PERFORMANCE OF STARTER BROILER CHICKENS FED ON A BLEND OF PHYTOBIOTICS AS IN-FEED ANTIBIOTIC REPLACERS

JULIUS KARANJA MAINA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for an award of a diploma or conferment of a degree in any other university or institution.

Signature: ……………………… Date: 8/9/2022…

Maina Julius Karanja
NM12/19135/15

Recommendation

This thesis has been examined, passed and submitted with our approval as the University supervisors.

Signature: ……………………… Date: 9/09/2022…

Dr. Roseline Kahindi, PhD
Chuka University

Signature: ……………………… Date: 8/09/2022

Dr. James Gitonga Kirimi, PhD
Directorate of Fisheries Development,
Meru County Government

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DEDICATION

This work is dedicated to my late parents Mr. Wilson Maina Karanja and Esther Wanjiru Mwangi; and to my siblings Beth Waitherero Maina, Stephen Ngure Maina and John Kambo Maina for their prayers, support and encouragement.

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ABSTRACT

The performance of starter Cobb 500 broiler chickens fed on stinging nettle leaf meal (NLM) or whole coriander seed meal (CSM) as feed additives singly and as a blend was investigated. The National Research Council (NRC) (1994) guidelines were followed in formulating the experimental diets. Each of the experiment lasted for 17 days. In experiment one, seven diets were prepared as follows: Control (0%), NLM at 1%, 1.5% and 2% and CSM at 0.5%, 1% and 1.5% dietary inclusion levels. A total of 84 unsexed chicks were weighed and randomly allocated the experimental diets with 4 replicates of 3 chicks each. The aim of the experiment was to get the level with highest Growth rate (GR) for each of the phytobiotic. For experiment two, three diets were utilised; basal diet supplemented with a blend of NLM at 1.5% and CSM at 0.5% inclusion levels (Blend), basal diet supplemented with oxytetracycline hydrochloride powder at the rate of 0.05g/kg of feed (Cox; the positive control) and the Control diet (negative control). A total of 36 unsexed chicks were weighed and randomly allocated to the experimental diets with 4 replicates of 3 chicks each. For both experiments, feed intake (FI) and body weight (BW) were weighed and recorded daily and weekly respectively. Feed conversion efficiency (FCE) and GR were also calculated. For experiment two, ileal nutrient digestibility (IND) and the populations of dominant ileal bacteria (DIB) were determined. Both experiments were laid out in a completely randomized design (CRD) with data being analysed using statistical analysis system (SAS version 9.4) software. Tukey pairwise comparisons were conducted to compare variations among diets where analysis of variance (ANOVA) showed differences at probability values (α = 0.05). Results from experiment one showed that the birds supplemented with NLM at 1% and CSM at 0.5% showed significant mean FI of 162.03g and 193g respectively. Significantly higher GR was recorded for all the dietary treatments; both for NLM and CSM supplemented groups ($p<0.05$) with the highest GR being 113.56g for NLM at 1.5% and 119.31g for CSM at 0.5%. Birds supplemented with NLM at 2% showed the highest FCE of 7.98 amongst the NLM group and CSM at 1.5% (8.35) for the CSM supplemented group. From the study, supplementing the diets of starter broiler chickens with NLM at 1.5% and CSM at 0.5% resulted to the highest GR. Results from experiment two showed that FI, GR and FCE were significantly ($p<0.05$) affected by the dietary treatments. The group of birds supplemented with Blend showed higher FI (133.08g), GR (117.10g) and FCE (5.35) as compared to the other dietary treatments. The IND of dry matter (DM) (85.21%), crude protein (CP) (89.86%) and crude fibre (CF) (67.65%) was significantly higher in the groups of birds fed on Blend. For the birds fed on Cox and control diets, the IND of DM and CF was numerically different but non-significant at $p<0.05$. The CP digestibility was significant for all the diets offered to the experimental animals at $p<0.05$. Additionally, all diets had a significant effect on the proliferation of ileal bacteria studied at $p<0.05$. The growth of *Escherichia coli* (*E. coli*) was significantly increased by the Control diet (4.56 cfu/ml), with Cox showing the least effect (3.13 cfu/ml). The populations of *Clostridium perfringens* (*C. perfringens)* were significantly increased with feeding Control diet (4.69 cfu/ml) but were decreased by Cox diet (2.09 cfu/ml). The Blend had the most significant effect on the growth of *Lactobacillus* species (spp.) (7.82 cfu/ml), with the Cox diet having the least effect (2.67 cfu/ml). From the study, dietary inclusion of a blend of NLM at 1.5% and CSM at 0.5% can be used as alterative to infeed oxytetracycline in growing of starter broiler chickens in Kenya.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CF	Crude Fibre
cfu	Colony Forming Unit
CP	Crude Protein
CSM	Coriander Seed Meal
Ctl	Control
DIB	Dominant Ileal Bacteria
DM	Dry Matter
EE	Ether Extract
FCE	Feed Conversion Efficiency
FI	Feed Intake
FW	Final Weight
GR	Growth Rate
IND	Ileal Nutrient Digestibility
IW	Initial Weight
MCP	Monocalcium Phosphate
ME	Metabolizeable Energy
N	Nitrogen
NACOSTI	National Commission for Science, Technology and Innovation
NFE	Nitrogen Free Extract
NLM	Stinging Nettle Leaf Meal
NRC	National Research Council
SAS	Statistical Analysis System
SEM	Standard Error of Mean
spp.	Species

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The amount of antibiotics used each year around the world have been estimated to range between 100,000 tonnes to 200,000 tonnes (Quek et al., 2022). The American animal production activities was approximated to have consumed 14,600 tonnes of antibiotics in 2012 (Wang et al., 2021). In China, the rise in intensification of livestock farming has led to antibiotics overuse with application in chicken production projected to rise by 143% in 2030 (Xu et al., 2020). Brazil has faced export restrictions for its poultry meat due to excessive use of antibiotics (Rabello et al., 2020). The sub-Saharan Africa faces the problem of antibiotic resistance due to antibiotic overuse (Kariuki et al., 2022). In Kenya, production of food animals uses about 14.6 tonnes of antibiotics yearly (Waithiru et al., 2022). Subtherapeutic levels of antibiotics such as tetracyclines, penicillins and sulphonamides are currently being used as growth promoters in broiler production (Muhammad et al., 2020). The first choice and most widely utilized antibiotics in broiler farming are tetracyclines as they are highly effective, affordable and with minimal side effects (Maddaleno et al., 2021; Quaik et al., 2020).

Different studies have attempted to explain the activity of in-feed antibiotics for the stimulation of broiler chickens' growth. Duan et al. (2022) reported that antibiotics tend to lower gut pathogenic bacterial populations thus allowing colonization by beneficial bacteria mainly *Lactobacillus* spp. *Lactobacillus salivarius*, have been reported to exhibit antibacterial activity in the ileum (Xu et al., 2022). This in turn have been found to help maintain integrity of intestinal mucosa leading to increased absorption of nutrients hence improved weight gain (Xiang et al., 2022). Alternatively, Robinson et al. (2019) and Lin (2014) explained that sub-therapeutic antibiotics promoted growth through reduction of bile salt hydrolase gut bacteria such as *Clostridium* and *Enterococcus* as they are the major producers of bile salt hydrolase enzyme which breaks down fats, reducing host animal's lipid digestion, energy harvest and utilization.

Recent research has indicated a rise in antibiotic resistance in broiler chickens in Kenya (Ndukui et al., 2022). This has been linked to two major reasons; The haphazard use of growth promoting antibiotics resulting to spread of resistant bacterial strains for example *Campylobacter jejuni* and *Escherichia coli* (Kamau, 2020; Carron et al., 2018). The problem is further compounded by the producers' failure to adhere to withholding periods of antibiotics (Kiambi et al., 2021; Mwangi et al., 2015). A case in point is a research investigation by Abubakar et al. (2019) who reported that isolates of *Campylobacter* species from broiler chicken farms in the environs of Thika town in Kenya had a 93.5% resistance to the antibiotic cotrimoxazole. *Campylobacter* species dominance in broiler farms in Thika town was reported as 22.5% (Abubakar et al., 2019). Withholding periods ranges from five to eight days for most antibiotics used in broiler chickens and majority of farmers do not comply with this leading to tissue deposition of residues of antibiotics in bones and meat of the birds (Van et al., 2020). By extension, the resistance is also spread to the consumers of products from such birds (Monir et al., 2021; Odore et al., 2015) as most antibiotic groups of drugs used in the growing of broiler chickens are also used for human medication (Silva et al., 2022; Muthuma et al., 2016).

There is a growing consumer food safety concerns in Kenya. For example, a study by Otieno and Ogutu (2020) reported that 82% of consumers of broiler chickens were against the application of synthetic growth promoters including antibiotics. Therefore, the problems arising from application of these drugs in growing of the birds can be alleviated through use of naturally occurring growth stimulants such as phytobiotics, as alternatives for antibiotics (Callaway et al., 2021; Rossi et al., 2020).

Phytobiotics are a class of natural growth promoters that can be obtained from spices, herbs or other plants (Khan et al., 2022). They are incorporated in livestock feed to enhance productivity through the improvement of feed intake, stimulation of digestive secretions, nutrient digestibility and absorption, immune stimulation, among other benefits (Raissy et al., 2022; Ni et al., 2016). Thus plant based phytobiotic feed additives are indispensable as they are locally available, effective and cheap (Alghirani et al., 2021; Odhiambo et al., 2011). However, the mechanisms behind their activity are yet to be completely studied and still an area of research interest for many animal nutritionists (Ali et al., 2021).

The leaves of stinging nettle (*Urtica dioica* L.) and whole seeds of coriander plants (*Coriandrum sativum* L.) have potential for use in broiler diets due to their impressive pharmacological activities. Stinging nettle leaves have been reported to contain thymol and carvacrol which are essential oils with great antibacterial action (Hashemi et al., 2018). Yin et al. (2017) reported that supplementing thymol and carvacrol to broiler chicks was found to favour the proliferation of *Lactobacillus* spp. bacteria and decreased the colonization of the ileum by *Clostridium perfrigens*, a pathogenic bacterium that causes necrotic enteritis in chickens. Moreover, supplementing diets of broiler chicks with 1% stinging nettle meal was observed to improve on feed conversion ratio (Safamehr et al., 2012). The plant is also rich in flavonoids notably quercetin, rutin and kaempferol (Ilhan et al., 2019). These compounds have also been shown to exhibit antibacterial action against common pathogens such as pathogenic strains of *Escherichia coli* (Ketema and Worku, 2020). Other beneficial compounds present in the nettle leaves include tannins and phenols (Al-Sal., 2020).

Whole coriander seeds have been shown to contain the essential oil linalool ranging from 60% to 80%, as the main active ingredient (Ali et al., 2021; Aćimović et al., 2016). Linalool functions through increasing liver functioning leading to improved production, release and utilization of nutrients such as proteins, carbohydrates and lipids (Al-Jaff, 2011). It has also been reported to exhibit antibacterial properties against disease causing bacteria including *Salmonella* and *Escherichia coli* (Satyal and Setzer, 2020). An investigation by Hosseinzadeh et al. (2014) reported that feeding ground coriander seeds at 2% dietary inclusion levels to broiler chicks improved the populations of beneficial bacteria in the ileum and lowered blood lipids such as cholesterols. There is little documented information on the use of stinging nettle leaves and whole coriander seeds when blended together and incorporated in the diets of starter broiler chickens. The current research was thus conducted to ascertain the growth, ileal digestibility of nutrients and the dominant ileal bacterial populations of starter broiler chickens supplemented with a blend of stinging nettle leaf and coriander seed meals as in-feed antibiotic replacers.

1.2 Statement of the Problem

Injudicious use of antibiotics as growth promotants for the growing of broiler chickens have amplified resistance and tissue deposition of residues in the edible portions of the birds such as meat and bones. Moreover, there is increased concern by broiler meat consumers of producers using in-feed antibiotic growth promoters in accelerating growth of broiler chickens. These problems can be reduced by application of phytobiotics which are plant based growth promoters that are natural, effective, readily available, have low risk of resistance and doesn't require any prescribed withholding periods. Therefore, the present study aimed to determine the growth rate, ileal digestibility of nutrients and the dominant ileal bacterial populations of starter broiler chickens supplemented with a blend of stinging nettle leaf and coriander seed meals as in-feed antibiotic replacers.

1.3 Objectives

1.3.1 Broad Objective

To determine the performance of starter broiler chickens supplemented with stinging nettle leaf or whole coriander seed meals as in-feed antibiotic replacers.

1.3.2 Specific Objectives

i. To determine the effects of supplementing different levels of stinging nettle leaf or whole coriander seed meals on the growth rate of starter broilers.

ii. To determine the effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the growth rate of starter broilers.

iii. To determine the effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the ileal digestibility of nutrients of starter broilers.

iv. To determine the effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the dominant ileal bacterial populations of starter broilers.

1.4 Hypotheses

Based on the specific objectives, the study tested the following null hypotheses:

H_{01}: There is no significant effects of supplementing different levels of stinging nettle leaf or whole coriander seed meals on the growth rate of starter broilers.

H_{02}: There is no significant effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the growth rate of starter broilers.

H_{03}: There is no significant effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the ileal digestibility of nutrients of starter broilers.

H_{04}: There is no significant effects of supplementing a blend of stinging nettle leaf and whole coriander seed meals on the dominant ileal bacterial populations of starter broilers.

1.5 Justification

One of the sustainable development goals of the Kenyan government is to have a food secure nation through increasing livestock productivity (GOK, 2017). Growing of broiler chickens have a capacity of improving food security as they give more meat in less time due to their high growth rates (Wambui et al., 2018). The meat is highly nutritious with high protein content and low cholesterol (Ndotono et al., 2022). Moreover, the increasing demand of broiler meat has a favourable effect on the future production of broiler chickens (Carron et al., 2018). Broiler farming also creates self-employment hence reducing poverty and improving standards of living (Wanjiku et al., 2020). Incorporation of a blend of stinging nettle leaves and whole coriander seeds in broiler feeds will help in production of broiler meat which is free from antibiotic residues. This will lead to production of safe meat hence safeguarding consumer health and reducing overreliance on growth promoting antibiotics by broiler producers (Muthuma et al., 2016). Stinging nettle leaves and whole coriander seeds contain bioactive compounds such as thymol and linalool that help improve on animal performance for example, in terms of feed intake, growth rate and improved feed utilization. They are also readily available, natural and safe. Therefore, good replacements of in-feed antibiotics in growing of starter broilers.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Poultry Production

Recent reports have projected that by the year 2050, the world demand for food will increase by 70 to 100% (Neupane et al., 2022). This will be due to increase in human population growth which is expected to exceed 9 billion (Robinson et al., 2015). Out of this, 8 billion will be found in the developing countries (Mountford and Rapoport, 2016). The high population growth will lead to consumption of greater amounts of meat (Velten et al., 2018). As a result, livestock production especially poultry, will intensify to satisfy the global meat demand (Iskandar et al., 2020). Poultry meat production and consumption has been steadily increasing throughout the world with chicken topping the list (Gunnarsson et al., 2020). The European Union, United States of America, China and Brazil are reported to be the world leaders in poultry production (Hautefeuille et al., 2020). Further, developing countries have been estimated to supply about 53% of the world's poultry (Moyo and Swanepoel, 2010).

Chicken production have been recorded to have lower environmental impacts compared to other meat producing livestock such as pigs and cattle thus their production is projected to increase further in the future (González et al., 2020). Their demand has been attributed to high rates of urbanization, diminishing land sizes and rising disposable incomes that enable buying and consumption of expensive diets like meat (Caekebeke et al., 2020). Advances in genetic enhancements, nutrition, management and disease control has made broiler chickens to be the most intensively grown of all livestock (Alghirani et al., 2021). Modern specialized broiler production utilizes high performing meat type hybrids that have been bred to have high feed conversion factors, growth rates and body conformation (Qaisrani et al., 2020).

2.2 Importance of the Poultry Industry in Kenya

In Kenya, chicken production is amongst the most successful ventures with a population of about 31 million birds of which 22% are broilers, 75% local chickens, 1% breeding stock and 2% other species of poultry such as geese, ducks, quails and turkeys (Chia et al., 2020; Lindahl et al., 2019). The sector is fundamental in food security, income and contributes 1.7% to the livestock gross domestic product (GDP) (Macharia et al., 2016) which in turn contributes 42% to the national agricultural GDP (Chesoo et al., 2020). Furthermore, about 70% of all the feeds manufactured in the country are meant for poultry production (Njoroge et al., 2015). This makes the industry the most popular and fastest growing of the livestock enterprises. High population growth of major cities and their vicinities including Kisumu, Nakuru and Nairobi has been the stimulus for the current surge in commercial chicken rearing (Korir et al., 2015). Hence farmers are looking for better methods of production and pricing such as contract farming. This arrangement improves on the farmer's income, production capacity and reduces market risks such as fluctuating prices (Ochieng et al., 2017).

Commercial strains of chicken for meat production such as broilers are favoured due to their high growth rates, high feed conversion efficiencies and greater output within a short period of time (Carron et al., 2017). Moreover, limited space requirement makes them easy to rear in an urban setting (Omondi, 2018). Improvements in breeding, nutrition and general management makes them more economically viable (Reverter et al., 2017). Thus most farmers opt for them as they are more profitable than local birds and their demand is rapidly growing (Carron et al., 2018). Chicken meat is the most preferred and consumed amongst the urban and rural families in Kenya (Carron et al., 2018). This is because it is white meat and highly nutritious especially with high protein content, has low cholesterol is palatable and affordable (Kariuki et al., 2013). Consumption is projected to rise by 300% by the year 2030 (Carron et al., 2017). Besides, the rise in health concerns where people prefer white meat have also contributed to the increased consumption (Omondi, 2018).

2.3 Effects of Unregulated Antibiotic Use by Broiler Chicken Farmers in Kenya

Oxytetracyclines and cotrimoxazole are the most popular antibiotics used in broiler production (Kariuki and Dougan, 2014). They are administered through drinking water or as feed additives (Odore et al., 2015) for improving performance such as daily gain and feed utilization and for controlling gastrointestinal pathogens (Langata et al., 2019). This ensures the birds achieve maximum market weights within the shortest time possible usually thirty-five days (Otieno and Ogutu, 2019). The uncontrolled use of antibiotics for growing of broilers has been the main reason for increased antibiotic resistance in chickens and consequently, in humans since the drugs are readily available and affordable in agrovets around the country (Mbugua et al., 2014). These problems necessitate the identification of cheap, locally available, safe, effective and nutritious natural growth promoters to substitute the antibiotic growth promoters in formulating home-made rations and commercially manufactured broiler feeds. The ideal alternatives should be able to replace the synthetic preparations without affecting the performance of broiler chickens (Martens et al., 2012).

2.4 The Role of Gut Microbes in Chicken Health

The microbiota found in the gastrointestinal tract of chickens are bacteria, protozoa, fungi, virus and archaea with bacteria being the most abundant having over 900 genera of distinct and varied colonies (Pourabedin and Zhao, 2015). The main genera of bacteria found in the ileum are *Lactobacillus, Escherichia, Clostridium* and *Enterococcus* (Bindari et al., 2021, Pourabedin and Zhao, 2015). The overall benefit of these microbes to the host animal include promotion of gut health by fighting pathogenic microbes and regulation of natural immunity. This in effect results to improved food digestion, disease resistance and growth (Stanley et al., 2014).

The intestinal bacteria coexist with the host animal through commensal and symbiotic associations. These relationships are vital for the survival of both species through acquisition of nutritional and protective benefits. For example, commensal bacteria protect the host animal from diseases through competitive exclusion of pathogens and foreign bacteria (Pan and Yu, 2014). Bacteria such as *Campylobacter, Salmonella* and pathogenic *coli* within a chicken's gut can proliferate and become pathogenic due to suitable conditions such as lowered host immunity leading to serious zoonotic diseases such as campylobacteriosis, salmonellosis and diarrhoea respectively (Shang et al., 2018). Treatment of the disease involves use of antibiotics such as erythromycin. The drug if misused, can lead to the bacteria becoming resistant making them transmitters of antibiotic resistance to humans who consume undercooked meat from such birds (Shang et al., 2018).

2.5 Interrelationship Between Antibiotic Resistance in Humans and in Livestock

Use of antibiotics to competitively exclude pathogenic bacteria and increase the population of desired ones for improved performance have been a widely spread practice until recently. This is because of increases in resistance to these synthetic growth promoters which are a hazard to public and livestock health (Oliveira et al., 2020). Most antibiotics administered to poultry are poorly broken down resulting to deposition of antibiotic residues in meat (Muhammad et al., 2020). In developed countries such as the European Union, disease preventing and growth boosting antibiotics in animals meant for human consumption has been banned (Devi et al., 2018). Despite this, it is predicted that by the year 2030, antibiotics usage in production of animals will surpass 67% (Van Boeckel et al., 2015) due to surge in intensification of livestock production systems especially for chickens resulting to increased incidences of diseases hence use of antibiotics for disease prevention (Blanco and Bautista, 2020).

The spread of zoonotic pathogenic microbes such as *Campylobacter* species and *Salmonella serovars* and rendering first choice antibiotics useless in treating simple diseases has resulted to serious public health challenge worldwide (Oliveira et al., 2020). The situation is further compounded by ordinary microbes such as *Streptococcus pneumoniae* developing multidrug resistances (Fernández et al., 2018). The pathogen causes life threatening diseases such as bacterial meningitis, sepsis and sinusitis in humans (Lynch, 2012). In Kenya, *Escherichia coli* is a problematic zoonotic pathogen resistant to tetracyclines; a popular antibiotic for the production of broiler chickens (Odwar et al., 2014).

The majority of antibiotics have been found to be undegradeable and water soluble (Alnajrani and Alsager, 2020). The unmetabolized antibiotics are mainly eliminated through faecal matter (Kairigo et al., 2020). This gives them the capacity to spread antibiotic resistant genes and causing potential environmental hazard to humans and animals (Blanco and Bautista, 2020). Moreover, these residues also enter the aquatic ecosystem through run offs of water, manure applications on farms (Younessi et al., 2022) and sewerage systems resulting to pollution of aquatic systems and agricultural soils (Yang et al., 2016; Muhammad et al., 2020).

2.6 Application of Phytobiotics in Livestock Nutrition to Enhance Productivity

Phytobiotics are also known as phytogenics or botanicals and collectively refers to herbs, plant extracts and spices which are prepared from lichens, algae, plants or fungi and are included in animal feed to improve productivity (Symeonidou et al., 2018). Preparation is by drying or extracting ingredients of interest such as oils (Johannah et al., 2018). Currently, other products including organic acids, prebiotics, enzymes, probiotics and are also being intensively researched for their potential application in livestock nutrition (Devi et al., 2018). Benefits of phytobiotics application in chicken nutrition include improved feed flavour and palatability, increased feed consumption (Petricevic et al., 2018), enhancing digestive enzymes activities leading to improved nutrient absorption (Wallace et al., 2010), lowered disease incidences and increased growth and consequently, higher profits (Barad et al., 2016). Plants such as sage, garlic, thyme, oregano and rosemary, together with their extracts, mostly essential oils, have been highly researched in broiler nutrition either singly or as blends (Puvača et al., 2014).

One phytobiotics' mechanism of activity is through boosting the populations of symbiotic gastrointestinal microbes (Alam et al., 2015). This reduces harmful pathogens resulting to increased nutrient bioavailability (Al-Tememy et al., 2011). Further, they increase gastric and intestinal motility and promote secretion of enzymes such as trypsin and pancreatic amylase that assist in the digestion of proteins and starch respectively (Mohammadi Gheisar and Kim, 2018). They have also been found to boost immunity thus increasing an animal's resistance to infectious agents (Devi et al., 2018). These benefits lead to improved animal health and performance in terms of meat, egg and milk production and reduced environment contamination (Stanton, 2013).

Growth promoting effects of phytobiotics is due to their constituents of secondary plant metabolites and essential oils (Symeonidou et al., 2018), known as phytonutrients (Haldar et al., 2014). Main bioactive constituents are phenolics among others eugenol, thymol and carvacrol (Christaki et al., 2012), terpenes such as linalool, menthol, geraniol and thujanol (Cheng et al., 2014), and aromatic glycosides for example cuminal, phellandral and cinnamaldehyde (Brenes and Roura, 2010). Phytobiotics also exhibits antioxidative (Alam et al., 2015), antihelminthic (Symeonidou et al., 2018), antibacterial (Solórzano-Santos and Miranda-Novales, 2012) and hypolipidemic activities (Puvača et al., 2015).

The ability of phytobiotics to achieve better performance is not completely clear (Agwah et al., 2018). Some ingredients may be harmful or irritating for example, capsaicin in chilli. Tissue deposition of phytogenic metabolites is a potential hazard which have not been fully elucidated (Grashorn, 2010). High levels of phytochemical feed additives have been shown to decrease feed intake especially for those with bitter taste (Valenzuela-Grijalva et al., 2017). Despite these shortcomings, they are largely acknowledged for use in animal nutrition as they are safe (Önder, 2018). The future of animal production hence pegs on the utilization of these natural elements in livestock nutrition (Alghirani et al., 2021; Christaki et al., 2012).

2.6.1 Stinging Nettle Leaves (*Urtica dioica* L.) as Feed Additives in Broiler Chickens

The word "nettle" is believed to have been coined from the word "noedl", an Anglo-Saxon word which means "needle" (Kregiel et al., 2018). The genus of stinging nettle plant is *Urtica* which originated from the Latin word "uro" meaning to burn or "urere" designating stinging (Joshi et al., 2014). The plant is perennial and classified in the family Urticaceae which boosts of over 1000 species (Ibrahim et al., 2018). The plant grows and thrives in the wilds of tropical and temperate countries (Joshi et al., 2014). Its popularly known as the common nettle is a celebrated medicine and food item in worldwide (Kukrić et al., 2012). In Kenyan rural households, it is popular as a source of healthy leafy vegetable and traditional medicine for the cure of ailments among others rheumatism, arthritis and diabetes (Kamau et al., 2016).

The plant grows to a height of 4 to 6 feet. Growth is characterized by stands comprised of thick, large clusters which increases in diameter annually (Baumgardner, 2016) through the root system which consists of yellow coloured stolons and rhizomes that spread far and wide giving rise to new shoots ensuring perpetuation of the species (Joshi et al., 2014). Concentrations of essential amino acids like lysine, valine, threonine and leucine in stinging nettle leaves have been recorded to be as high as 11.09g/100g (Rutto et al., 2013). The plant contains vitamins especially vitamins A and B, minerals including potassium, iron and calcium (Bekele et al., 2015). Beneficial compounds include essential fatty acids, carotenoids, phytosterols, flavonoids glycosides and tannins (Pliego et al., 2020). The plant has also been demonstrated to boost antibody reactions, reduce inflammatory reactions and to lower lipid levels in blood (Behboodi et al., 2021).

Different levels of dried stinging nettle meals have been investigated in different broiler age groups. For instance, Bekele et al. (2015) recorded that inclusion rate of 9% stinging nettle leaf meal in six-week Hubbard broilers diets exhibited the greatest performance with regards to daily, final and total body weight gains. Additionally, Nasiri et al. (2011) concluded that supplementing starter and grower broiler chickens with 1.5% meal of stinging nettle had no significant influence on FCE and weight gain but improved the carcass yields especially the breast and thigh. These studies left an opening for investigating the effects of combining stinging nettle with other phytobiotics such as coriander seeds in the diets of broiler chicks.

2.6.2 Coriander Seeds (*Coriandrum sativum* L.) as Feed Additives in Broiler Chickens

The name coriander comes from *koriannon*, which is originating from the ancient Greek words *annon* and *koris;* meaning bed bug. This was because of the foul, bed bug like smell of the plant (Pragalyaashree and Thirupathi, 2016). The use of coriander was documented as early as 1550 BC in Egypt where it was used for cooking and medicine for treating digestive upsets especially in children (Awas et al., 2016). Coriander is taxonomically classified in the order Apiales (Nyakudya et al., 2014) which have over 3000 species and around 300 genera of trees (Asgarpanah and Kazemivash, 2012). It is currently classified in the family *Apiaceae* but formerly umbelliferae (Önder, 2018; Jangra et al., 2017). The genus *Coriandrum* with the scientific name *Coriandrum sativum* is the most popular. The modern cultivated plant originated from the wild ancestor *Coriandrum tordylium* (Nadeem et al., 2013).

Pharmacologically, coriander seeds exhibit antioxidant (Aissaoui et al., 2011), antibacterial (Silva et al., 2011), and hypolipidemic properties **(**Sharopov et al., 2017**)**. Different levels of coriander seeds have been demonstrated to increase production of broiler chickens (Barad et al., 2016; Naeemasa et al., 2015). Barad et al. (2016) observed the best weigh gain in finisher broilers supplemented with 2% coriander seeds. Additionally, Taha et al. (2019) reported improved feed intake, growth rates, weight gains and lowered lipid levels of finisher broilers fed 0.4% coriander seed powder. Furthermore, Naeemasa et al. (2015) reported that supplementing starter, grower and finisher broilers with 1.2% coriander seed powder resulted to the best feed intake. Further, increases in feed conversion ratios was achieved with increased supplementation of coriander seed powder. Al-Jaff (2011) demonstrated increased final gain of body weights and better FCE of six-week grower broiler chickens supplemented with 2% coriander seeds. Similarly, Saeid and Al-Nasry (2010) reported higher feed conversion efficiency, carcass yield and body weight gain of 42-day old finisher broiler chickens supplemented with 0.3% dietary inclusion level of coriander seeds.

2.6.3 Benefits of Blending Phytobiotics in Improving Broiler Performance

Blending of two or more phytobiotics has have been observed to give better production performance effects in broiler chickens than singly used through utilizing their coaction (Kalantar et al., 2017). Glamoclija et al. (2016) recorded improved final weight gain, feed conversion efficiencies and carcass yields of fattening Cobb-500 broiler chickens supplemented a blend of phytobiotics consisting of mint, anise, clove and cumin as compared to the non-supplemented groups. In another study, Gilani et al. (2018) conducted a study to find out natural alternatives to antibiotics in starter and finisher hubbard broiler chickens using a blend of phytobiotics viz *Glycyrrhiza glabra* (liquorice), *Zingiber officinale* (ginger), *Withania somnifera* (Ashwagandha), *Nigella sativa* (black seed) and *Camellia sinensis* (green tea). They reported improved performance in terms of lowered blood cholesterol levels and improved immunity than the unsupplemented group.

Moreover, Tabari et al. (2016) carried out a study to assess the influence of blending pumpkin seed oil and root extract of stinging nettle on performance and gut microbiota of Ross 308 starter, grower and finisher broiler chickens. They reported improvement in body weight gain of the birds fed diets supplemented with the phytobiotics as compared to control groups which were offered corn and soya bean meal based basal diet. Likewise, Barad et al. (2016) demonstrated positive results of feeding a blend of turmeric powder, coriander seeds and black pepper promoters of growth in Cobb-400 broilers up to 42 days of age. They reported higher final body weights, increased FI and FCE of birds fed the feed additives than those that were fed the control diet without the feed additives. Similarly, Kumari et al. (2014) fed a mixture of coriander, linseed, neem leaf and sugar beet to Vanaraja broiler chickens up to 42 days old. They reported improved performance in terms of FI, body weight gain and FCE. Konkol et al. (2021) documented greater absorption of selenium in broilers supplemented with a phytobiotic blend and yeast (*Saccharomyces cerevisiae*) in contrast to the unsupplemented groups.

CHAPTER THREE

EFFECTS OF SUPPLEMENTING DIFFERENT LEVELS OF STINGING NETTLE LEAF OR WHOLE CORIANDER SEED MEALS ON THE GROWTH RATE OF STARTER BROILERS

3.1 Introduction

Many research works have described high residue concentrations of antibiotics such as tetracyclines and penicillins, used as growth promotants in the growing of broiler chickens, especially in tissues such as meat and bones of the birds (Odore et al., 2015; Ferdous et al., 2020; Monir et al., 2021; Mohammadzadeh et al., 2021). This has been associated with increased resistances to these medications which presents difficulties in preventing, treating and controlling of clinical cases in humans and livestock (He et al., 2020). Consequently, this has led to their ban in many countries across the globe (Ebeid et al., 2021). The recent rise in consumer health consciousness have resulted to an increase in demand for healthy, naturally produced broiler meat (Booth et al., 2021).

Phytobiotics such as stinging nettle, rosemary, coriander, thyme and neem have gained attention in present day growing of broiler chickens (Arain et al., 2022) due to their positive influence on the performance characteristics including increase in GR, FI and FCE, thus are potential candidates for safer and natural alternatives for growth boosters (Pliego et al., 2020; Oluwafemi et al., 2020). Additionally, they are reported to have high levels of active biomolecules including essential oils, that confers them with among others, antibacterial, growth promoting, lipid lowering, antioxidant and pharmacological activities (Arain et al., 2022). The bioactive content and function of phytobiotics are influenced by among other factors the different agro ecological conditions, variety and strain of the plants, processing methods, and growing seasons (Kačániová, 2020). Thus the goal of the present research was to get the level of stinging nettle leaf or whole coriander seed meals with the highest growth rate of starter broilers.

3.2 Materials and Methods

3.2.1 Study Site

This research was carried out at Chuka University's poultry unit which is situated in Chuka sub-county of Tharaka-Nithi County, Kenya. Chuka University is located 186 kilometres from Nairobi city along Nairobi-Meru highway and located between

Longitudes 37 18’37” and 37 28’33” East and Latitude 00 07’23” and 00 26’19” South. This area has an yearly rainfall average of 1373 mm and temperatures ranging from 16.0^0C to 24.0^0C.

3.2.2 Animals, Experimental Model and Housing

Eighty-four mixed sex day old broiler chickens (Cobb 500 strain) were bought from Kenchic Limited Meru depot, Kenya. The chicks are sold pre-vaccinated against Mareks disease. During the starter phase, young broiler chicks are usually susceptible to diseases such as necrotic enteritis and diarrhoea due to their lowered immunity. Prevention at early stage is critical to safeguard their health which results to improved growth rates, strong immunity and consequently, higher returns to the farmer (Dieryck et al., 2022).

The following linear model for a CRD was utilised:

$y_{ij} = \mu + t_i + + \epsilon_{ij}$

Where;

y_{ij} - is the response variables due to the dietary treatments (GR, FI and FCE).

μ - is the overall population mean of the starter broiler chickens.

t_i - is the effects (GR, FI and FCE) due to the NLM or whole CSM offered to the starter broiler chickens.

ϵ_{ij} - is the random error effect.

Each dietary treatment had twelve chicks (n = 12); where there were three chicks per pen with four replicates. The broiler chickens were randomly allocated to the experimental pens where they were allowed a total of 3 days for adaptation to experimental diets and conditions before commencement of the experiment. The research was carried out for a total of 17 days.

A total of twenty-eight experimental pens measuring 2 feet by 2 feet and a height of 1.5 feet were constructed using timber and chicken wire net. The chicks were raised in a deep litter system. The experimental house, pens, waterers and feeders were first cleaned with water. After drying, they were thoroughly disinfected with Noro cleanse® (contains the active ingredients Glutaraldehyde 15%w/v and Coco-benzyl-dimethylammonium chloride 10% w/v). Manufacturer's recommended dilution rate of 25 ml/100litres of water was adhered to.

In every pen, wood shavings (used as litter) was spread on the pen floor at a depth of four inches then disinfected with Noro cleanse®. Infrared brooding bulbs and fluorescent tubes were installed for heating and lighting the pens and experimental house respectively. Before arrival of the day old broiler chicks, the pens were pre-heated for six hours. Pen floor temperatures of 30 ^{0}C to 32 ^{0}C was maintained during the initial seven days. This was then steadily lowered by 5 ^{0}C weekly till the final day of the research (17). Temperatures were taken using a clinical thermometer. A lighting schedule of 24 hours was maintained using fluorescent tubes. Gunny bags were used to cover the openings on the sides of the experimental house. Biosecurity measures involving use of proper protective clothing (gumboots and laboratory coat) and foot bath containing the disinfectant Noro cleanse® (Manufacturer's recommended dilution rate of 30ml/100litres of water was used) at the entrance of the research house were observed. Newcastle disease and infectious bronchitis vaccine was administered on the seventh day through drinking water. Multivitamins were administered thereafter.

3.2.3 Formulation of Experimental Diets

Stinging nettle leaves and whole coriander seeds were sourced from Ol Joro Orok constituency, Weru ward. The upper four leaves were picked from stinging nettle plants as they are rich in the desired essential oils and flavonoids. Freshly harvested coriander seeds were also purchased from a farm within the same locality. Both were shade dried to a constant weight, ground and sieved using a mesh size of 1000 microns. The feed ingredients were manually mixed using a shovel till thoroughly and evenly mixed.

The NRC, (1994) guidelines on nutrient requirements of starter broiler diets were followed in formulating the experimental diets. The following feed ingredients were used: maize, soybean meal, sunflower meal, fish meal, monocalcium phosphate, wheat, vegetable oil, ground limestone, calcium carbonate, common salt, vitamin, mineral premixes and the following synthetic amino acids; DL-methionine, L-tryptophan and L-lysine. The amino acids were added in order to balance off any deficiency from the protein sources used. Maize, soybeans and wheat were ground using a mesh size of 1500 microns. Table 1 presents the amounts of each ingredient that was used in the formulation of the experimental diets. Seven diets were prepared as follows: Control at 0% which was not supplemented with any phytobiotic or antibiotics, NLM supplemented at the following inclusion levels of the total diet; 1%, 1.5% and 2%. The CSM was supplemented at the following inclusion levels of the total diet; 0.5%, 1% and 1.5%. The ingredients were balanced using excel software.

Table 1
Composition of Experimental Diets

	Ctl	NLM			CSM		
Ingredient	0%	1%	1.5%	2%	0.5%	1%	1.5%
Maize	49.25	49.21	46.49	46.26	47.93	47.42	47.09
Soybean meal	25.26	24.51	24.2	24.00	25.50	25.18	25.08
Sunflower meal	2.67	2.50	2.55	2.50	2.32	2.70	2.75
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
MCP	0.93	0.89	0.88	0.87	0.90	0.90	0.88
Wheat	7.49	7.50	10.00	10.00	8.44	8.40	8.30
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Ground limestone	0.30	0.30	0.30	0.30	0.33	0.30	0.30
Calcium carbonate	1.20	1.19	1.18	1.17	1.18	1.20	1.20
Common salt	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytobiotics	-	1.00	1.50	2.00	0.50	1.00	1.50
DL-methionine	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-tryptophan	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Total	100	100	100	100	100	100	100
Calculated Composition							
Crude protein	19.75	20.00	20.11	20.23	18.02	18.16	18.30
Crude fibre	3.27	5.24	5.27	5.29	26.05	26.19	26.32
ME (Kcal/Kg)	3176	3185	3194	3205	2980	3000	3055

3.2.4 Feeding and Data Collection

The experimental animals were offered feeds and water in an unrestricted approach. They were all put on the control diet for the first three days where old newspapers were spread on each pen floor and used as feeding papers to offer the feeds. Feeders were introduced on the second day to replace the feeding papers. On the fourth day, their weights were taken and recorded. Experimental diets were then offered to the respective groups of birds. Drinkers containing water mixed with multivitamins, glucose and liquid paraffin were also placed in each pen. The treated water was offered for the entire adaptation period which lasted three days.

On arrival, the chicks' individual BW were taken using an electronic scale with a precision of 0.000 (average weight per chick was 37.32g). The BW were then recorded and chicks randomly placed into the experimental pens. On the fourth day, their BW were taken and recorded. Experimental diets were then offered to the birds. Every morning, leftover feeds from each pen were weighed and recorded. Fresh feeds were then weighed and offered to the birds *ad libitum*. Waterers were also cleaned and fresh water offered. Weight taking and recording was done weekly at 0800hours using an electronic scale with a precision of 0.000. The BW of all three birds per pen were taken on days 7, 14 and 21. Average GR were calculated as the difference of final BW in grams and initial BW in grams divided by the length of the experiment (17 days).

3.2.5 Evaluation of Starter Broiler Chickens' Performance

The following variables were applied to ascertain the bird's responses to the dietary treatments: GR, FI and FCE.

Data on FI was collected for a period of 20 days. On day 21, which was the last day of the experiment, final leftover feeds from day 20 was weighed and recorded in order to get the FI of day 20. The final BW of the birds were also taken and recorded.

The mean GR was computed as the difference of mean final BW in grams and mean initial mean body weight in grams divided by the days of the experiment (17 days). The FCE was determined as the weight of all the feeds ingested (in grams) during the entire experiment divided by the weight gain in grams (final weight minus starting weight).

3.2.6 Laboratory Analysis

Proximate analysis was undertaken for all experimental diets at Chuka University laboratory to estimate their levels of crude protein (CP), ether extract (EE), moisture (MC), ash, crude fibre (CF) and nitrogen free extracts (NFE) according to AOAC 2000 methods. Diets were analysed before feeding to ensure they contained the right proportions of nutrients required by the birds especially CP, CF and the energy content.

The following equation was used in calculating the CP; % CP = % N × F (Factor). The F conversion factor used was 6.25.

3.2.7 Statistical Analysis

Statistical analysis system (SAS version 9.4) software (SAS Institute, Cary NC) was used to analyse all the data. Tukey pairwise comparison was conducted to compare variations among diets where ANOVA indicated differences at probability values (α = 0.05).

3.2.8 Ethical Considerations

The experiments were conducted in Chuka University's poultry unit. Ethical approval was done by the Chuka University Institutional Ethics Review Committee of application approval number NACOSTI/NBC/AC-0812 (Appendix 1). Research license was sought from NACOSTI license number NACOSTI/P/21/13883 (Appendix 2).

3.3 Results

3.3.1 Proximate Analysis of Experimental Diets

Proximate analysis carried out as indicated in Table 2. NLM had the highest CP content of 23.25% followed by the basal diet and CSM at 19.75% and 18.42% respectively. Crude fibre was highest in CSM at 25.93% and least in the basal diet at 3.27%. NLM diet had the highest energy content at 3400 Kcal/Kg, with CSM having the lowest value of 2960 Kcal/Kg.

Table 2

Proximate Analysis of Phytobiotics and Experimental Diets on a DM Basis (%)

Treatments	DM	CP	Ash	CF	EE	NFE	Energy
NLM	87.54	23.25	21.97	6.41	3.60	44.77	3400.00
CSM	85.63	18.42	5.10	25.93	5.65	44.90	2960.00
Control	90.40	19.75	5.33	3.27	6.40	65.25	3176.05
NLM 1%	90.05	20.00	19.76	5.24	7.15	47.85	3185.00
NLM 1.5%	89.00	20.11	19.70	5.27	7.12	47.80	3194.00
NLM 2%	90.01	20.23	19.73	5.29	7.16	47.59	3205.00
CSM 0.5%	94.02	18.02	4.85	26.05	11.40	39.68	2980.00
CSM 1%	93.20	18.16	5.01	26.19	11.60	39.04	3000.00
CSM 1.5%	94.00	18.30	4.98	26.32	11.50	38.90	3055.00

The estimated metabolizeable energy (ME) content expressed as Kcal/Kg

3.3.2 Effects of Supplementing Different Levels of Stinging Nettle Leaf Meal on the Feed Intake, Growth Rate and Feed Conversion Efficiency of Starter Broilers

The results of the feeding trial (Table 3) showed that the birds supplemented with NLM at 1% inclusion levels in their diets exhibited significantly higher mean FI of 162.03g, in comparison to the other groups. The birds in the control groups had the lowest mean FI of 148.93g. It was also observed that as the levels of NLM supplementation increased from 1% to 2%, the FI decreased significantly. The GR was significantly higher (113.56g) in birds supplemented with NLM at 1.5% inclusion levels. The control group had better GR (90.72g) compared to the group fed NLM at 2% level (88.48g). The FCE for the entire experimental period showed that higher levels of NLM (2%) resulted to higher FCE (7.98). The control group (NLM 0%) performed better (7.73) than NLM at 1% (7.59g) and 1.5% (6.44).

Table 3
Performance of Starter Broiler Chickens Supplemented with NLM

	Treatments				
Parameter	NLM 0%	NLM 1%	NLM 1.5%	NLM 2%	±SE
Mean IW	180.30	185.00	183.00	182.80	1.41
Mean FW	1722.50^{c}	1893.00^{b}	2113.50^{a}	1687.00^{d}	2.42
GR	90.72^{c}	100.47^{b}	113.56^{a}	88.48^{d}	0.42
FI	148.93^{c}	162.03^{a}	155.29^{b}	150.04^{c}	8.86
FCE	7.73^{b}	7.59^{c}	6.44^{d}	7.98^{a}	0.02

Data is represented as means ± SE (standard error of the mean).
Means not sharing a letter are different significantly at 95% confidence interval ($p<.05$).

3.3.3 Effects of Supplementing Different Levels of Whole Coriander Seed Meal on the Feed Intake, Growth Rate and Feed Conversion Efficiency of Starter Broilers

The results obtained from the feeding trial are summarized in Table 4. Results of statistical analysis of the mean FI showed that the birds supplemented with dietary inclusion levels of CSM at 0.5% showed the best mean FI of 193g with the control having the lowest mean FI of 148.93g. Dietary levels higher than 0.5% was observed to significantly decrease the FI of CSM. The group of birds fed on CSM at 0.5% inclusion levels showed significantly higher GR of 119.31g, with the control fed group having the lowest GR of 90.72g. As the inclusion levels of CSM was increased from

0.5%, 1% up to 1.5%, the GR significantly decreased from 119.31g, 107.68g and 99.96g respectively. Significant FCE was observed in the groups of birds fed diets supplemented with CSM at 1.5% (8.35). Those fed the control diet showed a better FCE (7.73) than those supplemented with CSM at 0.5% (7.61) dietary inclusion levels.

Table 4
Performance of Starter Broiler Chickens Supplemented with CSM

	Treatments				
Parameter	CSM 0%	CSM 0.5%	CSM 1%	CSM 1.5%	±SE
Mean IW	180.25	182.50	187.50	184.75	1.79
Mean FW	1722.50^{d}	2210.75^{a}	2018.00^{b}	1884.00^{c}	1.86
GR	90.72^{d}	119.31^{a}	107.68^{b}	99.96^{c}	0.01
FI	148.93^{d}	193.00^{a}	184.04^{b}	177.29^{c}	10.41
FCE	7.73^{c}	7.61^{d}	8.04^{b}	8.35^{a}	0.01

Data is represented as means ± SE (standard error of the mean). Means not sharing a letter are different significantly at 95% confidence interval (p<.05)

3.4 Discussion

3.4.1 Proximate Analysis of Experimental Diets

In the present study, analysed NLM was found to contain a CP of 23.25%. Alemayehu et al. (2016) reported 21.4% CP content after analysing *Urtica simensis* which is in the same genera with *Urtica dioica* L. The results also compare to 16.08% to 26.89% as determined in a study by Rafajlovska et al. (2013). Additionally, crude protein analysis of CSM yielded 18.42%. This was comparable to documented results of between 11.49% to 21.93% as reported by Kassahun (2020). The CP of experimental diets was within the NRC, (1994) recommendations for starter broilers. Adequate amino acids are required by broiler chickens to support their rapid muscle deposition in order to reach market weight early (Maynard et al., 2022). Excessive dietary crude proteins in the diets of broiler chickens have been found to increase faecal excretion of nitrogenous wastes like ammonia and nitrates leading to environmental pollution especially of soils, streams and rivers (Musigwa et al., 2020; Chrystal et al., 2020). The differences in contents of CP of the plants may be attributed to the varying agronomic conditions, variety and strain of the plants, postharvest handling and processing conditions (Milosevic et al., 2021).

From the study, the amounts of mineral in NLM (21.97%) and NLM diets was found to be quite high (average 19.73%). The leaves of stinging nettle plant have been recorded to be rich in minerals including calcium, zinc potassium and iron (Bhusal et al., 2022). These elements are especially important for the broiler chicks as they have a rapidly growing skeletal and muscle tissues (Zafari and Nazari, 2022). The dietary energy level was satisfactory in the growing of the chicks as it was within the NRC, (1994) recommended value of 3200 Kcal/Kg. The analysed crude fibre content of the basal diet was 3.27%. Chickens require a maximum of 5% of crude fibre for proper growth (Tejeda and Kim, 2021). Hence, the diet was satisfactory for the growth of the birds. The high levels of ether extract recorded for CSM diets (average 11.50%) is attributable to the presence of high amounts of essential oil linalool which is the main bioactive phytochemical found in CSM with amounts reported to range from 60% to 80% (Ali et al., 2021).

3.4.2 Effects of Supplementing Different Levels of Stinging Nettle Leaf Meal on the Feed Intake, Growth Rate and Feed Conversion Efficiency of Starter Broilers

In the present study, the low FI of birds fed NLM at 2% was attributed to the existence of natural products which in high levels, reduced feed intake. Additionally, high levels of the plant may result to high intake of crude fibre which has laxative effects on the gut leading to faster transit of nutrients along the gut resulting to poor retention and hence low digestibility (Alshelmani et al., 2021). Supplementing stinging nettle meal has been recorded to have no significant result ($p>0.05$) on the FI of broilers (Safamehr et al., 2012). However, more studies need to be carried out to find out negative effects of the plant's metabolites on nutrient palatability, intake, digestibility and use in broilers (Milosevic et al., 2021). Additionally, the low FI of chicks within the first few days of the experiment is attributed to the fact that young chicks have an immature digestive system hence low secretion of digestive enzymes and small sizes of absorptive organs with limited surface area which limits nutrient absorption (Ravindran and Abdollahi, 2021). But as the bird grows, and the digestive system matures, it is able to ingest and utilize more feed. However, low absorption rates of the ingested amounts of the phytobiotics may also be due to the lack of specific enzymes of digestion by the animals to fully break down the phytonutrients to bring about the desired growth (Kikusato, 2021).

In determination of GR, the outcomes of the current study were consistent with prior studies which reported improved GR of broiler chickens supplemented with stinging nettle leaves in their diets (Hashemi et al., 2018, Bekele et al. (2015), Safamehr et al., 2012). Enhanced GR is attributable to the high amount of phytochemicals such as flavonoids notably quercetin, rutin and kaempferol (Ilhan et al., 2019) which have been reported to have high levels of antibacterial compounds that help in reducing disease causing microbes resulting to increased nutrient digestibility and utilisation (Ketema and Worku, 2020). In contrast, the current study also reported a reduced GR at higher levels of NLM (2%) supplementation possibly attributable to the existence of elevated concentrations of secondary metabolites that limit nutrient digestibility and use. Previous studies have also recorded a decreased production of broilers fed increasing concentrations of stinging nettle leaf meal (Bekele et al. (2015), Nasiri et al. (2011)).

The low FCE exhibited by NLM at 1.5% may be attributed to the low levels of dietary inclusion hence low levels of beneficial bioactive compounds such as flavonoids. This was comparable to an investigation by Nasiri et al. (2011) who supplemented starter and grower broiler chickens with 1.5% stinging nettle meal in the basal diet. They described non-significant results of the additive on FCE and weight gain of the birds. Additionally, a significantly higher FCE was observed at inclusion levels of 2%. This was probably due to the increased amounts of flavonoids and essential oils such as thymol and carvacrol which have high antibacterial activity which promotes growth (Hashemi et al., 2018; Yin et al., 2017).

3.4.3 Effects of Supplementing Different Levels of Whole Coriander Seed Meal on the Feed Intake, Growth Rate and Feed Conversion Efficiency of Starter Broilers

The overall improved performance of the starter broilers offered diets enhanced with CSM as related to those offered control diet may be ascribed to the presence of high amounts of essential oil linalool which is the main bioactive phytochemical found in CSM with amounts reported to range from 60% to 80% (Ali et al., 2021). Linalool have been shown to function through increasing liver functioning (Al-Jaff, 2011) and the growth and functioning of villi of the small intestines (Ghazanfari et al. 2015) leading to improved feed intake, digestibility, absorption and utilization of nutrients (Irawan et al., 2021).

In the present research, high FI was recorded for the birds supplemented with CSM in their diets. Different studies have reported comparable results; Taha et al. (2019) and Naeemasa et al. (2015) narrated improved FI in broiler chickens with dietary supplementation of 0.4% and 1.2% coriander seed powder respectively.

Supplementation of CSM was recorded to significantly improve the GR of the starter broiler chickens. This was in agreement with previous research work by Ghazanfari et al. (2015) who narrated an increase in GR of broilers fed on a diet supplemented with 0.03% of coriander seed essential oil from starter to finisher stage at day 42, in comparison to the unsupplemented factions. Also, Taha et al. (2019) reported improved GR and weight gains of broiler chickens fed 0.4% coriander seed powder. Additionally, research works by Barad et al. (2016), Naeemasa et al. (2015), Al-Jaff, (2011) and Saeid and Al-Nasry, (2010) reported improved GR of broiler chickens supplemented with CSM in their diets.

In the current study, the chickens fed on 1.5% inclusion levels of CSM showed significant increase in the FCE. The results corresponded to those described by Ghazanfari et al. (2015) of high FCE in broilers fed coriander seed essential oil at the rate of 300mg/kg of feed as compared to control groups. In contrast, similar studies have reported negative results; Khubeiz and Shirif, (2020) reported that dietary supplementation of different levels of meals of coriander seeds to broilers did not have any significant effect on their FCE. Similarly, Rashid et al. (2014) reported inconsequential variances in broiler chickens fed on a diet containing increasing levels of CSM up to 1.5%. The diverse results may be attributed to the plant's different agro ecological conditions, variety and strain, postharvest handling and processing conditions (Milosevic et al., 2021).

CHAPTER FOUR

EFFECTS OF SUPPLEMENTING A BLEND OF STINGING NETTLE LEAF AND WHOLE CORIANDER SEED MEALS ON THE GROWTH RATE, ILEAL DIGESTIBILITY OF NUTRIENTS AND THE DOMINANT ILEAL BACTERIAL POPULATIONS OF STARTER BROILERS

4.1 Introduction

The microorganisms found in the chicken's gut contribute to their nutritional health and regulation of natural immunity (Biasato et al., 2020). *Escherichia coli* and *Clostridium perfringens* are normal flora found in the ileum of broiler chickens which under certain conditions such as stress and lowered immunity, they proliferate causing pathological diseases such diarrhoea and necrotic enteritis (Granstad et al., 2020). These causes huge economic impacts amongst broiler farmers through lowered production and animal deaths (Abd El-Hack et al., 2021). Hence the widespread utilization of antibiotics in animals meant for human consumption to help lower the clinical diseases and to improve production (Oliveira et al., 2020).

Antibiotics are extensively applied in the production of broiler chickens (Blanco and Bautista, 2020). As growth promoters, they have been recorded to mainly act by competitively excluding pathogens from the gut through preventing their adhesion and multiplication on the intestinal walls (Van et al., 2020). This helps maintain the integrity of the intestinal mucosa thereby improving nutrient digestibility and absorption (Pliego et al., 2020). Their sub-therapeutic use has led to development of resistant strains of bacteria through selective pressure of susceptible gut microbes within the affected animals thereby affecting the native bacteria (Alnajrani and Alsager, 2020). Additionally, most antibiotics administered to broiler chickens are poorly broken down in the gut resulting to deposition of antibiotic residues in products such as meat (Muhammad et al., 2020). This has made the drugs ineffective in managing of clinical illnesses in human beings who consume the contaminated products as most groups of antibiotics applied in production of animals are also utilized for human medication (Jafari et al., 2021). This is why their use in food animals is being phased out throughout most countries worldwide (Abd El-Ghany, 2020).

Phytobiotics have been reported to contain phytonutrients such as thymol, carvacrol and linalool which exhibit beneficial physiological properties such as antibacterial, growth promoting, antioxidant and lipid lowering activities (Raissy et al., 2022). This helps to reduce local inflammation in the small intestines leading to improved integrity of the intestinal mucosa which facilitates increased secretion of digestive juices, nutrient digestion and absorption (Alghirani et al., 2021). Stinging nettle leaves and whole coriander seeds are phytobiotics which are gaining research interest in chicken production as suitable replacement alternatives for growth promoting antibiotics. The main active ingredients reported for stinging nettle leaves are the essential oils thymol and carvacrol (Hashemi et al., 2018). They also have elevated amounts of flavonoids including rutin, kaempferol and quercetin (Ilhan et al., 2019). These molecules are also documented to exhibit high antibacterial effects against pathogenic bacteria such as *Clostridium perfringens* and also favour growth of *Lactobacillus* bacteria in the ileum of broilers (Yin et al., 2017). Linalool has been reported as the main bioactive molecule found in whole coriander seeds (Ali et al., 2021). The compound functions through increasing liver functioning leading to increased production, release and utilization of nutrients hence leading to improved growth performance in chickens (Al-Jaff, 2011). Additionally, the seeds also exhibit antibacterial properties against pathogenic *Escherichia coli* (Satyal and Setzer, 2020). Numerous studies have described enhanced functioning of broiler chickens supplemented with blends of two or more phytobiotics singly or as a mixture with other nutrients (Konkol et al. (2021), Gilani et al., (2018), Kalantar et al., (2017), Glamoclija et al., (2016)). The functioning of broilers supplemented with blends of phytobiotics has been reported to be better than singly used due to the synergistic effects of the plants extracts (Tavangar et al., 2021).\

4.2 Materials and Methods

4.2.1 Study Site

The study site was as indicated in chapter 3.2.1.

4.2.2 Animals, Experimental Model and Housing

A total of thirty-six day old broiler chickens were ought for this study. The response variables were GR, IND and DIB populations; and the number of experimental pens utilised were twelve. The rest of the information is as indicated in chapter 3.2.2.

4.2.3 Preparation of Experimental Diets

Three diets were then mixed as follows; basal diet supplemented with a blend of NLM at 1.5% and CSM at 0.5% inclusion levels (levels with the highest performance in terms of growth rate from experiment one) referred to as Blend; basal diet supplemented with oxytetracycline hydrochloride powder at the rate of 0.05g/kg of feed (positive control) (Metrocycline®, manufactured by Hightech Pharmaceuticals and Research Limited, Nairobi-Kenya) referred to as Cox and the basal diet (negative control) referred to as Control which was not supplemented with any phytobiotic or antibiotics. For the Blend and Cox, all additives were accurately weighed and manually mixed together until a homogeneous mixture was obtained (Table 5).

Table 5
Composition of Experimental Diets

Ingredient	Blend	Cox	Ctl
Maize	48.31	49.20	49.25
Soybean meal	24.40	25.26	25.26
Sunflower meal	2.50	2.67	2.67
Fish meal	5.00	5.00	5.00
MCP	0.89	0.93	0.93
Wheat	7.50	7.49	7.49
Vegetable oil	5.00	5.00	5.00
Ground limestone	0.30	0.30	0.30
Calcium carbonate	1.20	1.20	1.20
Common salt	0.10	0.10	0.10
Mineral premix	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50
Oxytetracycline	-	0.05	-
NLM	1.50	-	-
CSM	0.50	-	-
DL-methionine	0.50	0.50	0.50
L-tryptophan	0.20	0.20	0.20
L-lysine	1.10	1.10	1.10
Total	100.00	100.00	100.00
Analysed Composition			
Crude protein	23.55	19.73	19.75
Crude fibre	11.87	3.25	3.27
ME (Kcal/Kg)	3300.00	3185.00	3176.05

4.2.4 Feeding and Data Collection

The experimental animals were offered feeds and water in an unrestricted approach. They were all put on the control diet for the first three days where old newspapers were spread on each pen floor and used as feeding papers to offer the feeds. Feeders were introduced on the second day to replace the feeding papers. On the fourth day, their BW were taken and recorded. Experimental diets were then offered to the respective groups of birds. Drinkers containing water mixed with multivitamins, glucose and liquid paraffin were also placed in each pen. The treated water was offered for the entire adaptation period which lasted three days.

On arrival, the chicks' individual BW were taken using an electronic scale with a precision of 0.000 (average weight per chick was 37.0g). The BW were then recorded and chicks randomly placed into the experimental pens. On the fourth day, their BW were taken and recorded. Experimental diets were then offered to the birds. Every morning, leftover feeds from each pen were weighed and recorded. Fresh feeds were then weighed and offered to the birds *ad libitum*. Waterers were also cleaned and fresh water offered. Weight taking and recording was done weekly at 0800hours using an electronic scale with a precision of 0.000. The BW of all three birds per pen were taken on days 7, 14 and 21. Average GR (average daily gain) was calculated as the difference of final BW in grams and initial BW in grams divided by the days of the experiment (17 days).

In the last day of the study, a total of two broilers per replicate were selected at random (total of 8 chicks per dietary treatment) and humanely sacrificed through cervical dislocation and dissected to obtain ileal digesta samples by gently stripping the contents into pre-labelled sterile sample containers. The samples from each bird were completely mixed to achieve a uniform blend. The collected specimens were immediately placed in a cool box then transferred to a freezer set at -80^{0}C as they awaited analysis.

4.2.5 Evaluation of Starter Broiler Chickens' Performance

The following variables were applied to ascertain the bird's responses to the dietary treatments; GR, IND and DIB populations.

Data on FI was collected for a period of 20 days. On day 21, which was the last day of the experiment, final leftover feeds from day 20 was weighed and recorded in order to get the FI of day 20. The final BW of the birds were also taken and recorded. The mean GR was computed as the difference of mean final BW in grams and initial mean BW in grams divided by days of the experiment (17 days). FCE was determined as the weight of all the feeds ingested (in grams) during the entire experiment divided by the weight gain in grams (final weight minus starting weight).

Proximate analysis was carried out on the ileal digesta samples out and nutrient digestibility calculated using the following method: Digestibility (%) **= [**(Nutrient intake – Nutrient in ileal digesta) / Nutrient intake**]** ×100. The collected ileal digesta samples were cultured to determine the populations of *Lactobacillus* Species, *Escherichia coli* and *Clostridium perfringens* bacteria present in the ileum.

4.2.6 Laboratory Analysis

Proximate analysis for the diets and the ileal digesta was undertaken at the University of Nairobi Animal Nutrition laboratories to estimate their levels of MC, CP, EE, CF, ash, and NFE as outlined in the methods of AOAC 2000. Analysis of the diets was done before feeding to ensure they contained the right proportions of nutrients required by the birds. The following equation was used in calculating the CP; % CP = % N × F (Factor). The F conversion factor used was 6.25. Bacterial culture of the ileal digesta was done at the University of Nairobi veterinary laboratories using the standard plate count agar method. Bacterial colonies were calculated using the following method; cfu = number of colonies × dilution factor/volume of culture plate.

4.2.7 Statistical Analysis

Statistical analysis system (SAS version 9.4) software (SAS Institute, Cary NC) was used to analyse all the data. Tukey pairwise comparison was conducted to compare variations among diets where ANOVA indicated differences at probability values (α = 0.05).

4.2.8 Ethical Considerations

The ethical considerations were as indicated in chapter 3.2.8.

4.3 Results

4.3.1 Proximate Analysis of Experimental Diets

Table 6 indicates the results of proximate analysis carried out. The Blend had a CP content of 23.55%, a CF of 11.87% and a metabolizeable energy content of 3300 Kcal/Kg. Cox had a CP of 19.73%, CF of 3.25% and an energy content of 3185 Kcal/Kg. The control diet had a CP of 19.75%, CF of 3.27% and an energy content of 3176.05 Kcal/Kg. Proximate analysis of NLM and CSM is as described in chapter 3.3.1.

Table 6
Proximate Analysis of Phytobiotics and Experimental Diets on a DM Basis (%)

Treatments	DM	CP	Ash	CF	EE	NFE	Energy
NLM	87.54	28.17	21.97	6.41	3.60	39.85	3400.00
CSM	85.63	18.42	5.10	25.93	5.65	44.90	2960.00
Blend	89.10	23.55	10.80	11.87	5.21	48.57	3300.00
Cox	90.60	19.73	5.30	3.25	6.33	65.39	3185.00
Control	90.40	19.75	5.33	3.27	6.40	65.25	3176.05

The estimated metabolizeable energy (ME) content expressed as Kcal/Kg

4.3.2 Effects on the Growth Rate

The results of statistical analysis of the mean FI showed that all dietary treatments were significantly different at 95% level of significance. The group of birds supplemented with the Blend in their diets showed significantly higher mean FI of 133.08g in comparison to the other treatments. The broilers offered the control diet had better mean FI (123.15g) than those fed on Cox (117.24g). Further, the GR of birds fed on diets supplemented with the Blend was significantly higher (117.10g) as compared to the other diets. This was followed by the Cox supplemented groups (111.96g), with the control group having the lowest GR of 109.04g. Additionally, the groups of broilers offered diets supplemented with the Blend had higher FCE (5.35) as compared to the Cox (4.93) and control (5.31) supplemented groups (Table 7).

4.3.3 Effects on the Ileal Nutrient Digestibility

The groups of birds fed on diets supplemented with the Blend showed a significantly higher IND of DM (85.213%), CP (89.858%) and CF (67.645%) in comparison to the groups supplemented with the control and Cox. For the birds fed on Cox and Control diets, the IND of DM (78.398% for Cox and 76.393% for Control) and CF (56.438% for Cox and 54.830% for Control) was numerically different but non-significant at $p<0.05$. The CP digestibility was significant for all the diets offered to the experimental animals at $p<0.05$ (Table 7).

4.3.4 Effects on the Dominant Ileal Bacterial Populations

All diets had a significant effect on the proliferation of ileal bacteria studied; that is *Escherichia coli, Clostridium perfringens* and *Lactobacillus* species. The growth of *E. coli* was significantly increased by the Control diet (4.5578 cfu/ml), with Cox dietary treatment showing the least effect (3.1301 cfu/ml). The populations of *C. perfrigens* were significantly increased with feeding Control diet (4.6922 cfu/ml) but were decreased by Cox diet (2.0923 cfu/ml). The Blend had the most significant effect on the growth of *Lactobacillus* bacteria (7.8191 cfu/ml), with the Cox diet having the least effect (2.6667 cfu/ml). This information is summarised in Table 7.

Table 7
Performance of Starter Broiler Chickens Fed on Blend, Cox and Control Diets

Parameter		Treatments			
		Blend	Cox	Control	±SE
Growth	Mean IW	208.00	200.00	201.75	2.49
	Mean FW	2198.75^{a}	2103.25^{b}	2055.50^{c}	1.69
	GR	117.10^{a}	111.96^{b}	109.04^{c}	0.03
	FI	133.08^{a}	117.24^{c}	123.15^{b}	6.71
	FCE	5.35^{a}	4.93^{c}	5.31^{b}	0.01
IND (%)	DM	85.21^{a}	78.40^{b}	76.39^{b}	0.97
	CP	89.86^{a}	78.09^{b}	73.78^{c}	0.98
	CF	67.65^{a}	56.44^{b}	54.83^{b}	1.43
DIB (cfu/ml)					
	E. coli	4.25^{b}	3.13^{c}	4.56^{a}	0.03
	C. perfringens	3.44^{b}	2.09^{c}	4.69^{a}	0.01
Lactobacillus spp.		7.82^{a}	2.67^{c}	5.85^{b}	0.04

Data is represented as means ± SE (standard error of the mean).
Means not sharing a letter are different significantly at 95% confidence interval ($p<.05$).

4.4 Discussion

4.4.1 Proximate Analysis of Experimental Diets

In the current study, the Blend diet had a CP content of 23.55% (as compared to Cox's 19.73% and control's 19.75%) and a metabolizeable energy content of 3300 Kcal/Kg (as compared to Cox's 3185 Kcal/Kg and control's 3176.05 Kcal/Kg) (Table 6). An increase of CP in the Blend was followed by improvement of the content of energy hence the dietary treatment was balanced for the growth of the chicks. Chrystal et al. (2020) conducted an investigation on the impacts of CP and energy density reduction in broiler diets, the researchers concluded that an increase in the dietary CP should be followed with an increase in the dietary energy concentration and vice versa. Too much energy in the diets of broiler chickens have been reported to increase fat pads whilst low dietary CP levels negatively affects the efficiency indicators such as BW gain and GR (Maynard et al., 2022).

Although it has been reported that chickens require a maximum of 5% of crude fibre for proper growth (Ginindza et al., 2022; Tejeda and Kim, 2021), studies have shown that high crude fibre improves the abundance of beneficial microbes in the gut of broilers which improves nutrient digestibility (Mahmood and Guo, 2020). Phytobiotics have been recorded to have a positive influence on the multiplication of beneficial gut bacteria of chickens (Alghirani et al., 2021). Additionally, evidence of the beneficial influence of dietary supplementation of insoluble crude fibre to broilers on the growth of ileal *Lactobacillus* bacteria and the reduction of colonization by bacterial pathogens like *Salmonella* spp. and *Clostridium* exists (Peng et al., 2022; Van Hoeck et al., 2021; Rezaei et al., 2019; Sarbaz et al., 2018). The birds fed on the Blend diet with a 11.87% CF content exhibited better efficiency indicators viz FI, GR and FCE (Table 7). Toghyani et al. (2022) reported similar results while investigating the value of barley as replacement for maize as an energy source. They reported better performance of the groups of birds fed increased levels of barley (and hence high CF), in comparison to those offered the control diet. Nonetheless, investigations by Bornaei et al., (2022), Stokvis et al., 2021 and Onabanjo et al., 2021 have documented decreased efficiency of broilers supplemented with diets high in CF.

4.4.2 Effects on the Growth Rate

Results of the current research indicate that broiler chickens offered feeds with a dietary supplementation of Blend showed significant mean FI as compared to the other dietary treatments. The synergistic effects of the phytonutrients contained in both NLM and CSM was attributed to the high mean FI of the birds. Stinging nettle leaves are described to be high in bioactive molecules mainly flavonoids (Đurović et al., 2020). Whole coriander seeds are reported to be rich mainly in the essential oil linalool (Satyal and Setzer, 2020). These compounds helps to improve the palatability and appetite of animals leading to high feed intake (Abudabos et al., 2018). Earlier research works have described similar findings; Barad et al. (2016) investigated the influences of a mixture of phytobiotics consisting of turmeric powder, black pepper and coriander seeds, on the performance of broiler chickens, they reported an improved feed intake of the groups fed the blend of phytobiotics as growth promoters in comparison to the control diet. Better feed intake of broiler chickens was reported by Kumari et al. (2014) after feeding a mixture of coriander, linseed, neem leaf and sugar beet.

Birds fed on diets supplemented with Blend in their diets showed significantly higher GR at $p < 0.05$ in comparison to the other diets. This was agreeable to a similar experiment by Barad et al. (2016) who stated improved final body weights of broiler chickens fed a blend of phytobiotics as growth promoters in comparison to the unsupplemented groups of birds. Besides, Gilani et al. (2018) carried out an investigation on the natural replacements to antibiotics in starter and finisher broiler chickens using a blend of phytobiotics (Ashwagandha, ginger, black seed, green tea and liquorice). They reported improved performance in terms of lowered blood cholesterol levels and improved immunity than the control group. This led to a high growth rate of the birds.

Additionally, in the current study, dietary supplementation of Blend to the starter broilers was observed to give better GR as compared to the use of in-feed oxytetracycline. Different studies have produced similar results; Talabi et al. (2013) reported non-significant outcomes on the FI, weight gain and FCE of broilers offered a diet supplemented with 0.05g/kg of feed of oxytetracycline. Further, the results of the current research showed that the groups of broilers fed on diets supplemented with in-feed oxytetracycline performed better than those fed control diets in terms of GR. This was in agreement to a study by El-Deek et al., 2012 who augmented oxytetracycline at 0.1 g/kg diet to broilers and described improvements in the GR than the unsupplemented groups.

In the present study, groups of broiler chickens offered diets augmented with the Blend showed better FCE at $p < 0.05$ in comparison to the other dietary treatments. Previous studies had reported comparable observations; Barad et al. (2016) reported an improved FI and FCE of broilers offered a blend of phytobiotics as growth promoters in comparison to those that were offered the control diet without the phytobiotics. Kumari et al. (2014) documented improved FI and FCE of broilers fed a mixture of coriander, linseed, neem leaf and sugar beet. On the contrary, in a study by El-Deek et al. (2012) to determine the suitability of the phytobiotic hot pepper (*Capsicum annum*) as a replacement of oxytetracycline, they reported that the birds fed on the antibiotic supplemented diets showed significant FCE compared to the unsupplemented groups.

4.4.3 Effects on the Ileal Nutrient Digestibility

Ileal digestibility method of determining the utilization of nutrients in broiler chickens is widely used as it involves calculating the difference between the nutrients fed to the animals and those available in the ileum (Atchade et al., 2019). In the present experiment, dietary supplementation of broiler chicken's diet with the Blend of phytobiotics resulted to greater ileal digestibility of DM, CP and crude fibre as in comparison to those fed other dietary treatments (Table 7). This was agreeable to a study conducted by Aljumaah et al., (2020), where high digestibility of nutrients and hence better GR of broiler chickens supplemented with a mixture of phytobiotic feed additives as compared to the group fed with a diet supplemented with avilamycin antibiotic commonly used as a growth promoter was reported. Increased nutrient digestibility results to increased amounts of nutrients available at the small intestine for absorption. Phytobiotics are reported to proliferate the crypt depth and villus height in the ileum resulting to higher nutrient absorption hence better performance in terms of growth rate, BW gains and FCE (Gilani et al., 2021).

The synergistic effects of phytonutrients such as flavonoids, essential oils and tannins found in NLM and CSM supplemented in the Blend is thought to have contributed to increased stimulation of the production of digestive secretions within the gastro intestinal tract leading to increased release and absorption of feed nutrients hence better performance of the chicks fed with the diet (Table 7). There is documented evidence that phytobiotic feed additives results to stimulation of high production of digestive secretions such as enzymes and mucus leading to higher nutrient digestibility and availability (Krauze 2021; Abudabos et al., 2018). Nonetheless, some experiments have documented negative performance of broiler chickens supplemented with mixtures of phytobiotic feed additives; in a recent experiment by Hafeez et al., 2021 to determine the effects of *Carum copticum, Bunium persicum, Coriandrum sativum* and a protease enzyme on apparent digestibility of ileal nutrients of broiler chickens, they reported inconsequential effects ($p < 0.05$) of the phytobiotics on dry matter digestibility.

4.4.4 Effects on the Dominant Ileal Bacterial Populations

Dietary inclusion of blends of phytobiotic for growing of broilers have been recorded to increase the populations of beneficial bacteria while suppressing pathogenic ones thus promoting ileal health (Hussein et al., 2020). This have been shown to have a significant effect in the reduction of disease progression in the ileum (Hussein et al., 2020). Increases in the numbers of ileal *Lactobacillus* bacteria is critical as through competitive exclusion, they suppress the colonization of the ileum by populations of bacterial pathogens such as *Clostridium perfringens*, *Escherichia coli* and *Campylobacter jejuni*; to levels where they are unable to instigate clinical diseases for instance campylobacteriosis (Shang et al., 2018). This helps in protecting the ileal mucosa from damage thereby promoting feed digestion and nutrient absorption and utilization.

Most studies have recorded a positive effect of phytobiotics on the populations of *Lactobacillus* bacteria (da Silveira et al., 2021). In the present experiment, the group of broilers fed diets supplemented with a phytobiotic Blend comprising of NLM at 1.5% dietary inclusion level and CSM at 0.5% dietary inclusion level exhibited positive improvements in the ileal populations of *Lactobacillus* species and reduced the populations of *Escherichia coli* and *Clostridium perfrigens* (Table 7). This was comparable with prior research works that investigated the effects of feeding phytobiotic mixtures and antibiotic growth promotants on the efficiency of production of broilers; Gilani et al. (2021) documented an increase in the species of *Lactobacillus* which are beneficial bacteria, whilst reducing the populations of pathogenic *Escherichia coli*. This was observed to improve the BW gain of the birds. Similarly, a study by Al-Sal (2020) reported an increase in *Lactobacillus* bacteria and a decrease of coliforms including *Escherichia coli* in the ileum of broiler chickens treated with stinging nettle leaf extracts mixed with water and offered orally.

Additionally, a phytobiotic feed mixture comprising of cinnamon oil and citric acid was reported to give better production performance in terms of GR and FCE and decreased the levels of bacterial pathogens as compared to the group of birds supplemented with either of the additives (Krauze et al., 2021). Also, Iwiński et al. (2022) reported the bacterial inhibition potential of a mixture of phytobiotic feed additives consisting of among others linalool, menthol and thymol on *Salmonella* pathogens including *Salmonella typhimurium* and *Salmonella enteritidis*. Further, a study by Adeyemi et al. (2022) showed greater improvements in the populations of ileal *Lactobacillus* bacteria in the group of birds supplemented with phytobiotic feed additive comprising of guava leaf meal as compared to the group fed a diet augmented with 0.5g/kg of oxytetracycline.

The synergistic outcomes of NLM and CSM from the present study is thought to be responsible for the greater performance of the Blend diet due to their pharmacological properties. The young stinging nettle leaves have been published as rich in antibacterial properties owing to the existence of phytonutrients such as alkaloids, flavonoids and tannins (Đurović et al., 2020). They are also rich in the essential oils thymol and carvacrol already documented to have high antibacterial effects (Gholami- Ahangaran et al., 2022). Hence have been found to be effectual against a diversity of bacterial pathogens for example *E. coli* and *Salmonella* spp. (Grauso et al., 2020). Yin et al. (2017) supplemented thymol and carvacrol to broiler chicks and reported increased growth of ileal *Lactobacillus* bacteria and reduced growth of *Clostridium perfringens*. Whole coriander seeds are reported to be rich in essential oils mainly linalool which are responsible for their antibacterial properties (Satyal and Setzer, 2020). Studies by

Kassahun (2020) and Satyal and Setzer. (2020) reported linalool to be effective in cases of pathogens including *E. coli* and *Bacillus subtilis*. A study by Jeya et al. (2019) investigated the antibacterial effect of essential oil of *Coriandrum sativum* L. seeds and reported a high effectiveness against pathogenic microbes such as *Candida albicans, E. coli and Klebsiella pneumonia*.

Outcomes of the present research shows that the growth of *E. coli* and *C. perfrigens* was significantly decreased by feeding Cox diet and to a lesser extent, the Control diet (Table 7). This is because oxytetracycline; a broad spectrum bacteriostatic antibiotic, which act by inhibiting pathogens' cellular protein synthesis, inhibits intestinal inflammation caused by bacteria (Ramachanderan and Schaefer, 2021). This eliminates nutrient competition allowing the birds to have accelerated maturation of the digestive system and consequently, high nutrient absorption which results to high body weight gain and growth (Kairmi et al., 2022). This is the main reason why they are applied by most broiler chicken farmers at sub therapeutic levels for growth promotion. This poses a public health risk as consumption of products (meat) and by-products (bones, offals) contaminated with the drug residues encourages resistance of the bacterial microbes (Monir et al., 2021). Some experiments have described improved production efficiency of broiler chickens supplemented with infeed oxytetracycline; A study by Shah et al. (2022) to examine the merits of oxytetracycline as a growth booster in starter, grower and finisher broiler chickens detailed an increase of GR, FCE and BW gain of group of birds supplemented with the drug as compared to the non supplemented groups. The levels of pathogenic *Coliforms*, *Clostridium* spp. and *Salmonella* spp. were significantly reduced by application of 0.5g/kg of infeed oxytetracycline as reported by Adeyemi et al. (2022).

CHAPTER FIVE
SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The amount of antibiotics used each year in the production of food animals in Kenya is estimated to be about 14.6 tonnes. Most of these are used at subtherapeutic levels for growth promotion purposes. In the growing of broiler chickens, oxytetracyclines are the most widely applied because they highly affordable, effective and easy to apply through water or feed. Haphazard use and failure to adhere to manufacturer's recommended withholding periods have resulted to the tissue deposition of antibiotic residues in meat and bones of the broiler chickens leading to spread of resistant strains of bacteria such as *Escherichia coli* and *Campylobacter jejuni* among broiler flocks and by extension, to the consumers of products (mainly meat, offals and bones) from such birds. The current surge in consumer food safety concerns in Kenya have led to broiler chicken consumers rejecting the conventionally produced table birds in favour of organically produced ones. This have led to increased research interests in natural growth promoters such as phytobiotics as alternatives to antibiotics in the growing of broiler chickens.

Phytobiotics are a group of natural growth promoters derived from herbs, spices or other plants. They are incorporated in livestock feed to enhance productivity through the improvement of feed intake, stimulation of digestive secretions, nutrient digestibility and absorption, among other benefits. The feed additives are also locally available, cheap, easy to process and effective. In the current study, stinging nettle leaf and whole coriander seed meals were used as phytobiotics to replace in-feed oxytetracycline in the determination of the performance of starter broiler chickens in terms of GR, ileal nutrient digestibility and the dominant ileal bacterial populations.

The current study consisted of two experiments; the first experiment aimed to get the level of stinging nettle leaf and whole coriander seed meals with the highest growth rate of starter broiler chickens. Seven diets were prepared as follows: Control (0%), NLM at 1%, 1.5% and 2% and CSM at 0.5%, 1% and 1.5% dietary inclusion levels. From the results of this study, NLM at 1.5% and CSM at 0.5% resulted to the highest GR. For experiment two, the aim was to blend the levels from experiment one with the highest performance in terms of growth rate in order to investigate the effect of blending the two phytobiotics in determining the performance of starter broiler chickens in terms of GR, ileal nutrient digestibility and the dominant ileal bacterial populations. Three diets were utilised; basal diet supplemented with a blend of NLM at 1.5% and CSM at 0.5% inclusion levels (Blend), basal diet supplemented with oxytetracycline hydrochloride powder at the rate of 0.05g/kg of feed (Cox; the positive control) and the Control diet (negative control). From the current study, the application of a blend of NLM at 1.5% dietary inclusion level and CSM at 0.5% dietary inclusion level resulted to improved feed intake, growth rate, feed conversion efficiency, ileal nutrient digestibility of DM, CP and CF; and the populations of beneficial bacteria (*Lactobacillus* species) in the ileum of starter broiler chickens as compared to diets supplemented with oxytetracycline hydrochloride powder at the rate of 0.05g/kg of feed (positive control) and the basal diet (negative control).

5.2 Conclusion

From the results of the research, it can be concluded that: NLM can singly be supplemented in the diets of starter broiler chickens at 1.5% inclusion levels to improve on the FI, GR and FCE. Also, CSM at 0.5% dietary inclusion levels can be singly supplemented in the diets of starter broiler chickens to improve on the FI, GR and FCE. Additionally, supplementing the diets of starter broiler chickens with a phytobiotic blend comprising of NLM at 1.5% dietary inclusion level and CSM at 0.5% dietary inclusion level was reported to be effective in increasing feed intake, growth rate, feed conversion efficiency, ileal nutrient digestibility and the populations of beneficial bacteria (*Lactobacillus* species) in the ileum. In view of these findings, it was concluded that a blend of stinging nettle leaves and whole coriander seed meals can successfully be used as a replacement for infeed growth promoting oxytetracycline to promote productivity and health of starter broiler chickens, therefore, the use of oxytetracyclines as infeed growth promoter in the growing of starter broiler chickens by Kenyan farmers is unwarranted and unnecessary.

5.3 Recommendations

The researcher recommends further study on the following: -

i. The negative effects of either NLM or CSM on nutrient palatability, intake, digestibility and use in starter broiler chickens.
ii. The negative effects of blending NLM and CSM on nutrient palatability, intake, digestibility and use in starter broiler chickens.

5.4 Suggestions for Further Research

i. Evaluation of the organoleptic attributes of broiler chickens fed on either NLM or CSM.
ii. Evaluation of the organoleptic attributes of broiler chickens fed on a blend of NLM and CSM.
iii. The modes of action of phytogenic bioactive compounds.
iv. The effects of the NLM and CSM metabolites on nutrient palatability, intake, digestibility and use in all growing phases of broiler chickens.
v. Economic analysis for blending of NLM and CSM in growing of broiler chickens.

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APPENDICES

Appendix 1. Ethics Review Committee Approval Letter

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18
Direct Line: 0772894438 **Email: info@chuka.ac.ke,** **P. O. Box 109-60400, Chuka**
Website: www.chuka.ac.ke

REF: CUIERC/ NACOSTI/101
TO: MAINA JULIUS KARANJA **02/NOVEMBER/2020**

RE:. **Ileal Bacterial Populations of Broiler Chicks Fed on a Blend of Stinging Nettle Leaf And Coriander Seed Meals as Additives.**

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812.* The approval period is 02/November 2020 -02 November 2021.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used

ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC.*

iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification

iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours

v. Clearance for export of biological specimens must be obtained from relevant institutions.

vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

PROF. ADIEL MAGANA
CHAIRMAN CHUKA UNIVERSITY

Appendix 2. Research License from NACOSTI

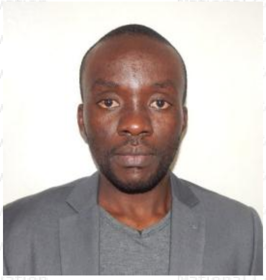
REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **188499**

Date of Issue: **28/October/2021**

RESEARCH LICENSE



This is to Certify that Mr.. Julius Karanja Maina of Chuka University, has been licensed to conduct research in Tharaka-Nithi on the topic: GROWTH, DIGESTIBILITY AND ILEAL BACTERIAL POPULATIONS OF BROILER CHICKS FED ON A BLEND OF STINGING NETTLE LEAF AND CORIANDER SEED MEALS AS ADDITIVES for the period ending : 28/October/2022.

License No: **NACOSTI/P/21/13883**

188499

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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