Intestinal Ecosystem of Broilers. *Animal, 5*(10), 1–18. https://doi.org/10.3389/fvets.2018.00245

Teng, P. Y., & Kim, W. K. (2018). *Review: Roles of prebiotics in intestinal ecosystem of broilers. Frontiers Media S.A.* https://doi.org/10.3389/fvets.2018.00245

Terie, K. A. (2016). *Studies on poultry management practices , marketing system and effect of feeding fish meal on growth performance and carcass characteristics of koekoek chickens in bora district , east shoa zone.* Awassa university.

Tewodros, A. (2019). Exotic chicken production performance, status and challenges in Ethiopia. *International Journal of Veterinary Science and Research, 5*(2), 39–45. https://doi.org/10.17352/ijvsr.000040

Tijani, L. A., Akanji, A. M. ., Agbalaya, K., & Onigemo, M. (2015). Haematological and Serum Biochemical Profiles of Broiler. *Journal of Tropical Agriculture, Food, Environment and Extension, 14*(3), 7–11. https://www.ajol.info/index.php/as/article/view/149053/138555

Tijani, L. A., Akanji, A. M., Agbalaya, K., & Onigemo, M. (2016). Comparative Effects of Graded Levels of Moringa Leaf Meal on Haematological and Serum Biochemical Profile of Broiler Chickens. *Journal of Agricultural Sciences, 11*(3), 137. https://doi.org/10.4038/jas.v11i3.8167

Tlili, H., Hanen, N., Arfa, A. Ben, Neffati, M., Boubakri, A., Buonocore, D., Dossena, M., Verri, M., & Doria, E. (2019). Biochemical profile and in vitro biological activities of extracts from seven folk medicinal plants growing wild in southern Tunisia. *PLoS ONE, 14*(9), 1–18. https://doi.org/10.1371/journal.pone.0213049

Tshabalala, T., Ncube, B., Madala, N. E., Nyakudya, T. T., Moyo, H. P., Sibanda, M., & Ndhlala, A. R. (2019). Scribbling the cat: A case of the "miracle" plant, Moringa oleifera. *Plants, 8*(11), 1–23. https://doi.org/10.3390/plants8110510

Tuohimaa, P. (2012). Vitamin D, aging, and chronic diseases. Vitamin D: Oxidative Stress. *Immunity, and Aging, 8*(3), 385–406. https://doi.org/10.1201/b13714

Udikala, M., Verma, Y., Sushma, S., & Lal, S. (2017). Phytonutrient and Pharmacological Significance of Moringa oleifera. *International Journal of Life-Sciences Scientific Research, 3*(5), 1387–1391. https://doi.org/10.21276/ijlssr.2017.3.5.21

Udofia, N. E., Onyancha, J. M., Mworia, M., William, N., & Apiri, M. G. (2020). Chemical Composition of Moringa oleifera Lam . and Moringa Stenopetala Bac . Leaves from Kenya. *International Journal of Plant Research, 10*(1), 1–10. https://doi.org/10.5923/j.plant.20201001.01

Ulfman, L. H., Leusen, J. H. W., Savelkoul, H. F. J., Warner, J. O., & van Neerven, R. J. J. (2018). Effects of Bovine Immunoglobulins on Immune Function, Allergy, and Infection. *Frontiers in Nutrition, 5*(6), 1–20. https://doi.org/10.3389/fnut.2018.00052

Ullrich, C., Langeheine, M., Brehm, R., Taube, V., Galera, M. R., Rohn, K., Popp, J., & Visscher, C. (2019). Influence of different methionine sources on performance and slaughter characteristics of broilers. *Animals, 9*(11), 1–20. https://doi.org/10.3390/ani9110984

Umaru, I. J., Badruddin, F. A., & Umaru, H. A. (2019). Phytochemical Screening of Essential Oils and Antibacterial Activity and Antioxidant Properties of Barringtonia asiatica (L) Leaf Extract. *Biochemistry Research International, 2*(1), 1-7. https://doi.org/10.1155/2019/7143989

United Nations. (2015). *Transforming our world: the 2030 Agenda for Sustainable Development. United Nations*. https://doi.org/10.1163/157180910X12665776638740

Valdez-Solana, M. A., Mejía-García, V. Y., Téllez-Valencia, A., García-Arenas, G., Salas-Pacheco, J., Alba-Romero, J. J., & Sierra-Campos, E. (2015). Nutritional content and elemental and phytochemical analyses of Moringa oleifera grown in Mexico. *Journal of Chemistry, 2*(15), 1-6. https://doi.org/10.1155/2015/860381

Vannucci, L., Fossi, C., Quattrini, S., Guasti, L., Pampaloni, B., Gronchi, G., Giusti, F., Romagnoli, C., Cianferotti, L., Marcucci, G., & Brandi, M. L. (2018).

Calcium Intake in bone health: A focus on calcium-rich mineral waters. *Nutrients, 10*(12), 1–12. https://doi.org/10.3390/nu10121930

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). *Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Frontiers in Microbiology.* https://doi.org/10.3389/fmicb.2019.00057

Voemesse, K., Teteh, A., Nideou, D., N'Nanlé, O., Gbeassor, M., Decuypere, E., & Tona, K. (2018). Effect of Moringa oleifera leaf meal on growth performance and blood parameters of egg type chicken during juvenile growth. *International Journal of Poultry Science, 17*(4), 154–159. https://doi.org/10.3923/ijps.2018.154.159

Walter, A., Samuel, W., Peter, A., & Joseph, O. (2011). Antibacterial activity of Moringa oleifera and Moringa Stenopetala methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *African Journal of Microbiology Research, 5*(2), 153–157. https://doi.org/10.5897/AJMR10.457

Wang, S., Zeng, X., Yang, Q., & Qiao, S. (2016). Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. *International Journal of Molecular Sciences, 17*(5), 1-7. https://doi.org/10.3390/ijms17050603

Wang, Z., Zhang, F., Xiao, F., Tao, Y., Liu, Z., Li, G., Wang, S., & Ding, Y. (2018). Contribution of mineral nutrients from source to sink organs in rice under different nitrogen fertilization. *Plant Growth Regulation, 86*(2), 159–167. https://doi.org/10.1007/s10725-018-0418-0

Wernicki, A., Nowaczek, A., & Urban-Chmiel, R. (2017). Bacteriophage therapy to combat bacterial infections in poultry. *Virology Journal, 14*(1), 1–13. https://doi.org/10.1186/s12985-017-0849-7

Wicks, J., Beline, M., Gomez, J. F. M., Luzardo, S., Silva, S. L., & Gerrard, D. (2019). Muscle energy metabolism, growth, and meat quality in beef cattle. *Agriculture (Switzerland), 9*(9), 1–10. https://doi.org/10.3390/agriculture9090195

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., De Vries, W., Majele Sibanda, L., … Murray, C. J. L. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet,*

393(10170), 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4

Wina, E. (2018). The Role of Saponin as Feed Additive for Sustainable Poultry Production. *Indonesian Bulletin of Animal and Veterinary Sciences, 27*(3), 117. https://doi.org/10.14334/wartazoa.v27i3.1588

Xie, M., Tang, J., Wen, Z., Huang, W., & Hou, S. (2014). Effects of pyridoxine on growth performance and plasma aminotransferases and homocysteine of White Pekin ducks. *Asian-Australasian Journal of Animal Sciences, 27*(12), 1744–1748. https://doi.org/10.5713/ajas.2014.14252

Xu, Z., & Howard, L. R. (2012). *Analysis of Antioxidant-Rich Phytochemicals. In Analysis of Antioxidant-Rich Phytochemicals.* https://doi.org/10.1002/9781118229378

Yadav, S., & Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization , performance , and health of poultry. *Journal of Animal Science and Biotechnolog, 10*(2), 1–11. https://dx.doi.org/10.1186%2Fs40104-018-0310-9

Yoo, J., Yi, Y. J., Koo, B., Jung, S., Yoon, J. U., Kang, H. B., Lee, D. H., & Heo, J. M. (2016). Growth performance, intestinal morphology, and meat quality in relation to alpha-lipoic acid associated with vitamin C and E in broiler chickens under tropical conditions. *Revista Brasileira de Zootecnia, 45*(3), 113–120. https://doi.org/10.1590/S1806-92902016000300005

Yoshii, K., Hosomi, K., Sawane, K., & Kunisawa, J. (2019). Metabolism of dietary and microbial vitamin b family in the regulation of host immunity. *Frontiers in Nutrition, 6*(4), 1–12. https://doi.org/10.3389/fnut.2019.00048

Younis, M. E. M., & Elbestawy, A. R. (2017). Water supplementation of Moringa oleifera and its effect on performance, blood antioxidant and immune response of two broiler breeds. *Journal of Biological Sciences, 17*(2), 52–60. https://doi.org/10.3923/jbs.2017.52.60

Yusuf. T, M., Tiamiyu S, A., & O, A. R. (2016). Financial analysis of poultry production in Kwara State, Nigeria. *African Journal of Agricultural Research, 11*(8), 718–723. https://doi.org/10.5897/ajar2015.10690

Zaefarian, F., Abdollahi, M. R., Cowieson, A., & Ravindran, V. (2019). Avian liver: The forgotten organ. *Animals, 9*(2), 1–23. https://doi.org/10.3390/ani9020063

Zaku, S. G., Emmanuel, S., Tukur, A. A., & Kabir, A. (2015). Moringa oleifera: An

underutilized tree in Nigeria with amazing versatility: A review. *African Journal of Food Science, 9*(9), 456–461. https://doi.org/10.5897/ajfs2015.1346

Zamxaka, C. W. (2016). *Socio-economic Contribution and Health Challenges of Indigenous Chickens in Smallholder Systems.* http://hdl.handle.net/20.500.11837/796

Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., Li, H. Bin, & Kitts, D. D. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules, 20*(12), 21138–21156. https://doi.org/10.3390/molecules201219753

APPENDICES

Appendix 1: Day-old Experimental chicks

The chicks from the Kenchic hatchery are packaged and delivered in boxes. Picture taken by Ebenezer Udofia

Appendix 2: Experimental Chicks after Acclimatization

Chicks were brooded before allotting to experimental units –Picture take by Ebenezer Udofia in the field.

Appendix 3: Feeding of Experimental Chicks

Appendix 4: Extract Being Administered to Experimental Chick

Moringa extracts administered to chicks by Elias and Gervason – two of the research Assistants. Picture taken by Ebenezer at the experimental site.

Appendix 5: Locating and Sterilizing the Brachial Vein for Blood Extraction

Extracting blood for haematological analysis by the two research Assistants. Picture was captured by Ebenezer

Appendix 6: Methods for the Determination of Biochemical Parameters

The calcium concentration in the broiler sera samples was determined by adopting the method provided in the calcium kit. The reaction mixture consisted of 0.5 ml of buffer and colour reagent respectively, followed by 0.02 ml of the sample and standard for the test and standard respectively. In the blank test tubes 0.02 ml of distilled water was added and then the content in all the test tubes mixed. The absorbance of the samples was read within the 60minutes at 570 nm. The calcium concentration was calculated as follows

$$CalciumConcentration = \frac{Abs.T}{Abs.S} \times 10$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Total Protein

The total protein was determined spectrophotometrically following the method in the total protein kit. Briefly 1ml of the biuret reagent was added in to the blank, standard and test tubes followed by 0.02 ml of the distilled water, standard protein and sample in the respective tubes. The content in the tubes was then mixed well and incubated at 37 ^{0}C for 10 minutes prior to reading the absorbance at 550nm against the blank within the 60minutes. The concentration of the total protein was calculated from the equation;

$$Totalprotein(g/dl) = \frac{Abs.T}{Abs.S} \times 8$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Creatinine

The creatinine content in the blood samples was determined following the alkaline picrate method as described in the creatinine kit. Briefly, into the 1.1 ml of the clear supernatant obtained by centrifuging the mixture of 2.0 ml of the picric acid reagent and 0.2ml of the blood serum sample, 0.1 ml of the buffer reagent (ii) was added, mixed well and incubated at room temperature for 20 minutes after which its absorbance was read at 520 nm against the blank that consisted of 1 ml and 0.1 ml of the picric acid, distilled water and buffer respectively. The absorbance of the standard was as well determined at this wavelength as the sample. The tests were conducted in triplicates and the creatinine concentration in percentage milligrams calculated by the following the equation:

$$Creatinine(mg\ \%) = \frac{Abs.T}{Abs.S} \times 2$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Albumin

The albumin levels in the blood serum samples of the broiler chicks was determined by method outlined in the albumin kit. Into the 0.1 ml of the sample/standard, 1ml of the bcc reagent was added, mixed well and then incubated at room temperature for 5mins. After incubation the absorbance of the sample test and standard was measured at 630 nm against the blank (1 ml bcc reagent + 0.1 ml distilled water). The albumin content in grams per deciliter was then calculated following the equation:

$$Albumin(g/dl) = \frac{Abs.T}{Abs.S} \times 4$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Urea

The urea content in the sera samples were determined by the kinetic method as per the protocols outlined in the urea kit. Into the clean test tubes 0.8 ml and 0.01 ml of the enzyme and standard/serum was added, mixed well incubated at room temperature. After the elapse of the 5 minutes, 0.2 ml of the starter reagent was added mixed well and the absorbance measured at interval of 30sec and 60sec respectively against the blank. The urea concentration in milligram per deciliter was calculated following the formula:

$$Urea(g/dl) = \frac{\Delta AT}{\Delta AS} \times 40$$

Where ΔAT is the change in the test absorbance and ΔAS is the change in the standard absorbance.

Glucose

The glucose levels in the blood sera samples were spectrophotometrically determined by the UV-Vis spectrophotometer following the method outlined in the blood glucose kit. Into the 1ml of the glucose reagent 0.01 ml of the sample/standard was added, mixed well and then incubated at room temperature. The absorbance of the samples and standard were then read at 505 nm within the 60 minutes against blank (1 ml glucose reagent (ii) + 0.01 ml distilled water). The glucose concentration in milligrams per deciliter in the sera samples were calculated following the formula:

$$Glucose(mg/dl) = \frac{Abs.T}{Abs.S} \times 100$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Triglycerides

The levels of the triglycerides in the blood sera samples of the broiler chicks was determined following the method in the triglycerides kit by use of UV-Vis spectrophotometer.Into the 0.01ml of the blood serum sample/standard 1 ml of working reagent was added, mixed well by shaking and incubated at room temperature for 15 minutes. The absorbance of the sample and standard were measured at 505 nm within the 60min against the blank. The triglycerides concentration in milligrams per deciliter was calculated from the formula:

$$Triglycerides(mg/dl) = \frac{Abs.T}{Abs.S} \times 200$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance.

Total cholesterol

The total cholesterol in the blood sera samples of the broiler chicks on the study was determined as per the method in the total cholesterol kit. Into the 1ml of the working reagent, 0.01 ml sample/ standard was added mixed well and incubated at room temperature for 15 minutes. After the incubation, the absorbance of the standard and sample were measured at 505nm against the blank (working reagent + distilled water) within the 60 minutes using the UV-Vis spectrophotometer. The cholesterol concentration using the formula below.

$$Cholesterol(mg/dl) = \frac{Abs.T}{Abs.S} \times 200$$

Where Abs.T is the test absorbance and Abs.S is the standard absorbance

Appendix 7: Procedure for qualitative phytochemical screening of *M. oleifera* and *M. stenopetala* powders

Test for Tannins

Five (5) grams of the powdered *M. oleifera* and *M. stenopetala* leaves were separately boiled in 20 ml of distilled water in test tubes and then filtered. Three drops of 0.1 % $FeCl_3$ were added to the resulting filtrates. The appearance of bluish-green precipitate indicated presence of tannins (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for Saponins (Frothing test)

Five grams of the powdered leaves of *Moringa oleifera* and *Moringa stenopetala* were separately boiled with 20 ml of distilled water respectively in test tubes and then allowed to cool. After shaking, the appearance of frothing was indicative of positive result for saponins (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for Alkaloids

Approximately 0.8 g of the powders of *Moringa oleifera* and *Moringa stenopetala* were separately mixed with about 10 ml of 1 % Hcl, warmed and filtered. Two millilitres of filtrates were treated with Mayer's reagent separately. The appearance of cream-colored precipitate was considered positive indication for alkaloids. Similarly, two millilitres of the filtrate were treated with Dragendorff's reagent. A reddish-brown precipitate was considered positive test for presence of alkaloids (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for Glycosides

(i)Keller-killiani test (test for deoxy sugars)

One gram of *Moringa oleifera* and *Moringa stenopetala* leaf powder were extracted with 10 ml chloroform and evaporated to dryness in test tubes. Glacial acetic acid (0.4 ml) containing trace amount of ferric chloride was added in each test tube followed by careful addition of 0.5 ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer showing a blue colour was considered positive test for presence of cardiac glycosides (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

(ii) Borntrager's test

0.5 gram of *Moringa oleifera* and *Moringa stenopetala* leaves were boiled separately in 1 ml of sulphuric acid in test tubes for 5 minutes. They were then filtered while hot, cooled and then shaken with equal volume of chloroform. The lower layers of chloroform were separated and shaken with half of their volumes with dilute ammonia. A rose pink to red colour produced in the ammoniacal layer was considered positive indication of the presence of glycosides (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

(iii) Modified Borntrager's test

Two drops of Kedde reagent were added to portions of extracts from Moringa oleifera and *Moringa stenopetala* leaves. The presence of purple colour was considered indicator for the presence of glycosides whose aglycone moiety had unsaturated lactone ring (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for steroids

One millilitre solution of ethanol extract was taken and then the Liebermann–Burchard reagent added. The reddish purple colour produced indicated the presence of steroids. (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for flavonoids

Five drops of concentrated hydrochloride acid was added to a small amount of an alcoholic extract of *Moringa oleifera* and *Moringa stenopetala* leaves. Immediate development of a red colour was considered as indicative of the presence of flavonoids. The presence of flavonoids was also confirmed by three other methods. 10 ml solution of the extract was hydrolysed with 10% sulfuric acid and divided into three portions: in the first portion dilute ammonia solution was added in one portion and the appearance of greenish yellow colour indicated the presence of flavonoids; into the second portion, 1ml dilute sodium carbonate solution was added and the appearance of the pale yellow indicated the presence of flavonoids; lastly in the third portion, 1 ml dilute sodium hydroxide solution was added and the appearance of a yellow colour indicated the presence of flavonoids (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for Phenols

One gram of *Moringa oleifera* and *Moringa stenopetala* leaves were boiled with 10 ml of 70 % ethanol in a water bath using boiling tubes for 5 minutes. The extracts were filtered while hot and cooled. To 2 ml of these extracts, 5 % Ferric chloride was added. The occurrence of a green precipitate was considered as indicative of the presence of phenols. (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Test for terpenoids

Two millilitres of *Moringa oleifera* and *Moringa stenopetala* leaves extracts were added to 1 ml of acetic acid anhydride followed by careful addition of concentrated sulphuric acid. The formation of blue-green ring was considered as indicative of the presence of terpenoids. (Savithrama, et al., 2011; Khandelwal and Vrunda, 2015).

Appendix 8: Methods for quantitative phytochemical analysis of *Moringa powder*

Determination of flavonoids

Moringa oleifera and *Moringa stenopetala* (5 g) were weighed into separate 250 ml titration flask, and 100 ml of the 80 % aqueous methanol was added at room temperature and shaken for 4 hours in an electric shaker. The entire solution was filtered through Whatman filter paper number 1. To the filtrate, 100 ml of the 80 % aqueous methanol was again added at room temperature, shaken for 4 hours in an electric shaker then filtered as before. The filtrates were later transferred into weighed crucibles and evaporated to dryness over a water bath and weighed again (Boham et al., 1994). The difference in weight gave the weights of flavonoids which were expressed as percentages of the weights of analyzed respective samples (Dabbou et al., 2018).

$$\% \, Flavonoidcontent = \frac{W2-W1}{W0} x100\%$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + flavonoids precipitate W0 = weight of sample

Determination of alkaloids

The quantities of alkaloids in *Moringa oleifera* and *Moringa stenopetala* leaf powders were determined by weighing 5 g of each samples into a 250 ml beaker. Two hundred millilitres of 20 % acetic acid in ethanol were added and allowed to stand for 4 hours. Thereafter the extracts were filtered concentrated using a water bath to evaporate about one-quarter of the original volume. Then concentrated ammonium solution was added dropwise to the extracts until precipitation was complete. The entire solutions were allowed to settle and washed using distilled

water then filtered. The weights of respective precipitates were recorded and used to calculate the percentage of alkaloids in the tested samples (Obadoni et al., 2001).

$$\% \, Alkaloidcontent = \frac{W2 - W1}{W0} x100\%$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + Alkaloids precipitate Wo=weight of sample

Determination of saponins

Twenty grams (20 g) of each of *Moringa oleifera* and *Moringa stenopetala* powders were weighed and added into 200 ml of 20 % ethanol. The respective suspensions were heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{0}C. The mixtures were filtered and the residues re-extracted with another 200 ml of 20 % ethanol. The combined extracts of each plant were reduced to 40 ml over water bath at about 90 ^{0}C. The concentrates were transferred into 250 ml separating funnels and 20 ml of diethyl ether added followed by vigorous shaking. Aqueous layers were recovered while the ether layer was discarded. 60 ml of n-butanol was added to the aqueous layer. The solution of n-butanol extracts was washed twice with 10 ml 5% aqueous sodium chloride. The remaining solutions were evaporated on a hot water bath and dried in the hot air oven to a constant weight. The saponins content was calculated in percentage (Obadoni et al., 2001).

$$\% \, Saponincontent = \frac{W2 - W1}{W0} x100\%$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + saponins precipitate Wo=weight of sample

Determination of phenols

To determine the total phenols, 5 g of each of *Moringa oleifera* and *Moringa stenopetala* powders were weighed into separate 250 ml titration flask and 100 ml n-hexane added twice at 4 hours interval each. The filtrates were discarded for fat free sample preparation. 100 ml of diethyl ether were added to the residues, heated for 15 minutes, cooled up to room temperature and filtered into a separating funnel. 100 ml of the 10 % NaOH solution were added and swirled to separate the aqueous layer from the organic layer. The aqueous layers were washed three times with 25 ml de-ionized water. The total aqueous layers were acidified up to pH 4.0 by adding 10 % HCl solution and 50 ml dichloromethane (CH_2Cl_2) thus acidifying the aqueous layers in the separating flasks. Consequently, the organic layers were collected, dried and then weighed (Iqbal et al., 2011).

$$\% \, Phenolcontent = \frac{W2 - W1}{W0} x100\%$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + Phenols precipitate Wo=weight of sample.

Appendix 9: Analysed Results

Growth Performance of broiler chicken
Aqueous and ethanolic leaf extracts of *M. oleifera* and *M. stenopetala*
EXTRACTS
Descriptive Statistics: Feed Intake
Statistics

Variable	Treatment	Mean	SE Mean
Feed Intake	MOE 1	2417.7	0.855
	MOE 25	3200.9	4.43
	MOE 5	2863.3	0.339
	MOW 1	2527.7	2.22
	MOW 25	3281.1	3.80
	MOW 5	2911.3	5.84
	MSE 1	2511.9	3.32
	MSE 25	3243.2	9.03
	MSE 5	2847.7	2.06
	MSW 1	2844.6	3.11
	MSW 25	3380.7	1.91
	MSW 5	3065.5	50.7
	NEGATIVE CONTROL	4414.3	3.56
	POSITIVE CONTROL	3133.8	6.14

EXTRACTS
One-way ANOVA: Feed Intake versus Treatment
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	14	MOE 1, MOE 25, MOE 5, MOW 1, MOW 25, MOW 5, MSE 1, MSE 25, MSE 5, MSW 1,MSW 25, MSW 5, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	13	9553175	734860	640.67	0.000
Error	28	32117	1147		
Total	41	9585291			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
33.8678	99.66%	99.51%	99.40%

Means

Treatment	N	Mean	StDev	95% CI
MOE 1	3	2417.72	1.48	(2377.67, 2457.77)
MOE 25	3	3200.88	7.67	(3160.83, 3240.93)
MOE 5	3	2863.29	0.59	(2823.24, 2903.35)
MOW 1	3	2527.74	3.85	(2487.69, 2567.80)
MOW 25	3	3281.11	6.59	(3241.06, 3321.17)
MOW 5	2	2911.26	8.25	(2862.20, 2960.31)
MSE 1	3	2511.86	5.75	(2471.81, 2551.92)
MSE 25	3	3243.16	15.65	(3203.11, 3283.21)
MSE 5	3	2847.71	3.56	(2807.66, 2887.76)
MSW 1	3	2844.64	5.39	(2804.59, 2884.70)
MSW 25	3	3380.75	3.31	(3340.70, 3420.80)
MSW 5	4	3065.5	101.4	(3030.8, 3100.2)
NEGATIVE CONTROL	3	4414.28	6.16	(4374.22, 4454.33)
POSITIVE CONTROL	3	3133.84	10.64	(3093.79, 3173.90)

Pooled StDev = 33.8678

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
NEGATIVE CONTROL	3	4414.28	A
MSW 25	3	3380.75	B
MOW 25	3	3281.11	BC
MSE 25	3	3243.16	C
MOE 25	3	3200.88	C D
POSITIVE CONTROL	3	3133.84	D E
MSW 5	4	3065.5	E
MOW 5	2	2911.26	F
MOE 5	3	2863.29	F
MSE 5	3	2847.71	F
MSW 1	3	2844.64	F
MOW 1	3	2527.74	G
MSE 1	3	2511.86	G H
MOE 1	3	2417.72	H

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOE 25 - MOE 1	783.2	27.7	(681.9, 884.4)	28.32	0.000
MOE 5 - MOE 1	445.6	27.7	(344.3, 546.9)	16.11	0.000
MOW 1 - MOE 1	110.0	27.7	(8.7, 211.3)	3.98	0.024
MOW 25 - MOE 1	863.4	27.7	(762.1, 964.7)	31.22	0.000
MOW 5 - MOE 1	493.5	30.9	(380.3, 606.8)	15.96	0.000
MSE 1 - MOE 1	94.1	27.7	(-7.1, 195.4)	3.40	0.088
MSE 25 - MOE 1	825.4	27.7	(724.2, 926.7)	29.85	0.000
MSE 5 - MOE 1	430.0	27.7	(328.7, 531.3)	15.55	0.000
MSW 1 - MOE 1	426.9	27.7	(325.6, 528.2)	15.44	0.000
MSW 25 - MOE 1	963.0	27.7	(861.7, 1064.3)	34.83	0.000
MSW 5 - MOE 1	647.8	25.9	(553.0, 742.5)	25.04	0.000
NEGATIVE CON - MOE 1	1996.6	27.7	(1895.3, 2097.8)	72.20	0.000
POSITIVE CON - MOE 1	716.1	27.7	(614.8, 817.4)	25.90	0.000
MOE 5 - MOE 25	-337.6	27.7	(-438.9, -236.3)	-12.21	0.000
MOW 1 - MOE 25	-673.1	27.7	(-774.4, -571.8)	-24.34	0.000
MOW 25 - MOE 25	80.2	27.7	(-21.1, 181.5)	2.90	0.235
MOW 5 - MOE 25	-289.6	30.9	(-402.9, -176.4)	-9.37	0.000
MSE 1 - MOE 25	-689.0	27.7	(-790.3, -587.7)	-24.92	0.000
MSE 25 - MOE 25	42.3	27.7	(-59.0, 143.6)	1.53	0.950
MSE 5 - MOE 25	-353.2	27.7	(-454.5, -251.9)	-12.77	0.000
MSW 1 - MOE 25	-356.2	27.7	(-457.5, -254.9)	-12.88	0.000

MSW 25 - MOE 25	179.9	27.7	(78.6, 281.2)	6.50	0.000
MSW 5 - MOE 25	-135.4	25.9	(-230.1, -40.7)	-5.23	0.001
NEGATIVE CON - MOE 25	1213.4	27.7	(1112.1, 1314.7)	43.88	0.000
POSITIVE CON - MOE 25	-67.0	27.7	(-168.3, 34.2)	-2.42	0.489
MOW 1 - MOE 5	-335.5	27.7	(-436.8, -234.3)	-12.13	0.000
MOW 25 - MOE 5	417.8	27.7	(316.5, 519.1)	15.11	0.000
MOW 5 - MOE 5	48.0	30.9	(-65.3, 161.2)	1.55	0.945
MSE 1 - MOE 5	-351.4	27.7	(-452.7, -250.1)	-12.71	0.000
MSE 25 - MOE 5	379.9	27.7	(278.6, 481.2)	13.74	0.000
MSE 5 - MOE 5	-15.6	27.7	(-116.9, 85.7)	-0.56	1.000
MSW 1 - MOE 5	-18.7	27.7	(-119.9, 82.6)	-0.67	1.000
MSW 25 - MOE 5	517.5	27.7	(416.2, 618.7)	18.71	0.000
MSW 5 - MOE 5	202.2	25.9	(107.4, 296.9)	7.82	0.000
NEGATIVE CON - MOE 5	1551.0	27.7	(1449.7, 1652.3)	56.09	0.000
POSITIVE CON - MOE 5	270.5	27.7	(169.3, 371.8)	9.78	0.000
MOW 25 - MOW 1	753.4	27.7	(652.1, 854.7)	27.24	0.000
MOW 5 - MOW 1	383.5	30.9	(270.3, 496.8)	12.40	0.000
MSE 1 - MOW 1	-15.9	27.7	(-117.2, 85.4)	-0.57	1.000
MSE 25 - MOW 1	715.4	27.7	(614.1, 816.7)	25.87	0.000
MSE 5 - MOW 1	320.0	27.7	(218.7, 421.3)	11.57	0.000
MSW 1 - MOW 1	316.9	27.7	(215.6, 418.2)	11.46	0.000
MSW 25 - MOW 1	853.0	27.7	(751.7, 954.3)	30.85	0.000

MSW 5 - MOW 1	537.7	25.9	(443.0, 632.5)	20.79	0.000
NEGATIVE CON - MOW 1	1886.5	27.7	(1785.2, 1987.8)	68.22	0.000
POSITIVE CON - MOW 1	606.1	27.7	(504.8, 707.4)	21.92	0.000
MOW 5 - MOW 25	-369.9	30.9	(-483.1, -256.6)	-11.96	0.000
MSE 1 - MOW 25	-769.3	27.7	(-870.5, -668.0)	-27.82	0.000
MSE 25 - MOW 25	-38.0	27.7	(-139.2, 63.3)	-1.37	0.978
MSE 5 - MOW 25	-433.4	27.7	(-534.7, -332.1)	-15.67	0.000
MSW 1 - MOW 25	-436.5	27.7	(-537.8, -335.2)	-15.78	0.000
MSW 25 - MOW 25	99.6	27.7	(-1.7, 200.9)	3.60	0.057
MSW 5 - MOW 25	-215.6	25.9	(-310.4, -120.9)	-8.34	0.000
NEGATIVE CON - MOW 25	1133.2	27.7	(1031.9, 1234.5)	40.98	0.000
POSITIVE CON - MOW 25	-147.3	27.7	(-248.6, -46.0)	-5.33	0.001
MSE 1 - MOW 5	-399.4	30.9	(-512.6, -286.2)	-12.92	0.000
MSE 25 - MOW 5	331.9	30.9	(218.7, 445.1)	10.74	0.000
MSE 5 - MOW 5	-63.5	30.9	(-176.8, 49.7)	-2.06	0.723
MSW 1 - MOW 5	-66.6	30.9	(-179.9, 46.6)	-2.15	0.661
MSW 25 - MOW 5	469.5	30.9	(356.2, 582.7)	15.19	0.000
MSW 5 - MOW 5	154.2	29.3	(46.8, 261.7)	5.26	0.001
NEGATIVE CON - MOW 5	1503.0	30.9	(1389.8, 1616.3)	48.61	0.000
POSITIVE CON - MOW 5	222.6	30.9	(109.3, 335.8)	7.20	0.000
MSE 25 - MSE 1	731.3	27.7	(630.0, 832.6)	26.45	0.000

MSE 5 - MSE 1	335.8	27.7	(234.6, 437.1)	12.15	0.000
MSW 1 - MSE 1	332.8	27.7	(231.5, 434.1)	12.03	0.000
MSW 25 - MSE 1	868.9	27.7	(767.6, 970.2)	31.42	0.000
MSW 5 - MSE 1	553.6	25.9	(458.9, 648.4)	21.40	0.000
NEGATIVE CON - MSE 1	1902.4	27.7	(1801.1, 2003.7)	68.80	0.000
POSITIVE CON - MSE 1	622.0	27.7	(520.7, 723.3)	22.49	0.000
MSE 5 - MSE 25	-395.5	27.7	(-496.7, -294.2)	-14.30	0.000
MSW 1 - MSE 25	-398.5	27.7	(-499.8, -297.2)	-14.41	0.000
MSW 25 - MSE 25	137.6	27.7	(36.3, 238.9)	4.98	0.002
MSW 5 - MSE 25	-177.7	25.9	(-272.4, -82.9)	-6.87	0.000
NEGATIVE CON - MSE 25	1171.1	27.7	(1069.8, 1272.4)	42.35	0.000
POSITIVE CON - MSE 25	-109.3	27.7	(-210.6, -8.0)	-3.95	0.025
MSW 1 - MSE 5	-3.1	27.7	(-104.4, 98.2)	-0.11	1.000
MSW 25 - MSE 5	533.0	27.7	(431.8, 634.3)	19.28	0.000
MSW 5 - MSE 5	217.8	25.9	(123.0, 312.5)	8.42	0.000
NEGATIVE CON - MSE 5	1566.6	27.7	(1465.3, 1667.9)	56.65	0.000
POSITIVE CON - MSE 5	286.1	27.7	(184.8, 387.4)	10.35	0.000
MSW 25 - MSW 1	536.1	27.7	(434.8, 637.4)	19.39	0.000
MSW 5 - MSW 1	220.8	25.9	(126.1, 315.6)	8.54	0.000
NEGATIVE CON - MSW 1	1569.6	27.7	(1468.3, 1670.9)	56.76	0.000
POSITIVE	289.2	27.7	(187.9, 390.5)	10.46	0.000

CON - MSW 1					
MSW 5 - MSW 25	-315.3	25.9	(-410.0, -220.5)	-12.19	0.000
NEGATIVE CON - MSW 25	1033.5	27.7	(932.2, 1134.8)	37.38	0.000
POSITIVE CON - MSW 25	-246.9	27.7	(-348.2, -145.6)	-8.93	0.000
NEGATIVE CON - MSW 5	1348.8	25.9	(1254.1, 1443.5)	52.14	0.000
POSITIVE CON - MSW 5	68.4	25.9	(-26.4, 163.1)	2.64	0.359
POSITIVE CON - NEGATIVE CON	-1280.4	27.7	(-1381.7, -1179.1)	-46.30	0.000

Individual confidence level = 99.90%

EXTRACTS

Descriptive Statistics: Body weight

Statistics

Variable	**Treatment**	**Mean**	**SE Mean**
Body weight	MOE 1	1638.3	0.855
	MOE 25	2490.3	4.43
	MOE 5	2130.1	0.339
	MOW 1	1673.4	2.22
	MOW 25	2533.5	3.80
	MOW 5	2135.8	5.84
	MSE 1	1752.6	3.32
	MSE 25	2514.3	9.03
	MSE 5	2098.6	2.06
	MSW 1	1943.7	3.11
	MSW 25	2674.2	1.91
	MSW 5	2271.8	44.6
	NEGATIVE CONTROL	1005.3	4.53
	POSITIVE CONTROL	2456.6	6.14

EXTRACTS

One-way ANOVA: Body weight versus Treatment

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	14	MOE 1, MOE 25, MOE 5, MOW 1, MOW 25, MOW 5, MSE 1, MSE 25, MSE 5, MSW 1, MSW 25, MSW 5, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	13	8245487	634268	703.89	0.000
Error	28	25230	901		
Total	41	8270718			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
30.0181	99.69%	99.55%	99.45%

Means

Treatment	N	Mean	StDev	95% CI
MOE 1	3	1638.25	1.48	(1602.75, 1673.75)
MOE 25	3	2490.27	7.67	(2454.77, 2525.77)
MOE 5	3	2130.05	0.59	(2094.55, 2165.55)
MOW 1	3	1673.36	3.85	(1637.86, 1708.86)
MOW 25	3	2533.53	6.59	(2498.03, 2569.03)
MOW 5	2	2135.84	8.25	(2092.36, 2179.31)
MSE 1	3	1752.64	5.75	(1717.14, 1788.14)
MSE 25	3	2514.30	15.65	(2478.80, 2549.80)
MSE 5	3	2098.61	3.56	(2063.11, 2134.11)
MSW 1	3	1943.70	5.39	(1908.20, 1979.20)
MSW 25	3	2674.16	3.31	(2638.66, 2709.66)
MSW 5	4	2271.8	89.3	(2241.1, 2302.6)

NEGATIVE CONTROL	3	1005.29	7.85	(969.79, 1040.79)
POSITIVE CONTROL	3	2456.61	10.64	(2421.11, 2492.11)

Pooled StDev = 30.0181

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
MSW 25	3	2674.16	A
MOW 25	3	2533.53	B
MSE 25	3	2514.30	B
MOE 25	3	2490.27	B
POSITIVE CONTROL	3	2456.61	B
MSW 5	4	2271.8	C
MOW 5	2	2135.84	D
MOE 5	3	2130.05	D
MSE 5	3	2098.61	D
MSW 1	3	1943.70	E
MSE 1	3	1752.64	F
MOW 1	3	1673.36	F G
MOE 1	3	1638.25	G
NEGATIVE CONTROL	3	1005.29	H

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOE 25 - MOE 1	852.0	24.5	(762.2, 941.8)	34.76	0.000
MOE 5 - MOE 1	491.8	24.5	(402.0, 581.6)	20.07	0.000
MOW 1 - MOE 1	35.1	24.5	(-54.7, 124.9)	1.43	0.969
MOW 25 - MOE 1	895.3	24.5	(805.5, 985.1)	36.53	0.000
MOW 5 - MOE 1	497.6	27.4	(397.2, 598.0)	18.16	0.000
MSE 1 - MOE 1	114.4	24.5	(24.6, 204.2)	4.67	0.004
MSE 25 - MOE 1	876.1	24.5	(786.3, 965.8)	35.74	0.000
MSE 5 - MOE 1	460.4	24.5	(370.6, 550.1)	18.78	0.000
MSW 1 - MOE 1	305.4	24.5	(215.7, 395.2)	12.46	0.000
MSW 25 - MOE 1	1035.9	24.5	(946.1, 1125.7)	42.27	0.000
MSW 5 - MOE 1	633.6	22.9	(549.6, 717.6)	27.64	0.000
NEGATIVE CON - MOE 1	-633.0	24.5	(-722.7, -543.2)	-	0.000

				25.82	
POSITIVE CON - MOE 1	818.4	24.5	(728.6, 908.1)	33.39	0.000
MOE 5 - MOE 25	-360.2	24.5	(-450.0, -270.4)	-14.70	0.000
MOW 1 - MOE 25	-816.9	24.5	(-906.7, -727.1)	-33.33	0.000
MOW 25 - MOE 25	43.3	24.5	(-46.5, 133.0)	1.77	0.874
MOW 5 - MOE 25	-354.4	27.4	(-454.8, -254.1)	-12.93	0.000
MSE 1 - MOE 25	-737.6	24.5	(-827.4, -647.9)	-30.10	0.000
MSE 25 - MOE 25	24.0	24.5	(-65.7, 113.8)	0.98	0.999
MSE 5 - MOE 25	-391.7	24.5	(-481.4, -301.9)	-15.98	0.000
MSW 1 - MOE 25	-546.6	24.5	(-636.3, -456.8)	-22.30	0.000
MSW 25 - MOE 25	183.9	24.5	(94.1, 273.7)	7.50	0.000
MSW 5 - MOE 25	-218.4	22.9	(-302.4, -134.5)	-9.53	0.000
NEGATIVE CON - MOE 25	-1485.0	24.5	(-1574.8, -1395.2)	-60.59	0.000
POSITIVE CON - MOE 25	-33.7	24.5	(-123.4, 56.1)	-1.37	0.978
MOW 1 - MOE 5	-456.7	24.5	(-546.5, -366.9)	-18.63	0.000
MOW 25 - MOE 5	403.5	24.5	(313.7, 493.3)	16.46	0.000
MOW 5 - MOE 5	5.8	27.4	(-94.6, 106.2)	0.21	1.000
MSE 1 - MOE 5	-377.4	24.5	(-467.2, -287.6)	-15.40	0.000
MSE 25 - MOE 5	384.3	24.5	(294.5, 474.0)	15.68	0.000
MSE 5 - MOE 5	-31.4	24.5	(-121.2, 58.3)	-1.28	0.987
MSW 1 - MOE 5	-186.4	24.5	(-276.1, -96.6)	-7.60	0.000
MSW 25 - MOE 5	544.1	24.5	(454.3, 633.9)	22.20	0.000
MSW 5 - MOE 5	141.8	22.9	(57.8, 225.8)	6.18	0.000
NEGATIVE CON - MOE 5	-1124.8	24.5	(-1214.5, -1035.0)	-45.89	0.000
POSITIVE CON - MOE 5	326.6	24.5	(236.8, 416.3)	13.32	0.000
MOW 25 - MOW 1	860.2	24.5	(770.4, 949.9)	35.09	0.000
MOW 5 - MOW 1	462.5	27.4	(362.1, 562.8)	16.88	0.000
MSE 1 - MOW 1	79.3	24.5	(-10.5, 169.0)	3.23	0.125
MSE 25 - MOW 1	840.9	24.5	(751.2, 930.7)	34.31	0.000
MSE 5 - MOW 1	425.2	24.5	(335.5, 515.0)	17.35	0.000
MSW 1 - MOW 1	270.3	24.5	(180.6, 360.1)	11.03	0.000
MSW 25 - MOW 1	1000.8	24.5	(911.0, 1090.6)	40.83	0.000
MSW 5 - MOW 1	598.5	22.9	(514.5, 682.4)	26.10	0.000
NEGATIVE CON - MOW 1	-668.1	24.5	(-757.8, -578.3)	-27.26	0.000

POSITIVE CON - MOW 1	783.2	24.5	(693.5, 873.0)	31.96	0.000
MOW 5 - MOW 25	-397.7	27.4	(-498.1, -297.3)	-14.51	0.000
MSE 1 - MOW 25	-780.9	24.5	(-870.7, -691.1)	-31.86	0.000
MSE 25 - MOW 25	-19.2	24.5	(-109.0, 70.5)	-0.78	1.000
MSE 5 - MOW 25	-434.9	24.5	(-524.7, -345.1)	-17.74	0.000
MSW 1 - MOW 25	-589.8	24.5	(-679.6, -500.1)	-24.07	0.000
MSW 25 - MOW 25	140.6	24.5	(50.9, 230.4)	5.74	0.000
MSW 5 - MOW 25	-261.7	22.9	(-345.7, -177.7)	-11.41	0.000
NEGATIVE CON - MOW 25	-1528.2	24.5	(-1618.0, -1438.5)	-62.35	0.000
POSITIVE CON - MOW 25	-76.9	24.5	(-166.7, 12.9)	-3.14	0.151
MSE 1 - MOW 5	-383.2	27.4	(-483.6, -282.8)	-13.98	0.000
MSE 25 - MOW 5	378.5	27.4	(278.1, 478.8)	13.81	0.000
MSE 5 - MOW 5	-37.2	27.4	(-137.6, 63.1)	-1.36	0.980
MSW 1 - MOW 5	-192.1	27.4	(-292.5, -91.8)	-7.01	0.000
MSW 25 - MOW 5	538.3	27.4	(438.0, 638.7)	19.65	0.000
MSW 5 - MOW 5	136.0	26.0	(40.8, 231.2)	5.23	0.001
NEGATIVE CON - MOW 5	-1130.5	27.4	(-1230.9, -1030.2)	-41.26	0.000
POSITIVE CON - MOW 5	320.8	27.4	(220.4, 421.1)	11.71	0.000
MSE 25 - MSE 1	761.7	24.5	(671.9, 851.4)	31.08	0.000
MSE 5 - MSE 1	346.0	24.5	(256.2, 435.7)	14.12	0.000
MSW 1 - MSE 1	191.1	24.5	(101.3, 280.8)	7.80	0.000
MSW 25 - MSE 1	921.5	24.5	(831.8, 1011.3)	37.60	0.000
MSW 5 - MSE 1	519.2	22.9	(435.2, 603.2)	22.65	0.000
NEGATIVE CON - MSE 1	-747.3	24.5	(-837.1, -657.6)	-30.49	0.000
POSITIVE CON - MSE 1	704.0	24.5	(614.2, 793.8)	28.72	0.000
MSE 5 - MSE 25	-415.7	24.5	(-505.5, -325.9)	-16.96	0.000
MSW 1 - MSE 25	-570.6	24.5	(-660.4, -480.8)	-23.28	0.000
MSW 25 - MSE 25	159.9	24.5	(70.1, 249.6)	6.52	0.000
MSW 5 - MSE 25	-242.5	22.9	(-326.4, -158.5)	-10.58	0.000
NEGATIVE CON - MSE 25	-1509.0	24.5	(-1598.8, -1419.2)	-61.57	0.000
POSITIVE CON - MSE 25	-57.7	24.5	(-147.5, 32.1)	-2.35	0.533

MSW 1 - MSE 5	-154.9	24.5	(-244.7, -65.1)	-6.32	0.000
MSW 25 - MSE 5	575.6	24.5	(485.8, 665.3)	23.48	0.000
MSW 5 - MSE 5	173.2	22.9	(89.3, 257.2)	7.56	0.000
NEGATIVE CON - MSE 5	-1093.3	24.5	(-1183.1, -1003.5)	-44.61	0.000
POSITIVE CON - MSE 5	358.0	24.5	(268.2, 447.8)	14.61	0.000
MSW 25 - MSW 1	730.5	24.5	(640.7, 820.2)	29.80	0.000
MSW 5 - MSW 1	328.1	22.9	(244.2, 412.1)	14.31	0.000
NEGATIVE CON - MSW 1	-938.4	24.5	(-1028.2, -848.6)	-38.29	0.000
POSITIVE CON - MSW 1	512.9	24.5	(423.1, 602.7)	20.93	0.000
MSW 5 - MSW 25	-402.3	22.9	(-486.3, -318.4)	-17.55	0.000
NEGATIVE CON - MSW 25	-1668.9	24.5	(-1758.6, -1579.1)	-68.09	0.000
POSITIVE CON - MSW 25	-217.6	24.5	(-307.3, -127.8)	-8.88	0.000
NEGATIVE CON - MSW 5	-1266.5	22.9	(-1350.5, -1182.6)	-55.24	0.000
POSITIVE CON - MSW 5	184.8	22.9	(100.8, 268.8)	8.06	0.000
POSITIVE CON - NEGATIVE CON	1451.3	24.5	(1361.5, 1541.1)	59.21	0.000

Individual confidence level = 99.90%

EXTRACTS

Descriptive Statistics: FCR

Statistics

Variable	Treatment	Mean	SE Mean
FCR	MOE 1	1.4758	0.000248
	MOE 25	1.2854	0.000507
	MOE 5	1.3442	0.000055
	MOW 1	1.5106	0.000678
	MOW 25	1.2951	0.000443
	MOW 5	1.3631	0.000992
	MSE 1	1.4332	0.000821
	MSE 25	1.2899	0.00105
	MSE 5	1.3570	0.000350
	MSW 1	1.4635	0.000741
	MSW 25	1.2642	0.000189
	MSW 5	1.3496	0.00437
	NEGATIVE CONTROL	4.3912	0.0213
	POSITIVE CONTROL	1.2757	0.000691

EXTRACTS

One-way ANOVA: FCR versus Treatment

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	14	MOE 1, MOE 25, MOE 5, MOW 1, MOW 25, MOW 5, MSE 1, MSE 25, MSE 5, MSW 1, MSW 25, MSW 5, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	13	25.8174	1.98596	18636.13	0.000
Error	28	0.0030	0.00011		
Total	41	25.8204			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0103230	99.99%	99.98%	99.97%

Means

Treatment	N	Mean	StDev	95% CI
MOE 1	3	1.47580	0.00043	(1.46359, 1.48800)
MOE 25	3	1.28536	0.00088	(1.27315, 1.29756)
MOE 5	3	1.34424	0.00009	(1.33203, 1.35644)
MOW 1	3	1.51058	0.00117	(1.49837, 1.52279)
MOW 25	3	1.29508	0.00077	(1.28287, 1.30729)
MOW 5	2	1.36306	0.00140	(1.34810, 1.37801)
MSE 1	3	1.43319	0.00142	(1.42098, 1.44540)
MSE 25	3	1.28989	0.00181	(1.27768, 1.30210)
MSE 5	3	1.35695	0.00061	(1.34474, 1.36916)
MSW 1	3	1.46352	0.00128	(1.45132, 1.47573)
MSW 25	3	1.26423	0.00033	(1.25202, 1.27644)
MSW 5	4	1.34960	0.00875	(1.33903, 1.36017)
NEGATIVE CONTROL	3	4.3912	0.0369	(4.3790, 4.4035)
POSITIVE CONTROL	3	1.27568	0.00120	(1.26347, 1.28789)

Pooled StDev = 0.0103230

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
NEGATIVE CONTROL	3	4.3912	A
MOW 1	3	1.51058	B
MOE 1	3	1.47580	C
MSW 1	3	1.46352	C D
MSE 1	3	1.43319	D
MOW 5	2	1.36306	E
MSE 5	3	1.35695	E
MSW 5	4	1.34960	E
MOE 5	3	1.34424	E
MOW 25	3	1.29508	F
MSE 25	3	1.28989	F
MOE 25	3	1.28536	F

POSITIVE CONTROL	3	1.27568	F
MSW 25	3	1.26423	F

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOE 25 - MOE 1	-0.19044	0.00843	(-0.22131, -0.15957)	-22.59	0.000
MOE 5 - MOE 1	-0.13156	0.00843	(-0.16243, -0.10069)	-15.61	0.000
MOW 1 - MOE 1	0.03478	0.00843	(0.00391, 0.06566)	4.13	0.017
MOW 25 - MOE 1	-0.18072	0.00843	(-0.21159, -0.14985)	-21.44	0.000
MOW 5 - MOE 1	-0.11274	0.00942	(-0.14726, -0.07822)	-11.96	0.000
MSE 1 - MOE 1	-0.04260	0.00843	(-0.07347, -0.01173)	-5.05	0.002
MSE 25 - MOE 1	-0.18590	0.00843	(-0.21678, -0.15503)	-22.06	0.000
MSE 5 - MOE 1	-0.11884	0.00843	(-0.14972, -0.08797)	-14.10	0.000
MSW 1 - MOE 1	-0.01227	0.00843	(-0.04314, 0.01860)	-1.46	0.965
MSW 25 - MOE 1	-0.21157	0.00843	(-0.24244, -0.18070)	-25.10	0.000
MSW 5 - MOE 1	-0.12620	0.00788	(-0.15508, -0.09732)	-16.01	0.000
NEGATIVE CON - MOE 1	2.91545	0.00843	(2.88458, 2.94632)	345.89	0.000
POSITIVE CON - MOE 1	-0.20012	0.00843	(-0.23099, -0.16924)	-23.74	0.000
MOE 5 - MOE 25	0.05888	0.00843	(0.02801, 0.08975)	6.99	0.000
MOW 1 - MOE 25	0.22522	0.00843	(0.19435, 0.25610)	26.72	0.000
MOW 25 - MOE 25	0.00972	0.00843	(-0.02115, 0.04059)	1.15	0.995
MOW 5 - MOE 25	0.07770	0.00942	(0.04318, 0.11222)	8.25	0.000
MSE 1 - MOE 25	0.14784	0.00843	(0.11696, 0.17871)	17.54	0.000

MSE 25 - MOE 25	0.00453	0.00843	(-0.02634, 0.03541)	0.54	1.000
MSE 5 - MOE 25	0.07160	0.00843	(0.04072, 0.10247)	8.49	0.000
MSW 1 - MOE 25	0.17817	0.00843	(0.14730, 0.20904)	21.14	0.000
MSW 25 - MOE 25	-0.02113	0.00843	(-0.05200, 0.00974)	-2.51	0.438
MSW 5 - MOE 25	0.06424	0.00788	(0.03536, 0.09312)	8.15	0.000
NEGATIVE CON - MOE 25	3.10589	0.00843	(3.07501, 3.13676)	368.49	0.000
POSITIVE CON - MOE 25	-0.00968	0.00843	(-0.04055, 0.02120)	-1.15	0.995
MOW 1 - MOE 5	0.16634	0.00843	(0.13547, 0.19722)	19.74	0.000
MOW 25 - MOE 5	-0.04916	0.00843	(-0.08003, -0.01829)	-5.83	0.000
MOW 5 - MOE 5	0.01882	0.00942	(-0.01570, 0.05334)	2.00	0.757
MSE 1 - MOE 5	0.08896	0.00843	(0.05808, 0.11983)	10.55	0.000
MSE 25 - MOE 5	-0.05434	0.00843	(-0.08522, -0.02347)	-6.45	0.000
MSE 5 - MOE 5	0.01272	0.00843	(-0.01816, 0.04359)	1.51	0.955
MSW 1 - MOE 5	0.11929	0.00843	(0.08842, 0.15016)	14.15	0.000
MSW 25 - MOE 5	-0.08001	0.00843	(-0.11088, -0.04914)	-9.49	0.000
MSW 5 - MOE 5	0.00536	0.00788	(-0.02352, 0.03424)	0.68	1.000
NEGATIVE CON - MOE 5	3.04701	0.00843	(3.01614, 3.07788)	361.50	0.000
POSITIVE CON - MOE 5	-0.06856	0.00843	(-0.09943, -0.03768)	-8.13	0.000
MOW 25 - MOW 1	-0.21550	0.00843	(-0.24637, -0.18463)	-25.57	0.000
MOW 5 - MOW 1	-0.14752	0.00942	(-0.18204, -0.11301)	-15.65	0.000
MSE 1 - MOW 1	-0.07739	0.00843	(-0.10826, -0.04651)	-9.18	0.000
MSE 25 - MOW 1	-0.22069	0.00843	(-0.25156, -0.18981)	-26.18	0.000

MSE 5 - MOW 1	-0.15363	0.00843	(-0.18450, -0.12275)	-18.23	0.000
MSW 1 - MOW 1	-0.04705	0.00843	(-0.07793, -0.01618)	-5.58	0.000
MSW 25 - MOW 1	-0.24635	0.00843	(-0.27722, -0.21548)	-29.23	0.000
MSW 5 - MOW 1	-0.16098	0.00788	(-0.18986, -0.13210)	-20.42	0.000
NEGATIVE CON - MOW 1	2.88066	0.00843	(2.84979, 2.91154)	341.77	0.000
POSITIVE CON - MOW 1	-0.23490	0.00843	(-0.26577, -0.20403)	-27.87	0.000
MOW 5 - MOW 25	0.06798	0.00942	(0.03346, 0.10250)	7.21	0.000
MSE 1 - MOW 25	0.13812	0.00843	(0.10724, 0.16899)	16.39	0.000
MSE 25 - MOW 25	-0.00519	0.00843	(-0.03606, 0.02569)	-0.62	1.000
MSE 5 - MOW 25	0.06188	0.00843	(0.03100, 0.09275)	7.34	0.000
MSW 1 - MOW 25	0.16845	0.00843	(0.13757, 0.19932)	19.98	0.000
MSW 25 - MOW 25	-0.03085	0.00843	(-0.06172, 0.00002)	-3.66	0.050
MSW 5 - MOW 25	0.05452	0.00788	(0.02564, 0.08340)	6.92	0.000
NEGATIVE CON - MOW 25	3.09617	0.00843	(3.06529, 3.12704)	367.34	0.000
POSITIVE CON - MOW 25	-0.01940	0.00843	(-0.05027, 0.01147)	-2.30	0.567
MSE 1 - MOW 5	0.07014	0.00942	(0.03562, 0.10465)	7.44	0.000
MSE 25 - MOW 5	-0.07316	0.00942	(-0.10768, -0.03865)	-7.76	0.000
MSE 5 - MOW 5	-0.00610	0.00942	(-0.04062, 0.02841)	-0.65	1.000
MSW 1 - MOW 5	0.10047	0.00942	(0.06595, 0.13499)	10.66	0.000
MSW 25 - MOW 5	-0.09883	0.00942	(-0.13335, -0.06431)	-10.49	0.000
MSW 5 - MOW 5	-0.01346	0.00894	(-0.04620, 0.01929)	-1.51	0.955
NEGATIVE CON - MOW	3.02819	0.00942	(2.99367, 3.06270)	321.34	0.000

5					
POSITIVE CON - MOW 5	-0.08738	0.00942	(-0.12189, -0.05286)	-9.27	0.000
MSE 25 - MSE 1	-0.14330	0.00843	(-0.17417, -0.11243)	-17.00	0.000
MSE 5 - MSE 1	-0.07624	0.00843	(-0.10711, -0.04537)	-9.05	0.000
MSW 1 - MSE 1	0.03033	0.00843	(-0.00054, 0.06120)	3.60	0.057
MSW 25 - MSE 1	-0.16897	0.00843	(-0.19984, -0.13809)	-20.05	0.000
MSW 5 - MSE 1	-0.08360	0.00788	(-0.11247, -0.05472)	-10.60	0.000
NEGATIVE CON - MSE 1	2.95805	0.00843	(2.92718, 2.98892)	350.95	0.000
POSITIVE CON - MSE 1	-0.15751	0.00843	(-0.18839, -0.12664)	-18.69	0.000
MSE 5 - MSE 25	0.06706	0.00843	(0.03619, 0.09793)	7.96	0.000
MSW 1 - MSE 25	0.17363	0.00843	(0.14276, 0.20451)	20.60	0.000
MSW 25 - MSE 25	-0.02566	0.00843	(-0.05654, 0.00521)	-3.04	0.181
MSW 5 - MSE 25	0.05971	0.00788	(0.03083, 0.08859)	7.57	0.000
NEGATIVE CON - MSE 25	3.10135	0.00843	(3.07048, 3.13223)	367.95	0.000
POSITIVE CON - MSE 25	-0.01421	0.00843	(-0.04509, 0.01666)	-1.69	0.904
MSW 1 - MSE 5	0.10657	0.00843	(0.07570, 0.13744)	12.64	0.000
MSW 25 - MSE 5	-0.09273	0.00843	(-0.12360, -0.06185)	-11.00	0.000
MSW 5 - MSE 5	-0.00735	0.00788	(-0.03623, 0.02152)	-0.93	0.999
NEGATIVE CON - MSE 5	3.03429	0.00843	(3.00342, 3.06516)	359.99	0.000
POSITIVE CON - MSE 5	-0.08127	0.00843	(-0.11215, -0.05040)	-9.64	0.000
MSW 25 - MSW 1	-0.19930	0.00843	(-0.23017, -0.16842)	-23.65	0.000

MSW 5 - MSW 1	-0.11393	0.00788	(-0.14281, -0.08505)	-14.45	0.000
NEGATIVE CON - MSW 1	2.92772	0.00843	(2.89685, 2.95859)	347.35	0.000
POSITIVE CON - MSW 1	-0.18785	0.00843	(-0.21872, -0.15697)	-22.29	0.000
MSW 5 - MSW 25	0.08537	0.00788	(0.05649, 0.11425)	10.83	0.000
NEGATIVE CON - MSW 25	3.12702	0.00843	(3.09614, 3.15789)	371.00	0.000
POSITIVE CON - MSW 25	0.01145	0.00843	(-0.01942, 0.04233)	1.36	0.980
NEGATIVE CON - MSW 5	3.04165	0.00788	(3.01277, 3.07052)	385.78	0.000
POSITIVE CON - MSW 5	-0.07392	0.00788	(-0.10280, -0.04504)	-9.38	0.000
POSITIVE CON - NEGATIVE CON	-3.11556	0.00843	(-3.14644, -3.08469)	-369.64	0.000

Individual confidence level = 99.90%

Leaf powders of *M. oleifera* and *M. stenopetala*

POWDERS

Descriptive Statistics: Feed Intake

Statistics

Variable	Treatment	Mean	SE Mean
Feed Intake	MOP 250	2655.3	9.44
	MOP 450	3055.7	10.3
	MOP 650	3450.1	9.27
	MSP 250	2813.7	6.74
	MSP 450	3147.5	16.2
	MSP 650	3542.6	15.6
	NEGATIVE CONTROL	3814.3	3.56
	POSITIVE CONTROL	3133.8	6.14

POWDERS

One-way ANOVA: Feed Intake versus Treatment

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	8	MOP 250, MOP 450, MOP 650, MSP 250, MSP 450, MSP 650, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	7	3093441	441920	1339.35	0.000
Error	16	5279	330		
Total	23	3098721			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
18.1645	99.83%	99.76%	99.62%

Means

Treatment	N	Mean	StDev	95% CI
MOP 250	3	2655.30	16.36	(2633.06, 2677.53)
MOP 450	3	3055.7	17.8	(3033.4, 3077.9)
MOP 650	3	3450.10	16.06	(3427.87, 3472.34)
MSP 250	3	2813.68	11.67	(2791.45, 2835.92)
MSP 450	3	3147.5	28.0	(3125.2, 3169.7)
MSP 650	3	3542.6	27.0	(3520.4, 3564.8)
NEGATIVE CONTROL	3	3814.28	6.16	(3792.05, 3836.51)
POSITIVE CONTROL	3	3133.84	10.64	(3111.61, 3156.07)

Pooled StDev = 18.1645

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
NEGATIVE CONTROL	3	3814.28	A
MSP 650	3	3542.6	B
MOP 650	3	3450.10	C
MSP 450	3	3147.5	D
POSITIVE CONTROL	3	3133.84	D
MOP 450	3	3055.7	E
MSP 250	3	2813.68	F
MOP 250	3	2655.30	G

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOP 450 - MOP 250	400.4	14.8	(349.0, 451.7)	26.99	0.000
MOP 650 - MOP 250	794.8	14.8	(743.4, 846.2)	53.59	0.000
MSP 250 - MOP 250	158.4	14.8	(107.0, 209.8)	10.68	0.000
MSP 450 - MOP 250	492.2	14.8	(440.8, 543.5)	33.18	0.000
MSP 650 - MOP 250	887.3	14.8	(835.9, 938.7)	59.83	0.000
NEGATIVE CON - MOP 250	1159.0	14.8	(1107.6, 1210.4)	78.14	0.000
POSITIVE CON - MOP 250	478.5	14.8	(427.2, 529.9)	32.27	0.000
MOP 650 - MOP 450	394.4	14.8	(343.1, 445.8)	26.60	0.000
MSP 250 - MOP 450	-242.0	14.8	(-293.4, -190.6)	-16.32	0.000
MSP 450 - MOP 450	91.8	14.8	(40.4, 143.2)	6.19	0.000
MSP 650 - MOP 450	486.9	14.8	(435.5, 538.3)	32.83	0.000
NEGATIVE CON - MOP 450	758.6	14.8	(707.2, 810.0)	51.15	0.000
POSITIVE CON - MOP 450	78.2	14.8	(26.8, 129.6)	5.27	0.002
MSP 250 - MOP 650	-636.4	14.8	(-687.8, -585.0)	-42.91	0.000
MSP 450 - MOP 650	-302.6	14.8	(-354.0, -251.3)	-20.41	0.000
MSP 650 - MOP 650	92.5	14.8	(41.1, 143.9)	6.24	0.000
NEGATIVE CON - MOP 650	364.2	14.8	(312.8, 415.6)	24.55	0.000
POSITIVE CON - MOP 650	-316.3	14.8	(-367.6, -264.9)	-21.32	0.000
MSP 450 -	333.8	14.8	(282.4,	22.50	0.000

MSP 250			385.2)		
MSP 650 - MSP 250	728.9	14.8	(677.5, 780.3)	49.15	0.000
NEGATIVE CON - MSP 250	1000.6	14.8	(949.2, 1052.0)	67.47	0.000
POSITIVE CON - MSP 250	320.2	14.8	(268.8, 371.5)	21.59	0.000
MSP 650 - MSP 450	395.1	14.8	(343.7, 446.5)	26.64	0.000
NEGATIVE CON - MSP 450	666.8	14.8	(615.4, 718.2)	44.96	0.000
POSITIVE CON - MSP 450	-13.6	14.8	(-65.0, 37.8)	-0.92	0.980
NEGATIVE CON - MSP 650	271.7	14.8	(220.3, 323.1)	18.32	0.000
POSITIVE CON - MSP 650	-408.7	14.8	(-460.1, -357.4)	-27.56	0.000
POSITIVE CON - NEGATIVE CON	-680.4	14.8	(-731.8, -629.0)	-45.88	0.000

Individual confidence level = 99.68%

POWDERS

Descriptive Statistics: Body weight

Statistics

Variable	Treatment	Mean	SE Mean
Body weight	MOP 250	1728.3	9.44
	MOP 450	2175.2	10.3
	MOP 650	2672.2	36.3
	MSP 250	1887.7	6.74
	MSP 450	2256.1	8.39
	MSP 650	2731.4	15.6
	NEGATIVE CONTROL	1005.3	4.53
	POSITIVE CONTROL	2456.6	6.14

POWDERS

One-way ANOVA: Body weight versus Treatment

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	8	MOP 250, MOP 450, MOP 650, MSP 250, MSP 450, MSP 650, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	7	6789873	969982	1340.61	0.000
Error	16	11577	724		
Total	23	6801449			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
26.8986	99.83%	99.76%	99.62%

Means

Treatment	N	Mean	StDev	95% CI
MOP 250	3	1728.33	16.36	(1695.41, 1761.26)
MOP 450	3	2175.2	17.8	(2142.3, 2208.1)
MOP 650	3	2672.2	62.9	(2639.3, 2705.2)
MSP 250	3	1887.72	11.67	(1854.80, 1920.64)
MSP 450	3	2256.12	14.53	(2223.20, 2289.04)
MSP 650	3	2731.4	27.0	(2698.5, 2764.3)
NEGATIVE CONTROL	3	1005.29	7.85	(972.37, 1038.21)
POSITIVE CONTROL	3	2456.61	10.64	(2423.69, 2489.54)

Pooled StDev = 26.8986

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping						
MSP 650	3	2731.4	A						
MOP 650	3	2672.2	A						
POSITIVE CONTROL	3	2456.61		B					
MSP 450	3	2256.12			C				
MOP 450	3	2175.2				D			
MSP 250	3	1887.72					E		
MOP 250	3	1728.33						F	
NEGATIVE CONTROL	3	1005.29							G

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOP 450 - MOP 250	446.9	22.0	(370.8, 523.0)	20.35	0.000
MOP 650 - MOP 250	943.9	22.0	(867.8, 1020.0)	42.98	0.000
MSP 250 - MOP 250	159.4	22.0	(83.3, 235.5)	7.26	0.000
MSP 450 - MOP 250	527.8	22.0	(451.7, 603.9)	24.03	0.000
MSP 650 - MOP 250	1003.0	22.0	(926.9, 1079.1)	45.67	0.000
NEGATIVE CON - MOP 250	-723.0	22.0	(-799.1, -646.9)	-32.92	0.000
POSITIVE CON - MOP 250	728.3	22.0	(652.2, 804.4)	33.16	0.000
MOP 650 - MOP 450	497.0	22.0	(420.9, 573.1)	22.63	0.000
MSP 250 - MOP 450	-287.5	22.0	(-363.6, -211.4)	-13.09	0.000
MSP 450 - MOP 450	80.9	22.0	(4.8, 157.0)	3.68	0.033
MSP 650 - MOP 450	556.2	22.0	(480.1, 632.3)	25.32	0.000
NEGATIVE CON - MOP 450	-1169.9	22.0	(-1246.0, -1093.8)	-53.27	0.000
POSITIVE CON - MOP	281.4	22.0	(205.3, 357.5)	12.81	0.000

450					
MSP 250 - MOP 650	-784.5	22.0	(-860.6, -708.4)	-35.72	0.000
MSP 450 - MOP 650	-416.1	22.0	(-492.2, -340.0)	-18.95	0.000
MSP 650 - MOP 650	59.1	22.0	(-17.0, 135.2)	2.69	0.194
NEGATIVE CON - MOP 650	-1666.9	22.0	(-1743.0, -1590.8)	-75.90	0.000
POSITIVE CON - MOP 650	-215.6	22.0	(-291.7, -139.5)	-9.82	0.000
MSP 450 - MSP 250	368.4	22.0	(292.3, 444.5)	16.77	0.000
MSP 650 - MSP 250	843.7	22.0	(767.6, 919.8)	38.41	0.000
NEGATIVE CON - MSP 250	-882.4	22.0	(-958.5, -806.3)	-40.18	0.000
POSITIVE CON - MSP 250	568.9	22.0	(492.8, 645.0)	25.90	0.000
MSP 650 - MSP 450	475.3	22.0	(399.2, 551.4)	21.64	0.000
NEGATIVE CON - MSP 450	-1250.8	22.0	(-1326.9, -1174.7)	-56.95	0.000
POSITIVE CON - MSP 450	200.5	22.0	(124.4, 276.6)	9.13	0.000
NEGATIVE CON - MSP 650	-1726.1	22.0	(-1802.2, -1650.0)	-78.59	0.000
POSITIVE CON - MSP 650	-274.8	22.0	(-350.9, -198.7)	-12.51	0.000
POSITIVE CON - NEGATIVE CON	1451.3	22.0	(1375.2, 1527.4)	66.08	0.000

Individual confidence level = 99.68%

POWDERS

Descriptive Statistics: FCR

Statistics

Variable	Treatment	Mean	SE Mean
FCR	MOP 250	1.5364	0.00294
	MOP 450	1.4048	0.00191
	MOP 650	1.2915	0.0161
	MSP 250	1.4905	0.00176
	MSP 450	1.3952	0.0119
	MSP 650	1.2970	0.00170
	NEGATIVE CONTROL	3.7944	0.0187
	POSITIVE CONTROL	1.2757	0.000691

POWDERS

One-way ANOVA: FCR versus Treatment

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Treatment	8	MOP 250, MOP 450, MOP 650, MSP 250, MSP 450, MSP 650, NEGATIVE CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	7	15.4344	2.20491	7648.57	0.000
Error	16	0.0046	0.00029		
Total	23	15.4390			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0169788	99.97%	99.96%	99.93%

Means

Treatment	N	Mean	StDev	95% CI
MOP 250	3	1.53637	0.00509	(1.51559, 1.55715)

MOP 450	3	1.40479	0.00331 (1.38401, 1.42557)
MOP 650	3	1.2915	0.0279 (1.2707, 1.3123)
MSP 250	3	1.49053	0.00304 (1.46975, 1.51131)
MSP 450	3	1.3952	0.0206 (1.3744, 1.4159)
MSP 650	3	1.29701	0.00295 (1.27623, 1.31779)
NEGATIVE CONTROL	3	3.7944	0.0323 (3.7736, 3.8152)
POSITIVE CONTROL	3	1.27568	0.00120 (1.25490, 1.29646)

Pooled StDev = 0.0169788

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping			
NEGATIVE CONTROL	3	3.7944	A			
MOP 250	3	1.53637		B		
MSP 250	3	1.49053		B		
MOP 450	3	1.40479			C	
MSP 450	3	1.3952			C	
MSP 650	3	1.29701				D
MOP 650	3	1.2915				D
POSITIVE CONTROL	3	1.27568				D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
MOP 450 - MOP 250	-0.1316	0.0139	(-0.1796, -0.0835)	-9.49	0.000
MOP 650 - MOP 250	-0.2448	0.0139	(-0.2929, -0.1968)	-17.66	0.000
MSP 250 - MOP 250	-0.0458	0.0139	(-0.0939, 0.0022)	-3.31	0.067
MSP 450 - MOP 250	-0.1412	0.0139	(-0.1892, -0.0932)	-10.19	0.000
MSP 650 - MOP 250	-0.2394	0.0139	(-0.2874, -0.1913)	-17.27	0.000
NEGATIVE CON - MOP 250	2.2580	0.0139	(2.2100, 2.3060)	162.88	0.000
POSITIVE CON - MOP 250	-0.2607	0.0139	(-0.3087, -0.2127)	-18.80	0.000
MOP 650 - MOP 450	-0.1133	0.0139	(-0.1613, -0.0652)	-8.17	0.000
MSP 250 - MOP 450	0.0857	0.0139	(0.0377, 0.1338)	6.18	0.000

MSP 450 - MOP 450	-0.0096	0.0139	(-0.0577, 0.0384)	-0.69	0.996
MSP 650 - MOP 450	-0.1078	0.0139	(-0.1558, -0.0597)	-7.77	0.000
NEGATIVE CON - MOP 450	2.3896	0.0139	(2.3416, 2.4376)	172.37	0.000
POSITIVE CON - MOP 450	-0.1291	0.0139	(-0.1771, -0.0811)	-9.31	0.000
MSP 250 - MOP 650	0.1990	0.0139	(0.1510, 0.2470)	14.36	0.000
MSP 450 - MOP 650	0.1036	0.0139	(0.0556, 0.1517)	7.48	0.000
MSP 650 - MOP 650	0.0055	0.0139	(-0.0425, 0.0535)	0.40	1.000
NEGATIVE CON - MOP 650	2.5029	0.0139	(2.4548, 2.5509)	180.54	0.000
POSITIVE CON - MOP 650	-0.0158	0.0139	(-0.0639, 0.0322)	-1.14	0.937
MSP 450 - MSP 250	-0.0954	0.0139	(-0.1434, -0.0473)	-6.88	0.000
MSP 650 - MSP 250	-0.1935	0.0139	(-0.2416, -0.1455)	-13.96	0.000
NEGATIVE CON - MSP 250	2.3038	0.0139	(2.2558, 2.3519)	166.19	0.000
POSITIVE CON - MSP 250	-0.2149	0.0139	(-0.2629, -0.1668)	-15.50	0.000
MSP 650 - MSP 450	-0.0981	0.0139	(-0.1462, -0.0501)	-7.08	0.000
NEGATIVE CON - MSP 450	2.3992	0.0139	(2.3512, 2.4473)	173.07	0.000
POSITIVE CON - MSP 450	-0.1195	0.0139	(-0.1675, -0.0714)	-8.62	0.000
NEGATIVE CON - MSP 650	2.4974	0.0139	(2.4493, 2.5454)	180.14	0.000
POSITIVE CON - MSP 650	-0.0213	0.0139	(-0.0694, 0.0267)	-1.54	0.777
POSITIVE CON - NEGATIVE CON	-2.5187	0.0139	(-2.5667, -2.4707)	-181.68	0.000

Individual confidence level = 99.68%

Appendix 10: Gut microbiota analysis results

CUSTOMER NAME AND ADDRESS			Ebenezer			
DATE OF SAMPLING			23/02/2019			
DATE OF RECEIPT			23/02/2019			
DATE OF ANALYSIS			23/02/2019			
		Total viable counts	*E. coli*	*Coliform*	*Salmonella*	*Clostridium spp*
Unit of measurement		Cfu/ gm	Cfu/ gm	Cfu/ gm	Present or Absent per/g	Present/Absent per/g
1	MOE 5mg A	1.87×10^{7}	1.7×10^{6}	2.3×10^{6}	Absent	Absent
2	MOE 5mg B	1.40×10^{7}	1.4×10^{6}	3.5×10^{6}	Absent	Absent
3	MOE 25 mg A	1.64×10^{7}	7.2×10^{6}	8.2×10^{6}	Absent	Absent
4	MOE 25mg B	1.94×10^{7}	5.9×10^{6}	9.1×10^{6}	Absent	Absent
5	MSE 1mg A	4.40×10^{6}	2.6×10^{6}	2.3×10^{6}	Absent	Absent
6	MSE 1mg B	4.40×10^{6}	4.2×10^{5}	7.0×10^{5}	Absent	Absent
7	MSE 5mg A	1.64×10^{7}	6.2×10^{6}	6.3×10^{6}	Absent	Absent
8	MSE 5mg B	1.82×10^{7}	5.9×10^{6}	8.6×10^{6}	Absent	Present
9	MSE 25mg A	1.62×10^{7}	5.3×10^{6}	7.2×10^{6}	Absent	Absent
10	MSE 25mg B	1.80×10^{7}	5.7×10^{6}	7.2×10^{6}	Absent	Absent
11	MSW 1mg A	1.78×10^{7}	1.9×10^{6}	2.2×10^{6}	Absent	Absent
12	MSW 1mg B	1.78×10^{7}	6.8×10^{6}	7.2×10^{5}	Absent	Absent
13	MSW 5mg A	1.84×10^{7}	3.5×10^{6}	4.3×10^{6}	Absent	Absent
14	MSW 5mg B	1.23×10^{7}	2.2×10^{6}	3.3×10^{6}	Absent	Absent
15	MSW 25mg A	1.98×10^{7}	4.0×10^{6}	6.3×10^{6}	Absent	Absent
16	MSW 25mg B	1.99×10^{7}	1.1×10^{6}	1.7×10^{6}	Absent	Absent
17	MSP 250mg A	2.4×10^{6}	2.1×10^{6}	2.0×10^{6}	Absent	Absent
18	MSP 250mg B	4.2×10^{6}	1.8×10^{6}	2.4×10^{5}	Absent	Absent
19	MSP 450mg A	1.94×10^{7}	6.2×10^{6}	9.4×10^{6}	Absent	Absent
20	MSP 450mg B	1.82×10^{7}	2.2×10^{6}	2.6×10^{6}	Absent	Present
21	MOW 1mg A	1.85×10^{7}	3.4×10^{6}	4.6×10^{6}	Absent	Absent
22	MOW 1mg B	1.83×10^{7}	1.7×10^{6}	2.0×10^{6}	Absent	Absent
23	MOW 5mg A	1.81×10^{7}	4.0×10^{6}	7.2×10^{6}	Absent	Absent
24	MOW 5mg B	8.0×10^{6}	1.4×10^{6}	1.8×10^{5}	Absent	Absent

Appendix 11: Certificates of analysis of *M. oleifera* and *M. stenopatala* feeds

UNGA CENTRAL LABORATORY
P.O BOX 30386 00100
NAIROBI

Lot no: 890000048578

Date: 12.02.19

CERTIFICATE OF ANALYSIS

Sample code/Serial number: MS/'037/19

Source: Nutrition

Customer Address/Contacts: pgichuru@unga.com

Samples description: FINISHERS OLEIFERA 2.5

Sample received on: 30.01.19

Samples analysed on: 31.01.19

PARAMETER.	RESULTS	TEST METHOD.
Protein*(As is)%	24.15	ISO5983-2:2005
Oil*(%)	3.36	ISO 6492:1999
Fibre*(%)	4.2	ISO 5498-1981(E)
Ash* (%)	6.0	ISO 5984:2002(E)
Calcium*(%)	1.15	ISO 5983-1:2005
Phosphorus (%)	0.57	AOAC 17th Edition
Nacl (%)	0.21	-
Acid Insoluble ash (%)	1.0	AOAC 17th Edition
Moisture*(%)	9.72	ISO 6496:1999
Aflatoxin(ppb)	5.0	ELISA

Tests with asterisk* are accredited

Unless otherwise stated the results shown above refer only to the samples tested

LABORATORY ANALYST (Sign) Date: 12.02.19

LABORATORY COORDINATOR (Sign) Date: 12.02.19

Appendix 12: Letter of approval to use MKU Research centre facilities

OUR REF: MKU00/R&I/004/VOL1/2019/011

MAY 13, 2019

Ebenezer Udofia Nathaniel
Admin. No: AGR-4-0422-1/2017
Kenya Methodist University

REF: APPROVAL FOR USE OF MOUNT KENYA UNIVERSITY RESEARCH CENTRE

Reference is made to your letter dated 15/01/2019 for a request to conduct assays in the Research Centre laboratories for Doctorate of Philosophy (PhD) degree project work.

This is to inform you that your request has been approved as from the month of **May to August 2019** subject to the adherence to the Research Centre Policy Guidelines

Yours faithfully,

Mount Kenya University
Head, Research Centre
P. O. Box 342 - 01000,
Thika

Dr. Jared Misonge Onyancha
HEAD RESEARCH CENTRE

Main Campus General Kago Road, P.O. Box 342-01000 Thika Tel: +254 67 2820 000,
Cell: +254 720 790 795, 0709 153 000
Email: info@mku.ac.ke, Web: www.mku.ac.ke
Chartered and ISO 9001 : 2008 Certified Institution.
Unlocking Infinite Possibilities

Appendix 13: Setup for the determination of crude lipid content.

At the Mount Kenya University biochemistry laboratory determining crude lipid content of *Moringa* leaves. Picture by Ebenezer Udofia

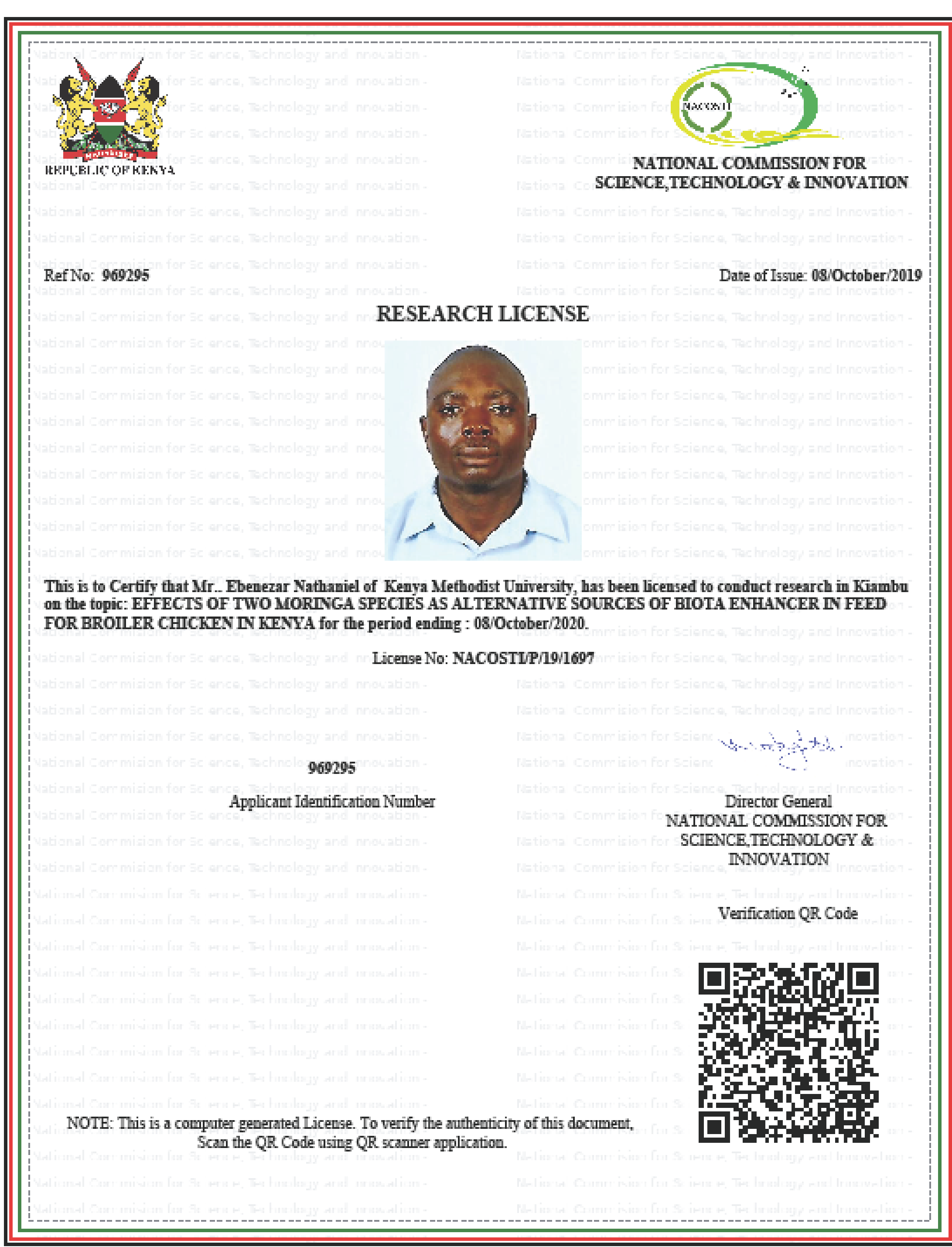
Appendix 14: Determination of Crude Protein the Studied Plants

At the Mount Kenya University biochemistry laboratory determining crude protein content of *Moringa* leaves. Picture by Ebenezer Udofia

Appendix 15: Map of *Moringa oleifera* Lam distributions in the world

Source (*Moringa*, Tree of life 2012).

Appendix 16: Research Permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 969295

Date of Issue: 08/October/2019

RESEARCH LICENSE

This is to Certify that Mr.. Ebenezar Nathaniel of Kenya Methodist University, has been licensed to conduct research in Kiambu on the topic: EFFECTS OF TWO MORINGA SPECIES AS ALTERNATIVE SOURCES OF BIOTA ENHANCER IN FEED FOR BROILER CHICKEN IN KENYA for the period ending : 08/October/2020.

License No: NACOSTI/P/19/1697

969295

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.