EVALUATION OF TWO HERBAL PRODUCTS (*FAGARA ZANTHOXYLOIDES* FRUIT MEAL AND *OCIMUM AMERICANUM* LEAF MEAL) AS GROWTH PROMOTERS IN BROILER DIETS

BY

ELVIS AMANOR

(10404632)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF PHILOSOPHY IN ANIMAL SCIENCE DEGREE

NOVEMBER, 2020

DECLARATION

I, ELVIS AMANOR, hereby declare that the work herein, submitted as a thesis for the award of Master of Philosophy in Animal Science degree is entirely my own conducted research. This thesis has not been presented elsewhere for another degree either in part or as a whole. The works of other researchers and authors used in this thesis, which served as sources of information, have duly been acknowledged and referenced.

.... 19/11/2020

ELVIS AMANOR DATE

(STUDENT)

.... 19/11/2020

DR. LEONARD KOFI ADJORLOLO DATE

(PRINCIPAL SUPERVISOR)

.... 19/11/2020

DR. JAMES EDINAM FUTSE DATE

(CO-SUPERVISOR)

ABSTRACT

This study evaluated *Fagara zanthoxyloides* fruit meal (FFM) and *Ocimum americanum* leaf meal (OLM) as growth promoters in broiler diets as replacements for antibiotics. A total of 400 one-day-old Cobb 500 broiler chicks were used for the experiment. The birds were initially raised together on a common starter diet for a week and on day 8, distributed into eight dietary treatment groups in a completely randomized design for six more weeks. The treatment diets were as follows: BD = Basal diet; 0.2FFM = BD + 0.2% FFM; 0.4FFM = BD + 0.4% FFM; 0.2OLM = BD + 0.2% OLM; 0.4OLM = BD + 0.4% OLM; 0.1FFM+0.1OLM = BD + 0.1% FFM + 0.1% OLM; 0.2FFM+0.2OLM = BD + 0.2% FFM + 0.2% OLM; PEN = BD + 0.01% Penicillin V. Each treatment was replicated five times with 10 birds in each replicate. Feed and water were provided *ad libitum*. The parameters measured include growth performance, carcass characteristics, apparent whole tract nutrient digestibility, nitrogen excretion, serum lipid profile, and faecal microbial count. Data collected were all subjected to analysis of variance using Genstat statistical software (12^{th} edition, 2009), and means with significant differences were separated with Student Newman-Keuls test at a probability of 5%. The results show no significant effects ($p>0.05$) of FFM, OLM, and penicillin on growth performance, carcass characteristics, digestibility of dry matter, crude protein, ash and crude fibre, nitrogen excretion, serum lipid profile, and counts of faecal pathogenic microbes when compared with birds fed BD. Fat digestibility and faecal microbial load were lowered ($p<0.05$) by FFM, OLM, and penicillin. Birds fed PEN recorded the least faecal count of yeasts and moulds similar ($p>0.05$) to that fed 0.4FFM and 0.4OLM. In conclusion, inclusion of FFM and OLM alone or in combination up to 0.4% in diets of broilers did not promote growth performance.

DEDICATION

This work is primarily dedicated to the Almighty God without whose help I could not have come this far, my parents (Mr. Eric Kwao Amanor and Mrs. Sarah Amanor), siblings (Chris Tetteh Kwao Amanor and Marilyn Adamki Amanor), Mr. Gideon Afolayan who encouraged me continually that God will help me to do my project when it looked like all hope was lost and finally, to my very good friend Mr. Daniel Bentum whose contribution towards the success of this work was so wonderful and much appreciated.

ACKNOWLEDGEMENT

My first and foremost gratitude goes to my heavenly Father, The Almighty God for His rich grace and love towards me. He has been my backbone throughout my graduate studies. When the going became tough and I felt like giving up, He strengthened and encouraged me, and here I stand today at the end of the tunnel by His grace. To Him be all the glory, praise, honour, and adoration forever.

My next appreciation goes to Dr. Leonard Kofi Adjorlolo, my principal supervisor, and Dr. James Edinam Futse, co-supervisor for their supervision, guidance, counsel, corrections, and instructions throughout the project and compilation of this thesis.

I am also indebted to the Livestock and Poultry Research Centre (LIPREC) for providing the research facility, broiler chicks, feed, medication, and water for the project, the Leventis Foundation Scholarship Fellowship for funding the project, and the senior members of the Animal Science Department, University of Ghana for their tutoring, training and contribution towards this work.

I also appreciate the wonderful family that God has blessed me with and the efforts of my parents in setting me up on the academic ladder and for their unflinching support throughout my education.

I also give God praise for making me meet the late Dr. Thomas Nii Narku Nortey, my former supervisor. Only God will understand how the training I received from him means to me. I learnt so much from him. May His soul rest in perfect peace.

Finally, my special gratitude to the following individuals and all who contributed in diverse ways to make this work a success; Prof. Boniface Kayang, Dr. Francis Dogodzi, Mr. Yussif Abdulai, Mr. Gilbert Gbafah, Mr. Christopher Tudeka, Mr. Gideon Afolayan, Mr. Daniel Bentum, Mr. Jonathan Quaye, Mr. Titus Kali, Mr. Solomon Boadu, Mr. Protase Yuorkuu, Mr. Matthew Nyovore, Mr. Benjamin Osei, Mr. Mohammed Bashiru, Mr. Amos Nyarko, Mr. Robert Ntreh, Mrs. Ruth Yeboah, and Mrs. Princess Anane.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

A. galli	*Ascaridia galli*
ADFI	Average Daily Feed Intake
ADG	Average Daily weight Gain
AGP	Antibiotic Growth Promoter
ALT	Alanine Aminotransferase
AMA	American Medical Association
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ASM	American Society for Microbiology
AST	Aspartate Aminotransferase
BD	Basal Diet
BMD	Bacitracin Methylene Disalicylate
BW	Body Weight
BWG	Body Weight Gain
CF	Crude Fibre
CFU	Colony-Forming Units

CP	Crude Protein
CRD	Completely Randomised Design
DCP	Dicalcium Phosphate
DM	Dry Matter
E. coli	*Escherichia Coli*
EE	Ether Extract
EO	Essential Oil
FAO	Food and Agricultural Organisation
FBW	Finishing/ Final Body Weight
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
FFM	*Fagara zanthoxyloides* Fruit Meal
FI	Feed Intake
GH	Growth Hormone
GPX	Glutathione Peroxidase
H: L	Heterophils to Lymphocytes
Hb	Haemoglobin
HDL	High-Density Lipoprotein

IBD	Infectious Bursal Disease
LDL	Low-Density Lipoprotein
LIPREC	Livestock and Poultry Research Centre
LW	Live Weight
MDA	Malondialdehyde
ME	Metabolisable Energy
MOLM	*Moringa oleifera* Leaf Meal
N	Nitrogen
NaCl	Sodium Chloride
ND	Newcastle Disease
OAB	Organic Acid Blend
OLM	*Ocimum americanum* Leaf Meal
PCV	Packed Cell Volume
PEN	Penicillin
PFA	Phytogenic Feed Additive
pH	power of Hydrogen
ppm	parts per million
PUFA	Polyunsaturated Fatty Acid

RBC	Red Blood Cell
SEM	Standard Error of Mean
SFA	Saturated Fatty Acid
SNK	Student Newman-Keuls
SOD	Superoxide Dismutase
SS	*Salmonella* and *Shigella*
T3	Tri-iodothyronine
T4	Thyroxine
VLDL	Very-Low-Density Lipoprotein
w.	weight
WBC	White Blood Cell
WHO	World Health Organisation
WP	Whole Plant
Y and M	Yeasts and Moulds

CHAPTER ONE

INTRODUCTION

Antibiotics have been used since the 1950s in meat poultry production for growth promotion (Thomke and Elwinger, 1998). They were incorporated into diets at sub-therapeutic levels primarily to enhance feed conversion efficiency and concurrently, decrease the population of pathogenic gut bacteria, fight infections, and reduce mortalities (Thomke and Elwinger, 1998; Gauer, 2004; Daramola, 2019). These functions allowed farmers to save on the production cost of chicken. Antibiotics, therefore, contributed significantly to the advancement and prosperity of the poultry industry (Castanon, 2007).

Nevertheless, over a decade ago, antibiotics were completely barred from being used for growth promotion by the European Union due to notable adverse effects on animals as well as humans (Dibner and Richards, 2005; Castanon, 2007). Antibiotics destroyed both harmful and beneficial gut microbes (Hernandez *et al.*, 2004; Guban *et al.*, 2006; Henry Ford, 2020). There were also major concerns about residual effects on the consumption of animal food products. Moreover, researchers reported increasing cases of continuous emergence and spread of resistant bacteria strains. The transfer of such germplasm to humans (cross-resistance) increases the risk of multiple drug resistance in humans who consume meat products of antibiotic-fed animals (Klotins, 2005; Owens *et al.*, 2008).

The exclusion of antibiotics from feeds however resulted in declines in growth performance, as well as increases in bacterial infections, disease severity, mortalities, and production cost (Castanon, 2007; Allen *et al.*, 2013; Zaunschirm, 2018). As a result, scientists began to diligently search for feed additives that can replace antibiotics. This led to the discovery of antimicrobial peptides, bacteriophages, herbal products, hyper-immune egg yolk antibodies, exogenous enzymes, metals, organic acids, prebiotics, probiotics, and synbiotics (Huyghebaert *et al.*, 2011; Upadhayay and Vishwa, 2014; Gadde *et al.*, 2017).

Of these alternatives to AGPs, herbal feed additives appear suitable because they are widely available, cheap and easy to obtain, eco-friendly, safe to use, free from toxins, can easily be planted, harvested and used, and have no residual effects on meat and eggs (Windisch *et al.*, 2008; Wan *et al.*, 2017; Vinus *et al.*, 2018). Numerous herbs and their bioactive components have been reported to have a broad range of antimicrobial activity (Dorman and Deans, 2000; Tucker, 2002). Tucker (2002) indicated that herbs, due to their antimicrobial properties, can replace antibiotic growth promoters. Not only do herbs and their extracts inhibit the activity of pathogenic gut bacteria and promote the growth of beneficial ones (Gill, 1999; Wenk, 2000), they also adapt chicken to environmental stress and improve feed intake, nutrient digestibility, and overall performance (Denli and Demirel, 2018; Vinus *et al.*, 2018).

Fagara zanthoxyloides and *Ocimum americanum* are aromatic herbal plants that are widespread in the tropics, especially in Ghana. They have diverse medicinal properties and are commonly used as condiments (Ngassoum *et al.*, 2004; Rady and Nazif, 2005; Guendéhou *et al.*, 2018). Extracts and metabolites from these plants have antimicrobial, anthelmintic, antioxidative, and anti-inflammatory properties (Adesina, 2005; Nascimento *et al.*, 2011; Adefisoye *et al.*, 2012, Azando *et al.*, 2017; Gberikon *et al.*, 2018). There are however no documented reports on the use of these herbs as additives in poultry feeds, hence this study was designed to evaluate the potential of the fruits of *Fagara zanthoxyloides* and leaves of *Ocimum americanum* as growth promoters in broiler diets as replacements for antibiotics.

If *Fagara zanthoxyloides* fruit meal (FFM) and *Ocimum americanum* leaf meal (OLM) can be shown to promote broiler growth and reduce loads of pathogenic gut microbes and mortalities, they will become suitable feed additives for local poultry farmers. This will save production costs and preclude the negative effects of antibiotics withdrawal from diets on birds.

1.1 Hypothesis

Supplementing diets of broiler chickens with *Fagara zanthoxyloides* fruit meal (FFM) or *Ocimum americanum* leaf meal (OLM) will promote growth performance and reduce loads of pathogenic gut microbes similar to conventional antibiotics.

1.2 Objective of the Study

To assess the growth-promoting effects and antimicrobial efficacy of FFM and OLM in broiler diets as potential alternatives to antibiotic growth promoters.

1.2.1 Specific objectives

To evaluate FFM and OLM in broiler diets as growth promoters and antimicrobials in comparison with penicillin on;

1. Growth performance
2. Carcass characteristics
3. Apparent whole tract nutrient digestibility
4. Nitrogen excretion
5. Serum lipid profile
6. Faecal microbial count

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Growth Promoters in Animal Diets

Growth promoters are substances, other than dietary nutrients, that are supplemented into nutritionally balanced feeds to increase the growth rate and feed conversion efficiency of farm animals (Upadhayay and Vishwa, 2014). Growth promoters increase nitrogen retention and utilization to form amino acids for growth enhancement (Wierup, 2001) and may improve the quality of animal products (Al-Dobaib and Mousa, 2009; Herago and Agonafir, 2017). The use of growth promoters is necessary because the traditional methods of raising farm animals allow little opportunity for rapid increases in feed conversion efficiency, weight gain, and profitability (Beermann, 1995). Inclusion of growth promoters in diets helps to produce food animals at cheaper costs to meet the ever-growing demand for animal products (Herago and Agonafir, 2017).

2.2 Antibiotics and their Benefits in Poultry Production

Antibiotics are chemical products manufactured synthetically or obtained from some strains of microorganisms to inhibit the growth or cause the death of other microbes (Geidam *et al.*, 2009; Mokhtari *et al.*, 2015). They are among the most commonly-used veterinary drugs in poultry husbandry (Lawal *et al.*, 2015). Antibiotics are used by veterinarians and farmers to treat poultry diseases (particularly, bacterial infections), improve growth performance, feed utilization, health status and egg production (Donoghue, 2003; Chattopadhyay, 2014; Sahu and Saxena, 2014), and reduce mortalities (Schjørring and Krogfelt, 2011).

Some examples of antibiotics that are commonly used in the poultry industry are presented in Table 2.1. However, most antibiotic products available on the market for poultry use are combinations of two or more of these antibiotics thus, making them extra potent, whilst other antibiotics are mixed with multivitamins (Lawal *et al.*, 2015).

Table 2. 1: Commonly-used antibiotics in poultry husbandry

No.	**Antibiotic**	**Reference**
1.	Avilamycin	(Eslami *et al.*, 2010)
2.	BMD	(Guban *et al.*, 2006)
3.	Bambermycin	(Diarra *et al.*, 2007)
4.	Chlortetracycline	(Proudfoot *et al.*, 1988)
5.	Colistin	(Jang *et al.*, 2007)
6.	Doxycycline	(Kana *et al.*, 2017)
7.	Flavomycin	(Attia *et al.*, 2011)
8.	Lincomycin	Proudfoot *et al.*, 1990)
9.	Monensin	(Guban *et al.*, 2006)
10.	Oxytetracycline	(Alhendi *et al.*, 2000)
11.	Penicillin	(Onu *et al.*, 2004)
13.	Salinomycin	(Diarra *et al.*, 2007)
14.	Streptomycin	(Onifade, 1997)
15.	Tylosin	(Onifade, 1997)
16.	Virginiamycin	(Baurhoo *et al.*, 2009)

BMD = Bacitracin methylene disalicylate

2.2.1 Uses of antibiotics in poultry husbandry

In poultry rearing, antibiotics are used for disease treatment (therapy), prophylaxis, and growth promotion.

Prophylactic use of antibiotics involves the use of antibiotics to prevent diseases. With this, birds are given sub-therapeutic doses of the drug either through feed or drinking water when signs and symptoms of infections are absent but suspected. This practice serves as a surety to prevent birds from coming down with diseases (Castanon, 2007; Nisha, 2008; Sahu and Saxena, 2014).

For growth promotion, antibiotics are administered in very low doses on regular basis to birds through feed, often over a lifetime, to increase growth rate and productivity. This practice is distinct from the use of antibiotics for therapy and prophylaxis which involve higher doses of the drugs administered generally through drinking water (Castanon, 2007; Geidam *et al.,* 2009; Sahu and Saxena, 2014). The growth-promoting effect of antibiotics was discovered in the United States in the 1940s when birds fed dried *Streptomyces aureofaciens* broth containing chlortetracycline residues grew at a faster rate (Moore *et al.*, 1946; Al-Dobaib and Mousa, 2009). It was realized that the antibiotic residues in the broth were responsible for the improvement in growth (Sahu and Saxena, 2014). It subsequently became a common practice to supplement poultry diets with antibiotics at sub-therapeutic doses for growth enhancement (Cook, 2004; Sahu and Saxena, 2014).

Antibiotics used as growth promoters (AGPs) increase feed utilization by 2 to 5% (Ewing and Cole, 1994) and growth rate by 1 to 10% (Chattopadhyay, 2014). They also promote uniform growth of farm animals and hence, reduce variations in carcass sizes at processing units (Robertsson and Lundeheim, 1994). Also, the quality of meat from animals fed diets containing AGPs is superior (with less fat and high protein content) to those fed diets without AGPs (Hughes and Heritage, 2002; Donoghue, 2003). AGPs also increase egg production and hatchability (Gustafson and Bowen, 1997). Moreover, some antibiotics, especially macrolides, have anti-inflammatory properties. They impede the production of pro-inflammatory cytokines from immune cells that cause anorexia and muscle catabolism (Buret, 2010; Chattopadhyay, 2014). This anti-inflammatory action of AGPs minimises energy wastage and instead, channels dietary energy for production (Niewold, 2007). Additionally, AGPs increase nutrient absorption and the production of growth factors (Prescott and Baggot, 1993).

Despite the numerous reported benefits, some researchers observed no effects of AGPs on poultry growth performance (Proudfoot *et al.*, 1990; Baurhoo *et al.*, 2009; Riyazi *et al.*, 2015). This is attributable to factors such as the health status of birds, rearing conditions, management practices, and composition of diets (Klotins, 2005; Sarica *et al.*, 2005). The growth-promoting effects of antibiotics are more noticeable when these factors are suboptimal (Sahu and Saxena, 2014; Biswas *et al.*, 2017). Consequently, when conditions of rearing environments are improved with infection control measures introduced, and overcrowding reduced, there will be no need for AGPs (Prescott and Baggot, 1993). Similarly, Anderson *et al.* (1999) indicated that animals that are well-nourished and reared in clean environments and at moderate stocking densities do not respond positively to AGPs. The effects of AGPs on broiler growth performance and other parameters observed in some studies are presented in Table 2.2.

Table 2. 2: Effects of antibiotic growth promoters (AGPs) in broiler chickens

No.	AGP	Effect(s)	References
1.	Monensin, BMD only or in combination (0.50g/kg)	• BMD: Increases in FI, BWG, FCE, and conjugated bile salt concentration • Reduction in population of *Lactobacillus salivarius* • Monensin: Increase in fat digestibility	(Guban *et al.*, 2006)
2.	Neomycin-oxytetracycline, procaine penicillin, streptomycin, tylosin (150ppm each)	• Increases in BWG, FBW, and carcass parameters • Improvement of FCR • Increase in FI except for procaine penicillin	(Onifade, 1997)
3.	Penicillin (50, 100, or 150ppm)	• Increases in FI, BWG, and FCE	(Onu *et al.*, 2004)
4.	Lincomycin (2.2ppm)	• No effects on FBW, FCR, mortality, and monetary indices	(Proudfoot *et al.*, 1990)
5.	Chlortetracycline (5.5mg/kg)	• No effects on BW and FCE	(Proudfoot *et al.*, 1988)
6.	Avilamycin (150ppm)	• No effects on FI, FCR, BWG, serum lipid metabolites, and carcass characteristics	(Riyazi *et al.* 2015)
7.	Avilamycin (10mg/kg)	• Increase in BW on days 21 and 42 • No effects on FI, FCR, and liveability • No effect on slaughter characteristics	(Bozkurt *et al.*, 2008)
8.	Avilamycin (150g/ton)	• No effects on concentrations of serum lipid metabolites.	(Eslami *et al.*, 2010)
9.	Bambermycin (2ppm), bacitracin (55ppm), penicillin (2.2ppm), salinomycin (60ppm), [Bacitracin (55ppm) + salinomycin (60ppm)]	• No effect on growth performance except for penicillin that caused an increase in FCE • No effects on intestinal, caecal, and litter bacterial counts	(Diarra *et al.*, 2007)

10.	Virginiamycin (16.5ppm), bacitracin (55ppm)	• No effects on growth and carcass characteristics • No effects on caecal counts of *E. coli* and *Campylobacter* (d 14 and 24) • No effect on caecal count of *Lactobacillus* (d 14, 24 and 34) • Reduction in caecal count of *Bifidobacteria* (d 14 and 24)	(Baurhoo *et al.*, 2009)

AGP = antibiotic growth promoter; BWG = body weight gain; BW = body weight; BMD = Bacitracin methylene disalicylate; FBW = finishing body weight; FCE = feed conversion efficiency; FI = feed intake; FCR = feed conversion ratio

2.2.2 Modes of action of antibiotic growth promoters (AGPs)

The results of several research works show that AGPs mediate growth through their antibacterial effects and hence, do not promote growth in germ-free animals (Feighner and Dashkevicz, 1987; Butaye *et al.*, 2003). AGPs promote growth by reducing intestinal use of nutrients by pathogenic gut microbes (Snyder and Wostmann, 1987), exhibiting antimicrobial activity on enteropathogens, inhibiting the incidence of subclinical infections (Brennan *et al.*, 2003; Humphrey and Klasing, 2004), minimising the production of metabolites that depress growth from Gram-positive bacteria (Knarreborg *et al.*, 2004), and thinning the wall of the small intestine to increase nutrient absorption and utilization (Feighner and Dashkevicz, 1987; Butaye *et al.*, 2003).

2.2.3 Adverse effects of AGPs on bird and human health

Despite the well-demonstrated advantages of AGPs, their use is associated with some deleterious effects in both poultry and humans. For instance, AGPs, in addition to destroying pathogenic gut microbes and reducing their populations, also do that to the beneficial ones (Engberg *et al.*, 2000; Knarreborg *et al.*, 2002; Hernandez *et al.*, 2004). For instance, Guban *et al.* (2006) fed broilers diets containing bacitracin methylene disalicylate and monensin alone or in combination and observed lower ileal count of *Lactobacillus salivarius.* According to Henry Ford (2020), antibiotics do not differentiate between beneficial and pathogenic bacteria and as such, may destroy beneficial bacteria in the gut.

Furthermore, indiscriminate use of AGPs results in the occurrence of drug residues in meat and eggs as well as the emergence and spread of antibiotic-resistant bacteria in birds (Monroe and Polk, 2000). These antibiotic-resistant bacteria strains can be transmitted to humans through food, environment, or direct contact with contaminated meat (Monroe and Polk, 2000). Due to these harmful effects, AGPs have been prohibited in several countries such as Sweden (Cogliani *et al.,* 2011), the United Kingdom, Denmark, Netherlands, and other European Union countries (Dibner and Richards, 2005; Castanon, 2007). Likewise, organizations such as American Public Health Association (APHA), American Medical Association (AMA), and American Society for Microbiology (ASM) have called for restrictions on antibiotic use in animal husbandry and a termination of all non-therapeutic uses of these drugs (Hashemi and Davoodi, 2011).

2.2.3.1 Effects of consuming poultry products containing antibiotic residues on human health

The continuous and abusive use of antibiotics results in the incidence of harmful concentrations of antibiotic residues in edible poultry products (Donoghue, 2003; Shareef *et al.,* 2009) which may persist in these foods several days even after cooking (Dipeolu, 2004). The consumption of meat and eggs containing antibiotic residues causes gastrointestinal syndromes (Jing *et al.,* 2009), carcinogenicity, immune-pathological effects, mutagenicity, loss of hearing, nephropathy, hepatotoxicity (Nisha, 2008; Prajwal *et al.,* 2017), imbalance of intestinal microflora (Olatoye and Ehinmowo, 2010), anaphylactic reactions, bone marrow toxicity, and reproductive disorders (Doyle, 2006; Shareef *et al.,* 2009).

2.3 Alternatives to Antibiotic Growth Promoters (AGPs) in Poultry Diets

The withdrawal of AGPs from poultry diets resulted in increases in bacterial infections, disease severity, mortalities, and reduction in profitability of poultry enterprise (Castanon, 2007; Allen *et al.*, 2013). Zaunschirm (2018) reported that antibiotic exclusion from diets will create a wide performance gap since sufficient amounts of energy will be channelled away from growth into fighting pathogens. These effects and the increasing awareness of the public about the dangers of AGPs on their health coupled with their quest for meat and eggs free from antibiotic residues encouraged scientists to investigate other products that can replace antibiotics in diets (Gadde *et al.*, 2017).

This led to the focus on feed additives such as antimicrobial peptides, bacteriophages, clay, exogenous enzymes, hyper-immune egg yolk antibodies, metals, organic acids, phytogenic products, prebiotics, probiotics, and synbiotics (Huyghebaert *et al.*, 2011; Upadhayay and Vishwa, 2014; Gadde *et al.*, 2017). These products were to enhance growth performance like AGPs but pose no harm to birds and consumers of poultry products (Huyghebaert *et al.*, 2011). The discovery of these products was to an extent guided by the apprehension of the modes of action of AGPs (Gadde *et al.*, 2017). The following section provides an overview of some of the alternatives to AGPs in poultry nutrition with details on their efficacies and mechanisms of action.

2.3.1 Probiotics

Probiotics, also called direct-fed microbial supplements, are live microbial organisms that are incorporated into feeds to suppress the growth of harmful microorganisms in the digestive tract and thus, improve the intestinal microbial balance of the animal (AFRC, 1989; Mokhtari *et al.*, 2015). They are administered through drinking water or feed alone or in combination with other additives (Thomke and Elwinger, 1998). Probiotics improve feed intake, enzyme secretion, nutrient digestion and absorption, and health status of farm animals (Wang and Gu, 2010; Ciorba, 2012). Probiotics administered to birds have no negative human health implications.

Different species of beneficial bacteria such as Bifidobacterium, Bacillus, Enterococcus, Lactococcus, Lactobacillus, and Streptococcus and sometimes yeast (Saccharomyces) have been used as probiotics in poultry nutrition (Simon *et al.*, 2001; Kabir, 2009; Gadde *et al.*, 2017). Numerous studies have explored and validated the potency of probiotics to improve growth performance of birds. For example, when broiler diets were supplemented with single species of Lactobacillus such as *L. bulgaricus, L. casei, L. reuteri,* and *L. fermentum,* live weight and feed conversion efficiency increased (Yeo and Kim, 1997; Apata, 2008). Similar outcomes were also observed when broiler rations were supplemented with multiple Lactobacillus strains (Jin *et al.*, 1998; Mookiah *et al.*, 2014). Furthermore, probiotics prepared with Bacillus species such as *B. licheniformis* and *B. subtilis* equally enhanced growth performance (Lee *et al.*, 2011a; Liu *et al.*, 2012). Likewise, inclusion of *Rhodopseudomonas palustris* (Xu *et al.*, 2014) and *Enterococcus faecium* (Kabir *et al.*, 2004) in diets significantly increased feed utilization and average daily weight gain of broilers. Karimi Torshizi *et al.* (2010) supplemented diet or drinking water of broilers with a probiotic mixture made up *Aspergillus oryzae, Bifidobacterium bifidum, Candida pintolopesii, Enterococcus faecium, L. acidophilus, L. bulgaricus, L. plantarum, L. rhamnosus,* and *Streptococcus thermophilus* and observed improvements in body weight gain and feed intake.

Blajman *et al.* (2014) performed a meta-analysis of some research trials conducted between 1980 and 2012 to examine the effects of probiotic supplementation on broiler growth performance. These authors reported that incorporating probiotics into rations improved feed conversion ratio and weight gain. Their study also showed that administering probiotics through drinking water is more effective than through feed. Additionally, Blajman *et al.* (2014) found no significant differences between the effects of single and multiple strain probiotics on broiler growth performance. Probiotic supplementation also improved laying performance and egg sizes of laying chickens (Lei *et al.*, 2013). Also in turkeys, dietary supplementation with probiotics increased daily weight gain and market body weight (Torres-Rodriguez *et al.*, 2007). However, Karaoglu and Durdag (2005) observed no impact of a dietary probiotic (*Saccharomyces cerevisiae*) on broiler growth and slaughter variables. This may be due to the strain and dosage of the probiotic used, composition of diet, and environmental factors (Gadde *et al.*, 2017).

Furthermore, probiotics produce lactic and short-chain fatty acids that lower the gut pH and make the gut microenvironment hostile to pathogenic bacteria. Also, when probiotic bacteria proliferate in the gut, they competitively exclude the pathogenic ones and compete with them for nutrients. Furthermore, some strains of probiotic bacteria (e.g. *Bacillus* and *Lactobacillus spp.*) synthesize and release antibacterial substances like bacteriocins that destroy pathogenic bacteria in the gut (Brown, 2011). The bacteria strains that produce bacteriocins have specific immunity to these peptides and hence, are not destroyed by the bacteriocins (Klaenhammer, 1993).

2.3.2 Prebiotics

Prebiotics are indigestible complex carbohydrates that selectively enhance the activity and/or growth of beneficial gut bacteria (Patterson and Burkholder, 2003). Some examples of oligosaccharides and non-starch polysaccharides that have been used as prebiotics in poultry diets are fructooligosaccharides, galactooligosaccharides, glucooligosaccharides, inulin, isomaltooligosaccharides, lactitol, lactulose, maltooligosaccharides, mannan oligosaccharides, oligofructose, pyrodextrins, soya-oligosaccharides, and xylooligosaccharides (Patterson and Burkholder, 2003; Steiner, 2006).

For instance, dietary supplementation with mannan oligosaccharides improved ($p<0.05$) feed conversion ratio in broilers (Mohammed *et al.*, 2008). Also, the inclusion of lactulose in broiler diets increased live weight, feed conversion efficiency, *Lactobacillus* population and concentrations of acetate, butyrate, and propionate in the caecum, goblet cell numbers, and intestinal villi heights (Calik and Ergün, 2015). In another study, isomaltooligosaccharide in broiler ration improved feed conversion ratio and body weight gain (Mookiah *et al.*, 2014). Hooge and Connolly (2011), concluded from a meta-analysis of broiler feeding trials, that prebiotics increase body weights by 5.41% and improve feed conversion ratio by 2.54%.

Furthermore, prebiotics stimulate the growth of beneficial gut microorganisms and their production of lactic acid and bacteriocins (Spring *et al.*, 2000). In addition to their effects on probiotics, some prebiotics directly inhibit the growth of certain pathogenic bacteria and hinder them from colonising the gut. For instance, mannan oligosaccharides bind to the type 1 fimbriae of enteropathogens and obstruct them from adhering to enteric epithelial cells (Spring *et al.*, 2000).

2.3.3 Synbiotics

Synbiotics are feed supplements that contain mixtures of prebiotics and probiotics such that both constituents act synergistically to improve animal performance (Patterson and Burkholder, 2003). The development of synbiotics was centred on the perception that a blend of prebiotics and probiotics will both implant beneficial microbes in the gut and stimulate their growth (Gibson and Roberfroid, 1995). Several feeding trials were carried out to investigate the efficacy of synbiotics to improve broiler growth performance. For example, Awad *et al.* (2009) observed that broilers fed a diet containing a synbiotic had significantly higher live weight, weight gain, feed conversion efficiency, and dressing percentage compared to the control group. Mohnl *et al.* (2007) also found that supplementing broiler diet with a symbiotic increased body weight by 2.04% and decreased mortality by 0.9%. Also, Mookiah *et al.* (2014) fed broilers a diet admixed with a symbiotic made up of an inulooligosaccharide and a probiotic mixture containing 11 *Lactobacillus sp.* These researchers observed significant improvements in feed conversion ratio and weight gain (Mookiah *et al.*, 2014). The synbiotic however did not cause a two-fold improvement effect on performance in comparison with birds that fed either a probiotic or prebiotic supplemented diet (Mookiah *et al.*, 2014). Likewise, feeding pullets a diet containing a combination of probiotics and carbohydrates derived from yeast increased body weight gain (Yitbarek *et al.*, 2015). Despite these reported benefits, Jung *et al.* (2008) supplemented broiler diet with a symbiotic and observed no effects on growth performance which may be due to the inclusion rate of the symbiotic, health status of birds, condition of rearing environment, and dietary factors or composition. The positive effects of synbiotics on poultry growth performance could be due to improvement in digestion and elimination of subclinical infections. The increase in the population of beneficial gut bacteria caused by symbiotics will cause an improvement in digestion and a competitive exclusion of pathogenic bacteria from the gut thus, eliminating the incidence of subclinical infections. These functions will allow birds to grow to their full genetic potential.

2.3.4 Organic acids

Dietary supplementation with organic acids (feed acidifiers) such as citric acid (Haque *et al.*, 2010), fumaric acid (Banday *et al.*, 2015), butyric acid, lactic acid (Adil *et al.*, 2010), and formic acid (Hernandez *et al.*, 2006) has been found to promote broiler growth performance. For instance, Banday *et al.* (2015) supplemented broiler diet with fumaric acid and observed increases in feed conversion efficiency and weight gain. Also, Adil *et al.* (2010) reported similar growth improvement effects of dietary supplementation with butyric or lactic acid in broilers. Samanta *et al.* (2010) tested the efficiency of an organic acid blend (OAB) composed of calcium propionate, formic acid, orthophosphoric acid, and propionic acid as growth promoter in broiler diet. These researchers observed increases in feed efficiency and protein accretion among birds that fed the OAB-supplemented diet.

Organic acids are administered either as organic acids, their salts or as a blend of several organic acids or their salts (Huyghebaert *et al.*, 2011). Gadde *et al.* (2017) reported that using a blend of organic acids is more effective and gives better results than using a single organic acid. Organic acids lower the gut pH and make the gut microenvironment unfavourable for pathogenic microbes but allow acid-tolerant beneficial ones like Lactobacilli to thrive. This reduces the load of pathogenic gut microbes and the competition between the beneficial and pathogenic microbes for nutrients (Boroojeni *et al.*, 2014). Organic acids also penetrate cell wall of pathogenic microbes and cause their death (Gadde *et al.*, 2017). Furthermore, organic acids increase protein retention, dry matter digestibility, and absorption and utilization of minerals (Nezhad *et al.*, 2011). Gadde *et al.* (2017) reported that some organic acids provide energy for the growth of enteric epithelial cells.

2.3.5 Exogenous enzymes

Exogenous or in-feed enzymes are protein supplements which when incorporated into feeds bind to feed particles (substrates) and accelerate their breakdown into minute particles for digestion and absorption (Thacker, 2013). Exogenous enzymes perform similar roles as endogenous ones and complement them to improve digestion (Choct, 2006). Some commonly-used exogenous enzymes in poultry nutrition are proteases, phytases, and carbohydrases (e.g. α-galactosidase, α-amylase, β-mannanase, cellulase, pectinase, and xylanase) (Gadde *et al.*, 2017). Exogenous enzymes increase digestibility of nutrients like phytate that are otherwise not digested by endogenous enzymes, eliminate the coating effect of plant cell wall polysaccharides on nutrients to increase nutrient availability, degrade anti-nutritive compounds, reduce digesta viscosity, and increase the solubility and caecal fermentation of non-starch polysaccharides (Choct, 2006; Kiarie *et al.*, 2013).

The growth-promoting effects of several types of in-feed enzymes have been reported in poultry (Choct, 2006). Also, several meta-analyses validate the growth-promoting effects of in-feed enzymes. For example, from a meta-analysis, Hooge *et al.* (2010) found that dietary supplementation with a multi-enzyme complex containing phytase and non-starch polysaccharide enzymes improved feed conversion ratio and finishing body weights of broilers by 2.64% and 3.73% respectively. From another meta-analysis, it was shown that supplementing male broiler diets with β-mannanase improved feed conversion ratio and weight gain by 4.8 points and 4.2% respectively (Jackson and Hanford, 2014). However, Nortey *et al.* (2015) supplemented broiler diets containing cocoa pod husk with phytase, an enzyme cocktail or both. The enzyme cocktail contained amylase, β-glucanase, cellulose, pectinase, phytase, protease, and xylanase. These scientists observed no effects of enzyme supplementation on broiler growth performance.

The effects of enzyme supplementation on poultry performance is affected by genetic variation among animals, enzyme type, inclusion rate, and composition of diets (Cheng *et al.*, 2014).

2.3.6 Egg yolk antibodies

Egg yolk antibodies are equally used as alternatives to antibiotic growth promoters. They are antibodies that are transferred from hens to chicks through egg yolks (Schade *et al.* 2005). With this, laying hens are inoculated with antigens of interest. The immune systems of the birds respond by producing antibodies that are transported to the yolks. The yolks are then separated from the albumens, and the antibodies are extricated from them, purified, and used as feed supplements (Yegani and Korver, 2010).

Cook (2004) reported that dietary supplementation with egg yolk antibodies improves poultry growth. Likewise, Mahdavi *et al.* (2010) observed that oral administration of an egg yolk antibody to broilers improved feed conversion efficiency and intestinal health. Furthermore, Tamilzarasan *et al.* (2009) found that some egg yolk antibodies have antibacterial effects on *Escherichia coli,* and *Campylobacter, Clostridium,* and *Salmonella spp*. Egg yolk antibodies bind to bacterial cell structures like the lipoglycans, pili, and flagella, and prevent bacteria from adhering to and colonizing the intestinal epithelium (Suresh *et al.,* 2018). Egg yolk antibodies also cause structural damages to the surfaces of bacterial cells after binding to them and neutralise their toxins (Xu *et al.*, 2011). According to Gadde *et al.* (2017), egg yolk antibodies are more effective, less toxic, and safer to use than antibiotics, and unlike antibiotics, bacteria cannot develop resistance to them. They are however expensive and susceptible to proteolytic degradation in the digestive tract (Mine and Kovacs-Nolan, 2002).

2.3.7 Metals

The use of metals like manganese, copper, iron, zinc, and selenium to improve animal growth performance and productivity, even though expensive, has gained acceptance in animal farming (Scott, 2012; Gadde *et al.,* 2017). Metals are supplemented into diets in inorganic or chelated forms or as inorganic salts, e.g sulfates, chlorides, and carbonates (Attia *et al.*, 2012; Gadde *et al.,* 2017). Metals do not only support biosynthetic, digestive, and physiological processes but also promote growth (Richards *et al.,* 2010). For example, copper, in addition to playing important roles in angiogenesis, haemoglobin synthesis, and bone development, acts as a growth promoter in poultry diets (Hoda and Maha, 1995; Vasanth *et al.*, 2015). The inclusion of copper sulphate pentahydrate into diet of broiler chickens at a dose of 250mg/kg improved body weight gain and feed conversion ratio (Pesti and Bakalli, 1996). Likewise, incorporating copper oxychloride, copper sulphate pentahydrate, and copper citrate into diets increased the weight gain of broiler chickens by 4.9, 4.9, and 9.1% respectively (Ewing *et al.*, 1998). Moreover, supplementing basal diet of broilers with zinc sulphate up to 80mg/kg significantly increased body weight gain (Burrell *et al.*, 2004). Also, feeding broilers for four weeks with a diet containing a combination of zinc oxide and sodium selenite resulted in an improvement in growth (Fawzy *et al.*, 2016).

Brainer *et al.* (2003) stated that the growth-promoting effects of metals, when supplemented into diets, is attributable to their antimicrobial properties. Accordingly, Yazdankhah *et al.* (2014) fed pigs with copper and zinc supplemented diets and observed significantly lower counts of pathogenic intestinal bacteria (Yazdankhah *et al.*, 2014).

2.4 Phytogenic Feed Additives in Poultry Nutrition

Phytogenic feed additives (PFAs), also known as botanicals or phytobiotic supplements, are products derived from plants, herbs, and spices that are supplemented into animal diets (Windisch *et al.*, 2008; Grela *et al.*, 2013). PFAs contain several bioactive compounds that affect several physiological functions of farm animals (Paskudska *et al.*, 2018). Some of these compounds improve the taste, flavour and palatability of feeds, thus stimulate the appetite of farm animals (Mirzaei-Aghsaghali, 2012). Others enhance the secretion of digestive juices and activity of endogenous enzymes, promote the growth of beneficial gut microbes, and inhibit the pathogenic ones. These effects culminate in improvements in nutrient absorption and utilization (Dorman and Deans, 2000; Windisch *et al.*, 2008; Mirzaei-Aghsaghali, 2012). Some phytochemicals improve the sensory properties of poultry products (Meineri *et al.*, 2016) and others have anthelmintic, anti-inflammatory, antimicrobial, antioxidative, anti-tumour, anticoccidial, hypoglycemic, and immune-modulatory properties (Grela *et al.*, 2013; Babak and Nahashon, 2014; Suganya *et al.*, 2016).

The bioactive compounds of medicinal plants are present in their leaves, fruits, flowers, seeds, stems, roots, and rhizomes (Mirzaei-Aghsaghali, 2012). These plant parts are therefore incorporated into diets often in dried and ground forms, or their extracts are used (Gadde *et al.*, 2017). Some botanicals that are commonly used in poultry diets and the effects of their phytochemicals are presented in Table 2.3.

Table 2. 3: Commonly used botanicals in poultry diets

Botanical	Parts used	Phytochemicals	Effects of phytochemicals
1. Aloe vera (*Aloe barbadensis*)	Leaves	Anthraquinones	Antimicrobial, anti-inflammatory, antioxidative, immunomodulatory, and anti-tumour
2. Aniseed (*Pimpinella anisum*)	Fruit	Anethole	Digestion stimulation
3. Amla (*Emblica officinalis*)	Fruit	Tannins, ellagic acid, gallic acid, vitamin C	Antioxidative
4. Black cardamom (*Amomum subulatum*)	Seeds	Cineol	Digestion and appetite stimulation
5. Cinnamon (*Cinnamomum zeylanicum*)	Leaves, Bark	Phenolic compounds, eugenol	Digestion and appetite stimulation, antiseptic, astringent, carminative, antiviral, antifungal, blood-purifying
6. Cloves (*Syzygium aromaticum*)	Cloves	Eugenol	Digestion and appetite stimulation, antiseptic
7. Coriander (*Coriandrum sativum*)	Seeds, leaves	Carvone, limonene, geraniol, linalool, flavonoids, elemol, camphor, borneol	Appetite and digestion stimulation, carminative
8. Cumin (*Cuminum cyminum*)	Seeds	Cuminaldehyde	Digestion stimulation, carminative

9. Fenugreek (*Trigonella foenum-graecum*)	Seeds	Trigonelline, trigoneoside, vamogenin saponins, protodioscin, diosgenin	Appetite stimulation, antimicrobial, cholesterol-reducing
10. Garlic (*Allium sativum*)	Bulb	Allicin	Digestion stimulation, antiseptic
11. Ginger (*Zingiber officinale*)	Rhizome	Zingerone, ar-curcumene, β-bisabolene, camphene	Gastric stimulation, methane-reducing
12. Horseradish (*Armoracia rusticana*)	Root	Allyl isothiocyanate	Appetite stimulation
13. Indian ginseng (*Withania somnifera*)	Leaves, seeds, root	Glycine, withanolides, withanine	Antistress, analgesic, hepatoprotective, immunomodulatory
14. Mint (*Mentha piperita*)	Leaves	Menthol	Digestion and appetite stimulation, antiseptic
15. Moringa (*Moringa oleifera*)	Leaves	Ascorbic acid, caffeic acid, carotenoids, chlorogenic acid, flavonoids, phenolics	Antioxidative, anti-bacterial
16. Mustard (*Brassica Nigra*)	Seeds	Allyl isothiocyanate	Digestion stimulation
17. Neem (*Azadirachta indica*)	Leaves	Nimbidin, nimbin	Antibacterial, antiviral, antifungal, anthelmintic, stimulation of fibre-degrading enzymes
18. Nutmeg (*Myristica fragrans*)	Seeds	Sabinene	Digestion stimulation
19. Parsley (*Petroselinum crispum*)	Leaves	Apiol	Digestion and appetite stimulation, antiseptic
20. Pepper (*Piper nigrum*)	Fruit	Piperine	Digestion stimulation
21. Rosemary (*Rosmarinus officinalis*)	Leaves	Cineol, resins, tannins	Digestion stimulation, antiseptic, antioxidative, anti-inflammatory

22. Thyme (*Thymus vulgaris*)	Whole plant	Thymol	Digestion stimulation, antioxidative, antiseptic
25. Tulsi (*Ocimum sanctum*)	Leaves	Ascorbic acid, beta-sitosterol, beta-carotene, eugenol, palmitic acid, tannins	Anti-microbial, antioxidative, analgesic, hepatoprotective, anthelmintic, cardioprotective, antispasmodic, antiulcerogenic
26. Turmeric (*Curcuma longa*)	Rhizome	Ar-turmerone, curcuminoids, curlone, zingiberene	Hypocholesterolemic, anti-inflammatory, anticarcinogenic, antioxidative, antihepatotoxic

Sources: Mirzaei-Aghsaghali, 2012; Vinus *et al.*, 2018

2.4.1 Effects of PFAs on feed intake, nutrient digestibility and gut morphology in poultry

The digestion process is influenced in several ways by phytogenic feed additives. Some herbal compounds act as sialagogues and hence, stimulate saliva secretion to facilitate swallowing (Suganya *et al.*, 2016). Others improve the palatability and flavour of feeds and excite the taste buds and olfactory nerves to induce feed intake (Al-Kassie, 2009; Muanda *et al.*, 2011). Accordingly, dietary supplementation with *Coriandrum sativum* (coriander) seeds, *Nigella sativa* (black seeds) (EL-Shoukary *et al.*, 2014), enzymatically-treated *Artemisia annua* (sweet annie) whole plant (Wan *et al.*, 2017), *Foeniculum vulgare* (fennel) seeds (Ragab *et al.*, 2013), *Origanum majorana* (marjoram) leaf powder (Ali, 2014), and a mixture of *Cuminum cyminum* (cumin) and *Curcuma longa* (turmeric) (Al-Kassie *et al.*, 2011a) increased feed intake in broilers.

However, increasing the supplementation rate of *Allium cepa* (onion) extract in broiler diets from 7.5 to 10g/kg significantly reduced feed intake (Aditya *et al.*, 2017). Likewise, a significant decrease in feed intake was observed when the level of *Allium sativum* (garlic) powder in broiler feed was increased from 3 to 5% (Mulugeta *et al.*, 2019). A similar effect was realized when the amount of *Tetrapleura tetraptera* (aridan; Prekese**)** fruit powder was increased from 0.2 to 0.4% in broiler diets

(Kana *et al.,* 2017). These depressions in feed intake may be attributed to the consequent increase in the strong odour of these spices which might have negatively affected acceptability. Odoemelam *et al.* (2013) explained that the beneficial effects of spices are sometimes concealed when they are supplemented in high amounts due to the consequent increase in the levels of anti-nutritional factors like tannins and saponins. These compounds interfere with nutrient utilization and depress growth (Kana *et al.*, 2017).

Also, herbs such as aniseed, cumin, fenugreek, ginger, onion, and *Capsicum annuum* (cayenne pepper) contain compounds that promote the synthesis of bile acid in the liver and its excretion into the bile for lipid digestion and absorption (Suganya *et al.,* 2016). Also, some phytochemicals increase bile flow, as well as the synthesis, secretion, and activity of pancreatic enzymes (Jang *et al.,* 2007; Hashemipour *et al.,* 2014). Others stimulate gastric secretions, activity of digestive enzymes, and digestion, absorption, and assimilation of nutrients (Frankič *et al.*, 2009). These effects lead to improvements in apparent whole tract nutrient digestibility (Hernandez *et al.*, 2004; Wang *et al.,* 2008; Issa and Omar, 2012). Accordingly, Issa and Omar (2012) observed better crude protein, dry matter, and fat digestibility in broilers fed diets containing garlic powder. Similarly, Hernandez *et al.* (2004) observed increases in apparent total tract and ileal digestibility of dry matter, ether extract, starch, and crude protein in broilers fed diets containing plant extracts. Nonetheless, Jamroz *et al.* (2003) observed no effects of 150ppm of a standardized mixture containing carvacrol, capsaicin, and cinnamaldehyde in broiler diet on apparent ileal digestibility of dry matter, crude fat, crude ash, and nitrogen. This may be due to the low dietary inclusion rate of the mixture since at a higher inclusion rate of 300ppm, the ileal digestibility of all the fore-mentioned nutrients increased significantly (Jamroz *et al.*, 2003).

Furthermore, some phytoconstituents improve gut microanatomy (Murugesan *et al.,* 2015). For example, Ahsan *et al.* (2018) demonstrated that supplementing broiler diets with a phytogenic product containing cumin, cinnamon, and essential oils of aniseed, garlic, fennel (*Foeniculum vulgare*), and *Mentha balsamea* (peppermint) significantly increased the number of goblet cells along with intestinal villus diameter and height. The increased number of goblet cells per villus implies an increase in the production of glycoproteins and mucins that bind to pathogenic microbes and prevent their attachment to the mucosa gut lining (Chacher *et al.,* 2017). Also, the increased villus diameter and height imply an increased intestinal surface area for nutrient digestion and absorption (Murugesan *et al.*, 2015). In addition to increasing goblet cell number and intestinal villus diameter and height, the phytogenic product decreased the thickness of the muscularis (Ahsan *et al.*, 2018). According to Ahsan *et al.* (2018), the thickness of the muscularis increases with increasing germ load in the gut. The thin muscularis observed among the birds fed the diet containing the phytogenic product, therefore, implies a lower germ load, hence, more dietary nutrients will be channelled for growth (Ahsan *et al.*, 2018).

2.4.2 Phytogenic feed additives as antimicrobial agents

Plant extracts in several *in vitro* and *in vivo* studies have exhibited strong antimicrobial activity against fungi, bacteria, viruses, and protozoa (Gupta and Charan, 2005; Swiatkiewicz *et al.,* 2009; Sood *et al.,* 2012; Babak and Nahashon, 2014; Suganya *et al.,* 2016).

For instance, Junaid *et al.* (2006) found African basil leaf extract to have antibacterial effects on isolates of *Aeromonas hydrophila, Bacillus cereus, Escherichia coli*, *Salmonella typhimurium,* and *Yersinia enterocolitica.* This antimicrobial effect is attributable to the eugenol content of the plant (Nakamura *et al.,* 1999). Also, cinnamon oil was shown to inhibit the growth of *Enterococcus faecalis, Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus* *aureus, Staphylococcus epidermis, Salmonella,* and *Vibrio parahaemolyticus* (Montes-Belmont and Carvajal, 1998; Chang *et al.*, 2001). Likewise, Tabak *et al.* (1999) demonstrated that cinnamon extract at an inclusion rate similar to common antibiotics represses *Helicobacter pylori* ascribable to the cinnamaldehyde, carvacrol, and eugenol contents of the extract. Furthermore, Goodarzi *et al.* (2014) studied the effects of onion bulbs on the intestinal microflora composition of broiler chickens. These scientists demonstrated that supplementing diets with 10 and 30g/kg of fresh onion bulb significantly decreased ($p<0.05$) ileal *Escherichia coli* population. Besides, the *Lactobacillus* population in the ilea of the birds that received the diet supplemented with 30g/kg onion bulb significantly increased (Goodarzi *et al.*, 2014). Similarly, *Aloe vera* gel in feeds decreased the count of *Escherichia coli* and increased that of *Lactobacillus* in the ilea of broilers (Darabighane *et al.*, 2012). Kollanoor-Johny *et al.* (2012) found two plant-derived compounds (i.e. eugenol and trans-cinnamaldehyde) at inclusion rates of 0.75 and 1% to be effective in reducing the colonization of the caeca of broilers by *Salmonella enteritis* by decreasing their motility and virulence genes. Also, *Artemisia sieberi* leaf meal in feed significantly reduced coliform and *Escherichia coli* counts in the caeca of broilers (Khalaji *et al.*, 2011). Similarly, addition of sage, thyme, or rosemary essential oil to diet of laying chickens reduced the counts of coliforms and *Escherichia coli* in faecal samples (Bölükbaşı *et al.*, 2008). Similarly, a commercial phytogenic product containing extracts of anise, clove, melissa balm, oak, peppermint, and thyme was shown to be as effective as bacitracin methylene disalicylate (BMD) in controlling *Escherichia coli*, *Salmonella*, and *Clostridium* in the caeca of broilers that were orally challenged with *Salmonella enteritidis* and *Escherichia coli* (Wati *et al.*, 2015). Moreover, the number of *Lactobacillus* in the caeca of the birds fed the diet containing the phytogenic product was significantly higher than those fed either the control or BMD-supplemented diet (Wati *et al.*, 2015). Also, Jamroz *et al.* (2003) reported that dietary supplementation with 300ppm of a standardized mixture containing carvacrol, capsaicin, and cinnamaldehyde significantly reduced *Escherichia coli* and *Clostridium perfringens* counts in the recta of broilers to the same extent as an antibiotic (avilamycin) in comparison with the control group. Similarly, Tucker (2002) reported a significant *Escherichia coli*-inhibition effect of a herbal mixture containing aniseed, cinnamon, garlic, thyme, and rosemary when incorporated into commercial swine diets. Despite the reported proliferation effect of phytogenic feed additives on beneficial gut bacteria, Anugom and Ofongo (2019) administered aqueous African basil leaf extract to broilers and observed significantly lower counts of *Lactobacillus* in digesta samples collected from the crop, proventriculus, caecum, and ileum on day 35 of the experiment in comparison with the non-treated group. These researchers concluded that the phytochemicals in the herb possibly have bactericidal effects also on beneficial microbes. Likewise, Hernandez *et al.* (2004) reported that plant extracts, in addition to inhibiting the growth and colonization of pathogenic gut bacteria, may inhibit the beneficial ones. Possibly, similar to antibiotics (Henry Ford, 2020), some phytochemicals do not differentiate between beneficial and pathogenic bacteria and hence, may destroy the beneficial ones.

Furthermore, dietary supplementation with *Ocimum sanctum* (tulsi) leaf powder inhibited *in vivo* replication of the infectious bursal disease virus in broiler chickens (Gupta and Charan, 2005). Also, extracts of sweet annie inhibited the proliferation of the Newcastle disease virus in chicken embryos (Liu *et al.*, 2009). Likewise, extracts of *Eugenia jambolana* (jambolan) exhibited very strong virucidal effect on the highly pathogenic avian influenza virus (H5N1) in embryonated eggs inoculated in tissue culture and *in ovo* (Sood *et al.*, 2012). The anti-viral property of most phytogenic products is attributable to their flavonoid content (Muanda *et al.*, 2011).

Furthermore, volatile oils extracted from the leaves of seven *Artemisia* species namely *Artemisia dracunculus*, *Artemisia cana*, *Artemisia absinthium*, *Artemisia biennis*, *Artemisia ludoviciana*, *Artemisia frigida*, and *Artemisia longifolia* showed inhibitory effects on the growth of *Candida albicans*, *Cryptococcus neoformans,* and *Aspergillus niger* (Lopes-Lutz *et al.*, 2008). Likewise, essential oil of oregano inhibited the mycelial growth of *Aspergillus ochraceus* and the production of ochratoxin A from the fungus attributable to its carvacrol and thymol contents (Basilico and Basilico, 1999).

2.4.2.1 Antimicrobial modes of action of phytochemicals

In addition to disrupting the membranes of pathogenic microbes and modifying their cell surfaces to reduce their virulence, some phytochemicals activate immune cells like macrophages, monocytes, natural killer cells, and T lymphocytes to destroy the pathogens. Besides, other phytochemicals stimulate the growth and proliferation of beneficial gut bacteria to competitively exclude the pathogenic ones (Diaz-Sanchez *et al.*, 2015; Suganya *et al.*, 2016). When loads of enteric pathogens decrease, nutrient availability increases, sub-clinical infections are eliminated, and mortality decreases (Wilson *et al.*, 2005; Brisbin *et al.*, 2008).

2.4.3 Phytogenic feed additives as growth promoters in poultry diets

Currently, herbs and spices are used as natural growth promoters in poultry diets in place of antibiotics (Gadde *et al.*, 2017). Several herbs and spices including *Achillea millefolium* (yarrow), cinnamon, coriander, ginger, garlic, marjoram, oregano, rosemary, and thyme have been incorporated into poultry diets for growth enhancement (Gadde *et al.*, 2017). Likewise, admixing diets with black cumin seeds (Khalaji *et al.*, 2011), fermented *Ginkgo biloba* (ginkgo) leaves (Cao *et al.*, 2012), fenugreek seeds (Azouz, 2001), *Stevia rebaudiana* (stevia) leaf meal (Atteh *et al.*, 2008), and extracts of aniseed (*Pimpinella anisum*) (Durrani *et al.*, 2007), purslane (*Portulaca* *oleracea*) (Zhao *et al.*, 2013), and weeping forsythia (*Forsythia suspense*) (Wang *et al.*, 2008) improved broiler growth performance. Also, inclusion of 0.2% aridan fruit powder in broiler ration improved feed conversion ratio, live weight, and weight gain (Kana *et al.*, 2017). However, increasing the inclusion rate of the aridan fruit powder from 0.2 to 0.4% depressed feed intake, live weight, weight gain, and carcass yield significantly (Kana *et al.*, 2017). The decline in productive traits could be due to the potential increase in the levels of anti-nutritive factors in the diet which might have negatively imparted nutrient utilization (Kana *et al.*, 2017). Also, Goodarzi *et al.* (2014) observed that birds fed a diet supplemented with 30g/kg fresh onion bulb had a significantly higher finishing body weight than those fed either a control or virginiamycin-supplemented diet. Incorporating *Ocimum basilicum* (sweet basil) seeds at a rate of 3g/kg in broiler diet also increased ($p<0.05$) finishing body weight (Abbas, 2010). Abbas (2010) observed that the broilers fed the sweet basil seed-supplemented diet had the least feed intake but the best feed conversion ratio.

Moreover, essential oils of herbs such as basil, clove, garlic, ginger, thyme, caraway, coriander, rosemary, sage, star anise, and turmeric have been used individually or as blends to boost poultry growth (Gadde *et al.*, 2017). For example, a blend of essential oils of cinnamon (ie. cinnamaldehyde) and thyme (ie. thymol), when incorporated into broiler diets improved body weight gain (Tiihonen *et al.*, 2010). Similar findings were made when diets were supplemented with essential oils of coriander (Ghazanfari *et al.*, 2015) and oregano (Basmacioğlu *et al.*, 2010), a blend of essential oils of thyme and star anise (Kim *et al.*, 2016), and a blend of cinnamaldehyde and essential oil of clove (Chalghoumi *et al.*, 2013). Likewise, a blend of cinnamaldehyde, carvacrol, and *Capsicum oleoresin* in broiler feed improved feed conversion efficiency and weight gain by 9.8% and 14.5% respectively (Bravo *et al.*, 2014).

The improvement in growth of birds fed diets containing phytogenic products is due to improvement in enzymatic function, stimulation of growth and proliferation of beneficial gut microflora, inhibition of pathogenic gut microbes, and decrease in the production of growth-depressing microbial metabolites such as biogenic amines and ammonia. These effects culminate in increases in nutrient absorption and utilisation (Jamroz *et al.*, 2003; Windisch *et al.*, 2008; Frankič *et al.*, 2009) which increases growth performance. Also, some phytogenic feed additives improve protein digestion in the intestine leading to growth improvement (Abbas, 2010). According to Fallah *et al.* (2013), the growth-promoting effects of phytogenic feed additives arise from the synergistic effect of their bioactive compounds.

However, some researchers fed diets admixed with phytogenic products and observed negative or no effects on growth performance. For instance, Goliomytis *et al.* (2014) supplemented broiler diets with 0.5 and 1g of quercetin (a plant flavonol) per kg of feed and observed no significant effects on cumulative feed intake and body weight. Furthermore, in that study, birds fed the quercetin-supplemented diets had a poorer feed conversion ratio (Goliomytis *et al.*, 2014). Also, other researchers who tested *Macleaya cordata* (plume poppy) extract (Juskiewicz *et al.*, 2011) and garlic powder (Issa and Omar, 2012) as growth promoters reported no impacts on broiler growth performance. Also, Hernandez *et al.* (2004) supplemented broiler diets with 5,000ppm Labiatae extract from rosemary, sage, and thyme mixture, and 200ppm essential oil of cinnamon, pepper, and oregano mixture, and observed no effects on feed intake, feed conversion efficiency, live weight, and body weight gain. Similarly, Jang *et al.* (2007) found no effects of dietary supplementation with a commercial essential oil product containing eugenol, piperine, and thymol on feed intake, body weight gain, and feed conversion ratio of broilers. The lack of response may be due to the inclusion rates of the phytogenic products, health status of the birds, and management practices. These factors influence the effects of phytogenic feed supplements on poultry performance (Yang *et al.*, 2009). Franz *et al.* (2010) also indicated that the inconsistency in the effects of essential oil on poultry performance could be due to variations in the type and composition of the oils as well as environmental factors.

The results of other research trials that examined the effects of phytogenic feed supplements on broiler growth performance are presented in Table 2.4.

Table 2. 4: Effects of phytogenic feed additives on broiler growth indices

Phytogenic Product	**Dietary inclusion rate**	**Effects on growth indices**	**Reference**
1. *Piper guineense* (Ashanti pepper) leaf or seed meal	0.2 or 0.4%	Increase in BWG Improvement of FCR	(Effiong and Ochagu, 2019)
2. Thyme extract	50, 100, 200, or 400ppm	Increase in BWG	(Alipour *et al.*, 2015)
3. Thyme extract	0.2, 0.4 or 0.6%	No effects on LW and FCR	(Pourmahmoud *et al.*, 2013)
4. EO from cinnamon or thyme	200ppm	Increases in FI and BWG Improvement of FCR	(Al-Kassie, 2009)
5. Turmeric powder	0.5%	Increase in BWG	(Yarru *et al.*, 2009)
6. Turmeric powder	2.5g/kg	Increase in BWG up to 21 d of age	(Akbari, 2014)
7. Mixture of turmeric and cumin	0.50, 0.75 or 1%	Increases in FI, BWG and FCE	(Al-Kassie *et al.*, 2011a)
8. Garlic powder	1, 3, or 5%	3%: Increase in FBW and improvement of FCR	(Mulugeta *et al.*, 2019)
9. Shatavari *(Asparagus racemosus*) root powder	0.5, 1 or 1.5%	Increases in BWG and FCE	(Rekhate *et al.*, 2004)
10. Marjoram leaf meal	0.5, 1 or 1.5%	Increases in FI, BWG and FCE	(Ali, 2014)
11. Green Tea extract	0.1 or 0.2g/kg	Increases in LW and FCE	(Erener *et al.*, 2011)
12. Blend of thymol and carvacrol	60, 100 or 200mg/kg	Increase in BWG	(Hashemipour *et al.*, 2013)
13. Oregano EO	100mg/kg	No effects on FI, BWG and FCR	(Avila-Ramos *et al.*, 2012)
14. Oregano EO	50 or 100 mg/kg	No effects on LW and FCR	(Botsoglou *et al.*, 2002)

15. Extract of cinnamon, clove, oregano, or red pepper	1000ppm	No effects on LW, FI and FCR	(Barreto *et al.*, 2008)
16. Sweet basil EO	200, 400 or 600mg/kg	No effects on FI, BWG and FCR	(Riyazi *et al.*, 2015)
17. Hesperidin, naringin	0.75 or 1.5g/kg	No effect on BW	(Goliomytis *et al.* 2015)
18. *Hibiscus sabdariffa* (Hs) calyx meal, *Ocimum gratissimum* (Og) leaf meal	0.25% Hs, 0.25% Og, or 0.125% Hs + 0.125% Og	No effects on FI, BWG and FCR	(Ojelade *et al.*, 2012)
19. Ginger powder	250, 500 or 750g/100kg	No effects on FI, BWG and FCR	(Mohammed and Yusuf, 2011)
20. *Moringa oleifera* leaf meal	3, 6 or 9%	No effects on ADG, FI, FCR and FBW	(Amevor, 2017)
21. *Moringa oleifera* leaf meal	50, 75 or 100%	Decreases in FI and LW	(Gadzirayi *et al.*, 2012)

ADG = Average daily gain; BWG = Body weight gain; EO = Essential oil; FCR = Feed conversion ratio; FBW = Final body weight; FCE = Feed conversion efficiency; FI = Feed Intake; LW = Live weight

2.4.3.1 Limitations in the use of phytogenic feed additives

The use of phytogenic feed additives is associated with some limitations. For instance, due to their complex compositions, they are not easily quantified and standardized (Suganya *et al.,* 2016). Also, their compositions are affected by soil type, stage of maturity of plants, geographical location, altitude, season of planting or harvesting, method of harvesting, climatic factors, environmental conditions, presence of anti-nutritional compounds, storage conditions, microbial contamination, and processing methods (Windisch *et al.*, 2008; Huyghebaert *et al.*, 2011; Nascimento *et al.*, 2011;

Suganya *et al.,* 2016). Also, some phytochemicals are liable to light and temperature variations hence, their availability and concentrations are unstable (Suganya *et al.,* 2016). Some phytogenic feed additives have antagonistic effects on animals when combined (Suganya *et al.,* 2016; Paskudska *et al.*, 2018) whilst others are rather effective when combined than when offered alone (Paskudska *et al.,* 2018). The efficacy of phytogenic feed supplements on poultry performance is also affected by nutritional status of birds as well as composition of diets (Giannenas *et al.,* 2003).

2.4.3.2 Benefits of phytogenic feed additives over antibiotic growth promoters

The following characteristics of phytogenic feed additives (PFAs) make them superior to antibiotic growth promoters (AGPs). They are natural, cheap, safe to use, less toxic, eco-friendly, and result in little to no cases of bacteria resistance **(**Hashemi and Davoodi, 2011; Sethiya, 2016; Nieto, 2017). Suresh *et al.* (2018) explained that, unlike AGPs, PFAs are composed of a complex blend of phytochemicals with each having a different antimicrobial activity which makes it very challenging for bacteria to gain resistance to them. Furthermore, PFAs have a multifactorial or broad spectrum of action on farm animals unlike AGPs (Steiner, 2006). PFAs also have no residual effects on poultry products and thus, increase the safety of these foods for human consumption (Sanjyal and Sapkota, 2011).

2.4.4 Phytogenic feed additives as antioxidative agents

Antioxidants, according to Muanda *et al.* (2011), are compounds that when added to feeds inhibit or impede lipid oxidation and rancidity thus, maintaining the nutritional quality of feeds. The health-promoting effects of antioxidants from plants possibly arise from their protective role in counteracting reactive oxygen species (Muanda *et al.*, 2011). Some herbs and spices also protect feeds from oxidative deterioration during storage. Owing to this property, essential oils of rosemary, sage, and thyme are commonly used in feed industries for feed preservation (Jacobsen *et al.,* 2008;

Nieto, 2017). The antioxidative property of herbs is related to the presence of phenolic compounds such as hydrolysable tannins, flavonoids, phenolic acids, proanthocyanidins, terpenes, and vitamins A, C, and E in them (Muanda *et al.*, 2011). The sulfur-containing molecules in onion and garlic also have antioxidative properties (Higuchi *et al.*, 2003).

In an experiment with broiler chickens, Reddy *et al.* (2009) observed that supplementing diet with tulsi leaf powder increased ($p<0.01$) the levels of the antioxidative enzymes - superoxide dismutase and catalase and thus, combated oxidative stress in the birds. Also, incorporating tulsi leaf extract into heat-stressed broiler diets significantly increased the activity of plasma glutathione peroxidase; an antioxidative enzyme that scavenges free radicals to prevent lipid peroxidation and maintain intracellular homeostasis (Vasanthakumar *et al.*, 2013). Steiner and Syed (2015) also reported that a blend of volatile oils of caraway, anise, and peppermint activated cellular pathways that protect animal cells from reactive oxygen species. Also, the leaves of *Moringa oleifera* contain large amounts of Vitamin E; an antioxidant that prevents oxidative spoilage of meat (Moyo *et al.*, 2012). Other herbs that contain antioxidative compounds are *Taraxacum officinale* (dandelion), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Matricaria chamomilla* (chamomile), *Ginkgo biloba* (ginkgo), *Tagetes erecta* (marigold) (Suganya *et al.*, 2016), *Capsicum annuum* (red pepper), *Piper nigrum* (black pepper), *Capsicum frutescens* (chili pepper) (Nakatani, 1997), *Prunus dulcis var. amara* (bitter almonds), *Cinnamomum verum* (cinnamon), *Mentha piperita* (peppermint), *Syzygium aromaticum* (clove), *Laurus nobilis* (laurel) (Deans *et al.*, 1993), *Carthamus tinctorius* (safflower), and *Brassica juncea* (mustard) (Lee *et al.*, 2007).

2.4.5 Phytogenic supplements as immune-stimulatory agents

Some examples of herbs that have immune-stimulatory properties are garlic, *Echinacea purpurea* (echinacea), *Glycyrrhiza glabra* (liquorice), and *Uncaria tomentosa* (cat's claw). Their phytoconstituents stimulate the synthesis of interferons and improve the activity of macrophages, lymphocytes, and natural killer cells to increase phagocytosis (Frankič *et al.*, 2009). Also, some phytochemicals increase the proliferation of immune cells, antibody titres to antigens, as well as cytokine expressions (Park *et al.*, 2011). Still, others induce the production of heat shock proteins that activate the Toll-like receptors to recognize pathogens and induce immune responses to them (Hashemi and Davoodi, 2012).

Supplementing broiler diets with essential oils of *Mentha* (mint), *Satureja hortensis* (savory), and *Hippophae rhamnoides* (sea buckthorn) stimulated the infiltration of leukocytes which suggested an improvement in immune response (Stef *et al.*, 2009). The immune-stimulatory effect of safflower extract on avian lymphocytes and mustard extract on macrophages have been demonstrated (Lee *et al.*, 2007). The safflower extract stimulated the proliferation of avian lymphocytes whilst the mustard extract stimulated the production of nitric oxide by macrophages (Lee *et al.*, 2007). Similarly, cinnamaldehyde, an organic compound derived from cinnamon, stimulated the proliferation of avian spleen lymphocytes and the production of nitric oxide by cultured macrophages (Lee *et al.*, 2011b). Islam *et al.* (2017) observed a significant improvement of the phagocytic activity of heterophils in broilers that were orally treated with non-dialyzable fraction of *Vaccinium macrocarpon* (cranberry) extract at a dose of 4mg/ml/bird. Also, oral administration of aqueous and ethanolic extracts of *Aloe vera* pulp at a rate of 300mg/kg of body weight for three consecutive days resulted in a significant increase in antibody titre to sheep red blood cells in broilers, suggesting an improvement in immune response (Akhtar *et al.*, 2012). Likewise, echinacea extract increased the concentration of immunoglobulins in the blood of laying chickens and improved their health status (Swiatkiewicz and Koreleski, 2007). Furthermore, feeding birds with a diet admixed with *Medicago sativa* (alfalfa) increased both the masses of the lymphatic organs and the number of lymphocytes produced by these organs (Skomorucha and Sosnówka-Czajka, 2012). Also, Guo *et al.* (2003) reported that *Astragalus membranaceus* (astragalus) polysaccharides in feed stimulates the growth of the bursa, spleen, and thymus.

2.4.6 Phytogenic feed additives as coccidiostatic agents

Coccidiosis, a very common cause of mortality in poultry, is a disease caused by a protozoa called Eimeria which damages the intestinal epithelium and impairs nutrient absorption (Paskudska *et al.*, 2018).

Sweet annie is one of the earliest plants used to combat coccidiosis in poultry. It contains artemisinin; a bioactive compound that inhibits the development of coccidiosis (Świątkiewicz *et al.,* 2009). As such, when dried fruits of sweet annie were incorporated into feed for chickens infected with *Eimeria tenella* oocysts, intestinal damages were reduced (Swiatkiewicz *et al.*, 2009). Also, *Origanum vulgare* (oregano) contains compounds that have coccidiostatic properties namely thymol, carvacrol, caffeic acid, rosmarinic acids, flavonoids, ursolic, and phytosterols (Waldenstedt, 2003). Accordingly, the incorporation of oregano extract into diets of broiler chickens infected with coccidia reduced both the counts of *Eimeria tenella* oocysts in excrement and the extent of intestinal damage (Waldenstedt, 2003). Another herb useful for alleviating the severity of coccidiosis is *Prunus salicina* (oriental plum) (Swiatkiewicz *et al.,* 2009). It contains phenolic acids, anthocyanins, and flavonoids that have coccidiostatic properties (Swiatkiewicz *et al.*, 2009). Incorporating dried oriental plum into feed for birds infected with *Eimeria* reduced the number of excreted *Eimeria* oocytes (Swiatkiewicz *et al.*, 2009). Other herbs that have anticoccidial effects are *Aloe vera* (Yim *et al.*, 2011), *Ocimum gratissimum* (Ogunleye, 2019), echinacea (Paskudska *et al.*, 2018), and *Nectaroscordum tripedale* (honey garlic) (Suganya *et al.*, 2016).

2.4.7 Phytogenic supplements as anthelmintic agents

The abusive use of conventional anthelmintic drugs such as piperazine and benzimidazoles (e.g. albendazole and fenbendazole) results in the occurrence of chemical residues in poultry products (Anthony *et al.*, 2005) as well as cases of resistance to these drugs by helminths (Abdelqader *et al.*, 2012). As a result, much attention has currently been placed on the use of botanicals as alternatives to conventional anthelmintic drugs in poultry production (Symeonidou *et al.*, 2018).

Some medicinal plants and herbs that have anthelmintic properties are *Fagara zanthoxyloides* (Athanasiadou *et al.*, 2007), *Ocimum gratissimum*, black caraway, tulsi, sweet annie, thyme, echinacea, ginkgo, *Melia azedarach* (chinaberry tree), *Carica papaya* (pawpaw), *Moghania vestita* (Sohphlang), *Juglan nigra* (black walnut), *Punica granatum* (pomegranate), *Embelia ribes* (false black pepper), *Picrasma excelsa* (Jamaican Quassia), *Chenopodium ambrosioides* (sweet pigweed), *Trifolium repens* (white clover), *Ficus insipida* (chalate), *Trachyspermum ammi* (ajowan caraway), and *Cucurbita maxima* (pumpkin) (Shifa, 2014). Several of these botanicals have been used as feed additives or water supplements to control helminths in farm animals (Athanasiadou *et al.*, 2007; Symeonidou *et al.*, 2018). Presented in Table 2.6 are the effects of phytogenic supplements on *Ascaridia galli;* a helminth of poultry known to inflict the most damage (Butcher and Miles, 1995).

Table 2. 5: Effects of phytogenic supplements on *Ascaridia galli (A. galli)* in chicken

No.	Botanical	Part used	Dose	Effect(s) on *A. galli*	Reference
1.	Neem (*Azadirachta indica*)	Leaves	Aqueous extract at 200mg/kg of BW	Death of parasite	(Khokon *et al.*, 2014)
2.	Squirrel's claws (*Caesalpinia crista*)	Seeds	Seed powder and methanol extract at 30, 40, and 50mg/kg of BW	Reduction in faecal egg count	(Javed *et al.*, 1994)
3.	Chinaberry tree *(Melia azedarach)*	Fruits	Fruit powder, aqueous, ethanol, and methanol extracts all at 20mg/kg of feed	Inhibition of egg development	(Akhtar and Riffat, 1985)
4.	Wild bauhinia (*Piliostigma thonningii*)	Bark	Ethanol extracts at 100, 200, and 400mg/kg of BW	Reduction in faecal egg count	(Asuzu and Onu, 1994)
5.	Bitter leaf (*Vernonia amygdalina*), Fish bean (*Tephrosia vogelii*)	Leaves	Aqueous extracts at 1kg/ litre	• Reduction in faecal worm egg count • Reduction in adult worm population	(Siamba *et al.*, 2007)

BW = Body weight

The modes of action of plant secondary metabolites against helminths are shown on Plate 2.1.

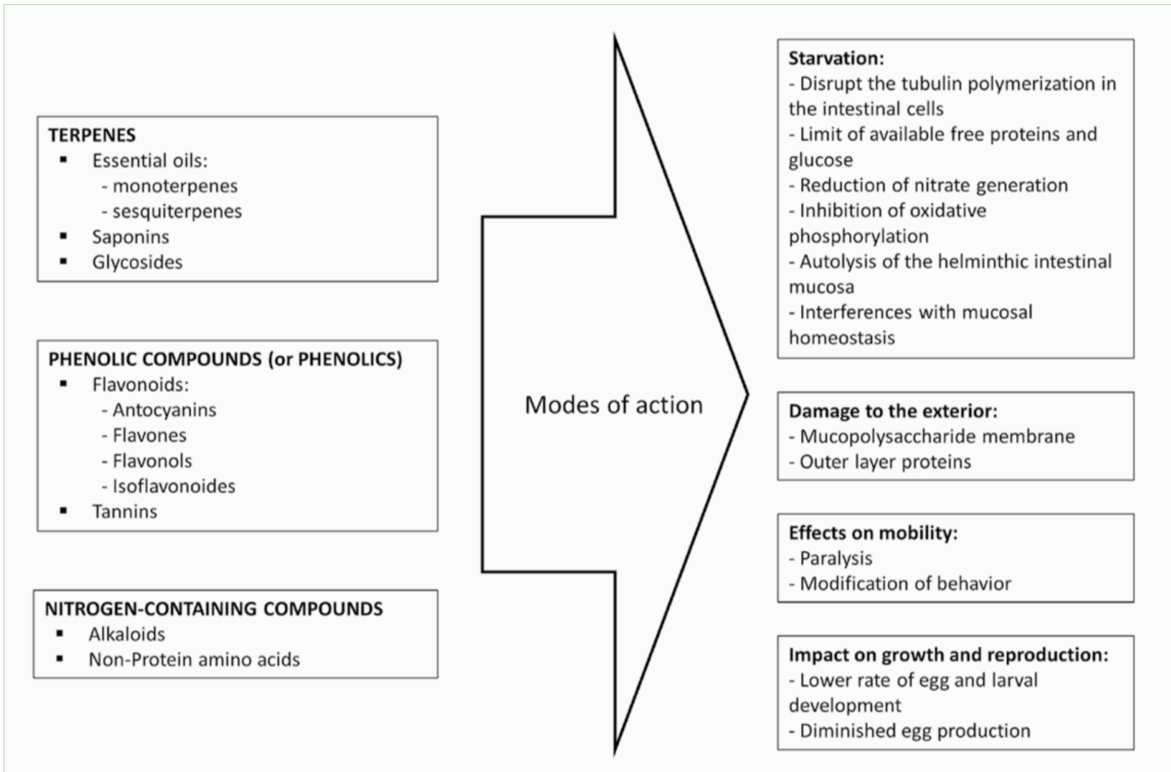

Plate 2. 1: Modes of action of phytochemicals against helminths

(Source: Symeonidou *et al.*, 2018)

2.4.8 Effects of phytogenic feed additives on some blood constituents in poultry

Supplementation of broiler diets with 3g/kg each of parsley, fenugreek, and sweet basil seeds reduced blood cholesterol but did not affect total protein, glucose, albumin, and globulin (Abbas, 2010). Fenugreek seeds at 1.5% in feed also reduced blood cholesterol concentration in Muscovi ducklings (El-Ghamry *et al.*, 2004). Also, a blend of cumin and turmeric supplemented at 0.75 and 1% in broiler diets decreased the concentrations of haemoglobin and cholesterol, counts of red and white blood cells, and heterophil to lymphocyte ratio (Al-Kassie *et al.*, 2011a). Moringa leaf powder, when supplemented into broiler diets, equally reduced ($p<0.05$) serum triglyceride and cholesterol levels (Mandal *et al.*, 2014). Moringa leaves contain large amounts of alkaloids, flavonoids, phenolic compounds, and polyphenols with hypocholesterolemic properties (Moyo *et al.*, 2012; Mandal *et al.,* 2014). Furthermore, supplementing broiler diets with *Ocimum gratissimum* leaf meal significantly reduced ($p<0.05$) blood cholesterol but did not affect albumin, creatinine, and total protein (Olumide *et al.,* 2018). Likewise, Prasad *et al.* (2009) observed that dietary supplementation with garlic powder significantly reduced the concentrations of triglycerides, cholesterol, very-low-density lipoprotein, and low-density lipoprotein, but increased that of high-density lipoprotein in broilers. Qureshi *et al.* (1983) also supplemented broiler diets with garlic paste and recorded significantly lower serum cholesterol levels. This hypocholesterolaemic effect is caused by the depressing effect of some compounds in garlic namely 3-hydroxyl-3-methyl-glutaryl-CoA reductase, glucose-6-phosphatase dehydrogenase, fatty acid synthase, and malic enzyme on the activities of the cholesterogenic and lipogenic enzymes of the liver (Qureshi *et al.*, 1983; Qureshi *et al.*, 1987). However, dietary inclusion of onion (*Allium cepa*) extract at the rates of 5, 7.5, and 10g/kg did not affect total cholesterol, high-density lipoprotein, triglycerides, and glucose levels in broilers (Aditya *et al.*, 2017) whilst thyme powder at a concentration of 1g/kg in broiler feed caused increases ($p>0.05$) in plasma cholesterol and triglycerides (Demir *et al.*, 2005). The effects of phytogenic feed supplements on some blood parameters observed in other studies are presented in Table 2.7.

Table 2. 6: Effects of PFAs on blood haematology and biochemical indices of broilers

PFA	Dietary inclusion rate	Effects	Reference
1. *Emblica officinalis* (amla) fruit powder	0.25, 0.50, 0.75 or 1%	• Decreases in serum cholesterol and heterophil count • Increase in Hb	(Dalal *et al.*, 2018a)
2. Garlic bulb, garlic husk	2 or 4%	• Decreases in total cholesterol and LDL • No effect on HDL	(Kim *et al.*, 2009)
3. Garlic extract	2, 4 or 6%	• Decreases in total cholesterol and LDL • Increases in HDL and HDL: LDL ratio	(Utami *et al.*, 2018)
4. *Ocimum basilicum* EO	200, 400 or 600mg/kg	• Increase in triglycerides • No effects on cholesterol, HDL, and LDL	(Riyazi *et al.*, 2015)
5. *Ocimum basilicum* leaf powder	0.5 or 1g/kg	• 0.5 g/kg: Increase in glucose • 1 g/kg: Increase in lymphocytes • Both: Increase in RBC count but no effects on plasma cholesterol and triglycerides	(Osman *et al.*, 2010)

EO = Essential oil; LDL = Low-Density Lipoprotein; Hb = Haemoglobin; H: L = Heterophils to Lymphocytes; HDL = High-Density Lipoprotein; PFA = Phytogenic feed additive; RBC = Red blood cell; WBC = White blood cell

2.4.9 Effects of phytogenic feed additives on the quality of poultry products

Lipid oxidation is the primary cause of meat deterioration (Mandal *et al.*, 2014). It is a complex process that occurs in aerobic cells due to the interaction between polyunsaturated fatty acids and molecular oxygen (Verma *et al.*, 2009). Lipid oxidation causes meat spoilage particularly when meat is exposed to oxygen, light, or heat (Mandal *et al.*, 2014). Supplementing animal diets with phytogenic feed additives with antioxidative properties is an effective means to increase the oxidative stability, quality, and shelf life of their meat (Tavárez *et al.,* 2011). Furthermore, Kim *et al.* (2009) evaluated the sensory and physicochemical characteristics of thigh muscles of broilers fed diets containing different levels of garlic bulb or garlic husk. Garlic supplementation increased the protein content, flavour, and texture of the meat, and reduced the fat content (Kim *et al.,* 2009). Also, increasing the inclusion rate of garlic bulb from 2 to 4% reduced the shear force and thiobarbituric acid reactive substances in the thigh muscles (Kim *et al.*, 2009). Also in that study, the highest level of garlic husk supplementation (4%) decreased ($p<0.05$) the concentrations of saturated fatty acids and increased ($p<0.05$) that of unsaturated fatty acids in the meat (Kim *et al.,* 2009). A similar distribution of saturated and unsaturated fatty acids was observed in the meat of broilers fed diets containing peppermint and *Viola tricolor var. hortensis* (pansy) (Kapica *et al.*, 2006).

Furthermore, the flowers of *Tagetes erecta* (tagetes) and *Calendula officinalis* (calendula) when added to broiler diets, improved carcass colour (Grela *et al.*, 2013). These flowers added a yellow tint to the skin of the carcasses (Grela *et al.,* 2013). Also, dietary inclusion of *Allium sativum* and *Ocimum gratissimum* significantly improved the tenderness and juiciness of the meat of broilers (Odoemelam *et al.*, 2017).

Moreover, phytogenic feed additives are used as colouring agents in diets of laying hens to improve egg yolk colour (Nobakht and Moghaddam, 2013). For example, incorporating 2% dried aerial parts powder of *Tanacetum balsamita* (costmary) into diets of laying chickens did not only lower ($p<0.05$) blood cholesterol level but also positively imparted egg yolk colour (Nobakht and Moghaddam, 2013). A similar observation was made when diets were supplemented with marigold extract (Sirri *et al.*, 2007). However, Yildirim *et al.* (2013) and Kopřiva *et al.* (2014) observed no effects of dietary supplementation with *Panax ginseng* (Korean ginseng) root extract and dried *Beta vulgaris* (beetroot) respectively on egg yolk colour.

Also, Azeezah *et al.* (2019) supplemented cockerel diets with *Moringa oleifera* and *Ocimum gratissimum* leaf meals and observed increases in meat protein and mineral contents (calcium, magnesium, iron, and phosphorus), and a decrease in cooking loss (Azeezah *et al.*, 2019).

Additionally, some phytochemicals have hypocholesterolaemic effects and hence, reduce the levels of cholesterol in meat and eggs. This make these foods safe for human consumption (Grela *et al.*, 2013). For instance, Grela *et al.* (2013) reported that dietary supplementation with garlic reduces cholesterol levels in meat and eggs. Likewise, adding 1% *Ocimum sanctum* leaf powder to broiler diet was shown to reduce cholesterol in breast and thigh muscles (Lanjewar *et al.*, 2009).

2.4.10 Phytogenic supplements as anti-stress agents in poultry

Heat stress, a commonly-faced problem in the tropics, has been widely reported to deleteriously affect poultry growth performance and productivity (Lara and Rostagno, 2013; Bhadauria *et al.*, 2016). Heat stress occurs when the amount of heat generated from the animal's body exceeds that released into the environment (Lara and Rostagno, 2013). Of all livestock species, poultry is most negatively affected by heat stress because they lack sweat glands in their skin to allow easy release of internally-generated heat (Bhadauria *et al.*, 2016). Heat stress causes declines in feed consumption, feed utilization, body weight gain, egg production, egg quality, and carcass yield (Whitehead, 1998; Zeferino *et al.*, 2016). The reduction in feed intake leads to reductions in nutrient uptake and utilization and thus, results in poor productivity (Shokryazdan *et al.*, 2017). Chronic heat stress during the growing period of broilers negatively affects the quality of their meat (Lu *et al.*, 2007). Also, heat stress adversely affects the immunity, welfare, and behavior of birds, and ultimately results in mortalities and economic losses to farmers (Lara and Rostagno, 2013; Ranjan *et al.*, 2019).

Supplementing drinking water or rations with some phytogenic products is one of the ways to combat heat stress in poultry (Grela *et al.*, 2013, Abd El-Hack *et al.*, 2020). The products of herbs such as black seed, chicory (*Cichorium intybus*), dill (*Anethum graveolens*), moringa, ginger, red pepper (*Capsicum annuum*), thyme, sweet annie, fennel, and rosemary have been successfully used as anti-stress agents in poultry (Abd El-Hack *et al.,* 2020). Herbs with antioxidative properties can equally be used to combat heat stress (Shokryazdan *et al.*, 2017). Heat stress causes oxidative stress in the body, and the oxidation of substrates generates free radicals and other reactive oxygen species that can damage body cells and organs. Hence, antioxidants are neceassary to counteract these effects (Halliwell and Gutteridge, 1984). The effects of some phytogenic feed additives on poultry performance, physiology, and productivity under heat stress conditions are presented in Table 2.8.

Table 2. 7: Effects of phytogenic feed additives on poultry performance, physiology, and productivity under heat stress conditions

No.	PFA	Poultry species	Inclusion rate	Effects	Reference
1.	Black seed (*Nigella sativa*)	Pigeons	2%	• Increases in FI and BWG • Improvement of FCR • Decrease in aggressive behavior • Decreases in blood cholesterol, catalase, and glucose levels	(Shoukary *et al.*, 2018)
2.	Black seed (*Nigella sativa*)	Broilers	1%	• Increases in FI, ADG, and dressing percentage • Decreases in panting and water/feed ratio • Decreases in T3 and corticosterone concentrations	(EL-Shoukary *et al.*, 2014)
3.	Thyme (*Thymus vulgaris*) EO	Broilers	100, 150 or 200mg/kg	• Improvement of growth performance • Increases in relative weights of bursa of Fabricius, spleen, and thymus • Increase in lymphocyte count • Decrease in H: L ratio	(Olfati and Mojtahedin, 2018)
4.	Ginger (*Zingiber officinale*) root powder or EO	Broilers	7.5g or 150mg/kg	• Increases in BW, BWG, and superoxide dismutase • No effects on blood variables and carcass traits • Reduction in MDA level in the liver	(Habibi *et al.*, 2014)
5.	Sweet annie (*Artemisia annua*) Enzymatica lly-treated WP	Male broilers	1 or 1.25g/kg	• Increases in FI, BWG, and carcass traits • Decreases in blood pH and serum levels of corticosterone, AST, MDA, and ALT • Increases in serum SOD, T3, and T4 levels	(Wan *et al.*, 2017)
6.	Fennel (*Foeniculu m vulgare*) fruits	Laying chickens	10 or 20g/kg	• Improvement of egg quality traits • Reduction in number of broken eggs • Reductions in carboxyl and MDA in eggs • Reductions in yolk cholesterol and triglyceride levels	(Gharaghani *et al.*, 2015)

7.	Fennel (*Foeniculum vulgare*) seeds	Broilers	1 or 2%	• Increases in FI, breast meat yield, and circulating leukocytes • Decreases in body temperature and mortality	(Ragab *et al.*, 2013)
8.	Sweet basil (*Ocimum basilicum*) seed powder	Broilers	5g/kg	• Increases in BWG and dressing percentage • Improvement of FCR • Decreases in body temperature, water intake, and mortality • Increases in retention of CP, CF, and ME • Increase ($p>0.05$) in intestinal villi size • No effects on RBC count and PCV • Increases in Hb and WBC count • Increase ($p>0.05$) in ND antibody titre	(Jahejo *et al.*, 2019)
9.	Coriander (*Coriandrum sativum*) seeds	Broilers	2%	• Increases in FI, ADG, and dressing percentage • Decreases in panting behavior and water/feed ratio • Decreases in T3 and corticosterone concentrations • No effects on drinking behavior, FCR, slaughter weight, and T4 level	(EL-Shoukary *et al.*, 2014)
10.	Javanese turmeric (*Curcuma xanthorrhiza*) EO	Broilers	200 or 400mg/kg	• Increases in erythrocyte GPX and SOD activity, serum total protein, and plasma GH concentration • Decreases in cholesterol, LDL, phosphorus, and chloride concentrations • Increase in bronchitis antibody titre	(Akbarian *et al.*, 2015)

Source: Abd El-Hack *et al.* (2020)

ADG = Average daily gain; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; *BWG* = Body weight gain; CP = Crude protein; CF = Crude fibre, EO = Essential oil; FCR = Feed conversion ratio; FI = Feed intake; GH = Growth hormone; GPX = Glutathione peroxidase; Hb = Haemoglobin; H: L = Heterophils to lymphocytes; HDL = High-density lipoproteins; LDL = Low density lipoprotein; MDA = Malondialdehyde; ME = Metabolisable energy; ND = Newcastle disease; PCV = Packed cell volume; PFA = Phytogenic feed additive; PUFA = Polyunsaturated fatty acid; RBC = Red blood cell; SFA = Saturated fatty acids; SOD = Superoxide dismutase; T3 = Tri-iodothyronine; T4 = Thyroxine; WBC = White blood cell; WP = whole plant

2.5 *Fagara zanthoxyloides*

2.5.1 Biology of *Fagara zanthoxyloides*

Fagara zanthoxyloides is an important medicinal plant in Africa that belongs to the Rutaceae family (Joshua *et al.*, 2016). It is known by other names such as artar root, candlewood, lime-prickly ash, Senegal prickly-ash, wild lime, *Zanthoxylum polyganum*, *Zanthoxylum senegalensis*, and *Zanthoxylum zanthoxyloides* (Ngassoum *et al.*, 2003; Ogwal-Okeng *et al.*, 2003; Joshua *et al.*, 2016; Guendéhou *et al.*, 2018). In Ghana, it is locally called "Haatso". *Fagara zanthoxyloides* is woody and grows to a height of about 12m (TPD, 2020a). It has a rough trunk that develops to a diameter of about 0.25m. The bark is gray and has large, sharp, and claw-shaped prickles on it (Plate 2.2). The prickles are widely distributed at regular intervals on the stem and branches. The branches are irregularly shaped with pinnate leaves on them. The leaves are also garnished with thorns. The fruits are capsules with diameter of about 5 to 6mm, as shown on Plate 2.3. Each fruit contains a single seed with a shiny black colour (Guendéhou *et al.*, 2018).

Plate 2. 2: *Fagara zanthoxyloides* plant

Plate 2. 3: Fruits of *Fagara zanthoxyloides*

2.5.2 Chemical composition of *Fagara zanthoxyloides*

Fagara zanthoxyloides is rich in several bioactive compounds such as alkaloids, amides, coumarins, lignins, flavonoids, glycosides, polyphenols, polyterpenes, saponins, sterols, and tannins (Adesina, 2005; Adefisoye *et al.*, 2012; Guendéhou *et al.*, 2018). Enechi *et al.* (2019) analysed the phytochemical constitution of the leaf extract and observed high levels of alkaloids, flavonoids, phenols, and terpenoids, moderate levels of glycosides and tannins, and low levels of saponins and steroids in the extract. In agreement with this report, Elujoba *et al.* (2005) reported that *Fagara zanthoxyloides* contains high amounts of the alkaloids - berberine, canthin-6-one, chelerythrine, fagaronine, and benzoic acid derivatives. Also, Jirovetz *et al.* (1997) reported the presence of limonene, myrcene, α-phellandrene, α-terpinolene, α-pinene, β-pinene, trans-β-ocimene, sabinene, citronellyl acetate, geraniol, terpinen-4-ol, p-cymene, and methyl citronellate in volatile oil extract of the seeds.

2.5.3 Uses of *Fagara zanthoxyloides*

The bark and leaves are often crushed into powder and used as condiments with smells similar to that of lime (Elbert, 1980). Traditionally, the bark, leaves, stem, and roots are used in medicinal preparations for the treatment of cold, colic, cough, dysentery, digestive disorders, fever, hypertension, dental caries, oral infections, sore gums, toothache, scabies, sickle cell disease, snakebites (Ngassoum *et al.*, 2003; Elujoba *et al.*, 2005; Guendéhou *et al.*, 2018), abdominal pain, dysmenorrhea, elephantiasis, gonorrhoea, lumbago, malaria, sexual impotence, and urinary diseases (Ogwal-Okeng *et al.*, 2003; Adesina, 2005). Also, the root extracts are used in embrocation to treat headaches, and intercostal, lumbar spine, and rheumatic pains (Guendéhou *et al.*, 2018). Moreover, the root powder is sniffed as an emmenagogue likewise the leaf powder to treat migraines

(Guendéhou *et al.*, 2018). In West Africa, parts of the plant are used as chewing sticks for tooth cleaning (Ogwal-Okeng *et al.*, 2003).

Enechi *et al.* (2019) demonstrated the antimalarial property of methanolic extract of the leaves. In that study, treatment with the extract normalized the biochemical and haematological anomalies caused by malaria in *Plasmodium berghei*- parasitised mice. Esan *et al.* (2014) reported that the root extract is equally effective in controlling malaria due to its inhibitory effects on the *in-vitro* growth and development of *Plasmodium falciparum*; a species of plasmodium reported to cause majority of deaths from malaria in Africa (WHO, 2020). The antimalarial property of the extract is attributable to its fagaronine content (Esan *et al.*, 2014).

Moreover, the root bark extracts have insecticidal properties (Denloye *et al.*, 2010). They effectively controlled insect pests of cowpea and maize grains such as *Callosobruchus maculatus*, *Sitophilus zeamais*, and *Tribolium castaneum* (Denloye *et al.*, 2010). Similarly, topical application of the root bark powder to cowpea increased ($p<0.05$) mortality of the seed beetle *Callosobruchus maculatus* and protected the grains from damage during storage (Musa, 2012).

Still, other researchers reported the analgesic, anticonvulsant, antidiabetic, anti-sickling, antioxidative, anthelmintic, antitumor, antitrypanosomal, antiviral, anticancer, hypolipidaemic, hypotensive, and molluscicidal properties of extracts of the plant (Prempeh, 2008; Guendéhou *et al.*, 2018; Dofuor *et al.*, 2019; Enechi *et al.*, 2019).

2.5.4 Antimicrobial properties of *Fagara zanthoxyloides* extracts

The antibacterial and antifungal effects of several extracts of the plant have been reported. For instance, in some *in vitro* studies, extracts of the fruits exhibited inhibitory effects on *Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes* (Gardini *et al.*, 2009), *Bacillus subtilis, Salmonella typhimurium, Streptococcus mutans, Micrococcus luteus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* (Misra *et al.*, 2013). This antimicrobial activity is attributable to the high geraniol content of the extracts (Gardini *et al.*, 2009; Sado Kamdem *et al.*, 2015). Another compound isolated from the fruit with antibacterial activity is 3,4,5,7-tetrahydroxy-1-methoxy-10-methyl-9-acridone. This compound inhibited the growth of *Pseudomonas aeruginosa* and *Micrococcus luteus* (Misra *et al.*, 2013; Wouatsa *et al.*, 2013). Similarly, Ngassoum *et al.* (2003) found volatile oil extract of the dried fruits to be effective against *Bacillus subtilis*, *Bacillus cereus*, *Corynebacterium glutamicum, Enterococcus faecalis*, *Escherichia coli*, *Streptococcus faecalis,* and *Staphylococcus aureus*. Also, the crude extract of the powdered stem displayed antibacterial effects on *Escherichia coli, Lactobacillus plantarum*, *Lactobacillus brevis*, and *Proteus vulgaris* (Adefisoye *et al.*, 2012). In an investigation by Ynalvez *et al.* (2012), the defatted ethanolic extract of the root bark showed great zones of inhibition against *Escherichia coli, Staphylococcus aureus*, and *Enterococcus faecium*. Likewise, other researchers reported the antibacterial effects of ethanolic and methanolic extracts of the plant against *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Staphylococcus aureus* (Adebiyi *et al.*, 2009; Adegbolagun and Olukemi, 2010). Similarly, the chemical and chromatographic fractions of the powdered root had effects on *Escherichia coli* and *Staphylococcus aureus* which is attributable to the berberine, canthin-6-one, chelerythrine, and flavonoid contents of the extracts (Odebiyi and Sofowora, 1979).

Besides, Osho and Adelani (2012) assayed aqueous and ethanolic extracts of chewing sticks made from the plant for their antifungal efficacy against the oral pathogens - *Candida tropicalis*, *Candida albicans,* and *Candida krusei*. Both extracts inhibited the growth of all the tested fungi.

2.5.5 Anthelmintic properties of *Fagara zanthoxyloides* extracts

In vitro studies with crude leaf extracts displayed anthelmintic effects on the sheep parasites *Haemonchus contortus* and *Trichostrongylus colubriformis* (Hounzangbe-Adote *et al.*, 2005; Azando *et al.*, 2011). Also, ethanolic and aqueous leaf extracts had effects on *Ascaris lumbricoides*, *Haemonchus contortus*, and *Trichostrongylus colubriformis* (Barnabas *et al.*, 2010; Azando *et al.*, 2011). Similarly, essential oil extract of the seeds affected *Strongyloides ratti*; a gastrointestinal parasite of rats (Olounladé *et al.*, 2012). Regular consumption of small amounts of the leaves by sheep reduced faecal *Haemonchus contortus* egg counts (Hounzangbe-Adote *et al.*, 2005). The ethanolic extract of the root bark was found to be effective against *Ascaris suum*; an endoparasite of pigs (Williams *et al.*, 2016). Owing to this property, extracts of the plant are used to treat ascariasis (Guendéhou *et al.*, 2018). Even in humans, methanolic extract of the root-bark is used to treat worm infections (Ogwal-Okeng, 1990). The flavonoid, terpene, and tannin contents of the extracts are responsible for the anthelmintic effects. These compounds inhibit the development of the larvae of helminths (Prempeh, 2008; Olounladé *et al.*, 2012). Additionally, tannins inhibit egg production from adult helminths (Athanasiadou *et al.*, 2001).

2.6 *Ocimum americanum*

2.6.1 Biology of *Ocimum americanum*

Ocimum americanum is an aromatic herb with lavender or white flowers that grows to a height of about 20 to 30cm (TPD, 2020b). Despite its name, *Ocimum americanum* is innate to Africa, the Indian subcontinent, Southeast Asia, and China (Junaid *et al.,* 2006). It is variously called American basil, English camphor basil, basilic camphor, hairy or hoary basil, lime or lemon basil, and *Ocimum canum* (Chagonda *et al.,* 2000; Anuradha, 2014; Gberikon *et al.*, 2018). In Ghana, it is locally known as "akokobesa" or "eme" (Berhow *et al.*, 2012). It belongs to the kingdom Plantae, phylum Tracheophyta, division Magnoliophyta, class Magnoliopsida, order Lamiales, family Lamiaceae, genus Ocimum, and specie americanum (Anuradha, 2014).

Plate 2. 4: *Ocimum americanum* plants

2.6.2 Chemical composition of *Ocimum americanum*

Anuradha (2014) analysed aqueous leaf extract of the plant for its phytochemical composition and found alkaloids, amino acids, carbohydrates, flavonoids, phenolic compounds, steroids, and terpenoids in it. In another study, Gberikon *et al.* (2018) observed significant concentrations of cardiac glycosides, steroids, and flavonoids in both flower and leaf extracts. The leaf extract additionally contained tannins that were absent in the flower extract (Gberikon *et al.*, 2018). Also, volatile oil extracted from aerial parts and leaves of the plant contained 1,8-cineole, bornyl acetate, bicyclogermacrene, borneol, cadinol, camphor, cedrol, endo-fenchyl acetate, eugenol, germacrene A, germacrene D, linalool, methyl chavicol, terpinen-4-ol, trans-caryophyllene, α-bergamotene, trans-β-terpineol, α-terpineol, β-elemene, and γ-cadinene (Nascimento *et al.*, 2011).

Furthermore, Mustafa and El-kamali (2019) performed proximate analyses on dried leaves and flowers of the plant and reported the results shown in Table 2.9.

Table 2. 8: Proximate composition of the foliage (at flowering) of *Ocimum americanum*

Nutrient (%)	Leaves	Flowers
Moisture	5.5±0.1	5.53±0.1
Crude protein	18.43±0.31	10.15±0.9
Total ash	19.09±0.1	8.87±0.05
Crude fibre	13.8±0.1	29.23±0.2

Source: Mustafa and El-kamali (2019)

2.6.3 Uses of *Ocimum americanum*

The leaves are used for cooking and in traditional medicines to treat cold, diabetes, fever, headaches, inflammation of joints, malaria, as well as genitourinary and skin diseases (Ngassoum *et al.,* 2004; Asase *et al.,* 2005; Berhow *et al.,* 2012; Gberikon *et al.,* 2018). Also, essential oils extracted from the plant are used to manufacture perfumes (Rady and Nazif, 2005). Furthermore, the phytoconstituents of *Ocimum americanum* have analgesic, anticancer, antiasthmatic, antistress, antibacterial (Nascimento *et al.,* 2011), antioxidative (Gberikon *et al.,* 2018), antidiabetic (Nyarko *et al.*, 2002), nematicidal, insecticidal, and fungicidal properties (Karuppusamy *et al.*, 2002).

2.6.4 Antimicrobial activity of *Ocimum americanum* extracts

Silver nanoparticles derived from aqueous *Ocimum americanum* leaf extract exhibited tremendous antibacterial activities against *Escherichia coli* and *Staphylococci aureus* (Anuradha, 2014). The antibacterial property of *Ocimum americanum* extracts is attributable to the cardiac glycosides and tannins in them (Gberikon *et al.,* 2018).

The antifungal property of extracts of *Ocimum americanum* has also been reported (Anuradha, 2014; Gberikon *et al.,* 2018). In a study conducted by Gberikon *et al.* (2018), the leaf and flower extracts had effects on *Trichophyton mentagrophytes*; a fungus that causes skin infections in animals and humans. Anuradha (2014) also found silver nanoparticles obtained from the aqueous leaf extract to inhibit the mycelia growth of *Aspergillus niger*. This antifungal effect is attributable to the methyl chavicol content of the leaves (Anuradha, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site and Duration of Study

This research work was carried out at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana. LIPREC is in the coastal savannah area of Ghana and on latitude $05^{0}40$'N and longitude $00^{0}16$'W. The area experiences a bimodal rainfall distribution. The major rainy season begins from March to July whilst the minor rains begin from August to November. The annual mean rainfall of the area ranges from 128 to 1709mm, and the annual mean temperature is 26.9°C. The relative humidity at LIPREC lies between 58 and 83.7% at 1500h.

3.2 Experimental Birds and Their Management

3.2.1 Brooding of birds

A total of four hundred unsexed one-day-old Cobb 500 broiler chicks were used for the trial. Before the birds arrived, the brooder house was cleaned and fumigated to eradicate pests in the building. Clay pots containing live charcoal and electric lamps were used to provide warmth for the birds. The floor of the brooder house was covered with wood shavings. The chicks on arrival were first introduced to clean drinking water mixed with glucose D and vitamins (see appendix A for composition). The glucose and vitamin mixture was given to relieve the birds of the stress they might have suffered during transportation. The glucose further supplied them with energy. The birds were allowed to drink for about two hours before feed was offered. They were brooded for seven days together on a common starter diet and fed *ad libitum* throughout this period. Not knowing how birds will react or respond to FFM and OLM since they are novel feed additives, it was planned for trial to start in week two about which time their body systems will be quite developed to handle such additives.

3.2.2 Distribution of birds after brooding

On the 8th day, the birds were transferred into an open-sided poultry house and kept there until the end of the feeding trial. A total of forty deep litter pens measuring 1.5m x 2.5m were used for raising the birds. Wood shavings served as bedding materials in the pens. Ten birds were randomly selected from the lot, weighed, and distributed into each of these pens in a completely randomised design. There were eight dietary treatment groups with five replicates each, and each replicate had 10 birds. Plate 3.1 is an image of the birds in one of the pens after distribution.

Plate 3. 1: Birds in a pen after distribution

3.2.3 Vaccination schedule

The birds were orally vaccinated against infectious bursal disease (IBD) on days 7, 21, and 35, and Newcastle disease on days 14 and 28.

3.2.4 Lightning

The birds were provided with lights in the evenings such that they had 24 hours of continuous light throughout the experiment.

3.2.5 Litter management

Wood shavings were used as litter materials throughout the experiment. They covered the floor to a depth of about 4cm during the brooding stage and between 6 and 8cm as the birds grew. The wood shavings were treated with insecticide, disinfected, and allowed to dry before use. The litter was turned every two to three days to avoid litter compaction and allow the escape of ammonia gas from it. Occasionally, wet and caked portions of the litter were scooped out and replaced with fresh ones. Wood shavings were changed every two weeks.

3.3 Preparation of Experimental Diets

The *Fagara zanthoxyloides* fruits and *Ocimum americanum* leaves were harvested from plants growing at LIPREC. They were air-dried under shade for five to seven days, ground in a Retsch mill through a one-mm-sieve into meals, stored, and used to prepare the experimental diets. Obtaining *Ocimum americanum* leaves was fairly easy. However, it was quite difficult to obtain *Fagara zanthoxyloides* fruits due to the thorns on the stems and leaves of the plant.

Eight experimental diets were formulated and fed to the birds. Diet BD was the basal diet and served as the negative control diet. The remaining diets were supplemented with FFM or OLM alone or in combination at different inclusion rates, or antibiotic (Penicillin V), as illustrated in Table 3.1. The basal diet (BD) served as the negative control diet whilst that supplemented with Penicillin V (PEN) was the positive control diet. The inclusion rates of FFM, OLM, and Penicillin V, shown in Table 3.1, were maintained for both the experimental starter and finisher diets.

Table 3. 1: Experimental diets and treatment layout

Dietary treatment	**Composition**	**No. of Replicates**	**No. of birds/replicate**
BD	Basal Diet (BD)	5	10
0.2FFM	BD + 0.2% FFM	5	10
0.4FFM	BD + 0.4% FFM	5	10
0.2OLM	BD + 0.2% OLM	5	10
0.4OLM	BD + 0.4% OLM	5	10
0.1FFM+0.1OLM	BD + 0.1% FFM + 0.1% OLM	5	10
0.2FFM+0.2OLM	BD + 0.2% FFM + 0.2% OLM	5	10
PEN	BD + 0.01% Penicillin V	5	10

The experimental starter diets were fed from day 8 to 28 and finisher diets from day 29 to 49. The ingredient compositions of the experimental starter and finisher diets are presented in Tables 3.2 and 3.3 respectively. The diets were supplied *ad libitum* throughout the trial.

Table 3. 2: Ingredient composition of experimental starter diets

	Percentage (%) inclusion of ingredients							
Ingredient	**BD***	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**
FFM	0.00	0.20	0.40	0.00	0.00	0.10	0.20	0.00
OLM	0.00	0.00	0.00	0.20	0.40	0.10	0.20	0.00
Penicillin V	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Maize	50.00	49.90	49.80	49.90	49.80	49.90	49.80	50.00
Soya bean meal	35.55	35.48	35.41	35.48	35.41	35.48	35.41	35.55
Wheat bran	12.00	11.98	11.95	11.98	11.95	11.98	11.95	12.00
Limestone	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DCP	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Broiler premix+	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	Calculated proximate composition (% DM)							
Dry matter	90.79	91.22	91.14	90.78	90.96	91.38	91.60	91.02
Ash	6.55	7.55	7.03	7.10	7.20	6.86	8.07	7.33
Ether extract	3.39	3.44	3.84	4.05	3.84	3.69	3.55	3.37
Crude protein	23.45	23.40	23.36	23.40	23.36	23.40	23.36	23.45
Crude fibre	3.90	4.04	4.12	4.32	4.59	3.93	4.51	3.89
ME (kcal/kg)	3048	3043	3037	3042	3037	3042	3037	3049
Cost (GH¢ 100kg)	199.22	200.04	200.86	199.84	200.46	199.94	200.66	207.22

FFM = *Fagara zanthoxyloides* fruit meal; OLM = *Ocimum americanum* leaf meal; DCP = Dicalcium phosphate; NaCl = Sodium chloride; ME= Metabolisable energy. Toxin binder (Biomin®) was added to diets at the manufacturer's recommended rate. +Broiler premix composition is shown in Appendix B. BD* served as the common starter diet fed to the birds during the brooding stage (from day 1 to 7).

Table 3. 3: Ingredient composition of experimental finisher diets

	Percentage (%) inclusion of ingredients							
Ingredient	**BD**	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**
FFM	0.00	0.20	0.40	0.00	0.00	0.10	0.20	0.00
OLM	0.00	0.00	0.00	0.20	0.40	0.10	0.20	0.00
Penicillin V	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Maize	56.61	56.50	56.39	56.50	56.39	56.50	56.39	56.61
Soya bean meal	23.55	23.50	23.45	23.50	23.45	23.50	23.45	23.54
Wheat bran	17.03	17.00	16.97	17.00	16.97	17.00	16.97	17.03
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DCP	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Broiler premix+	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	Calculated proximate composition (% DM)							
Dry matter	87.74	87.96	88.03	87.55	87.40	87.53	87.92	88.00
Ash	5.45	5.46	5.71	5.51	5.37	5.57	5.22	5.49
Ether extract	3.09	3.27	3.85	3.26	3.48	3.47	3.40	3.08
Crude protein	19.08	19.04	19.00	19.04	19.00	19.04	19.00	19.07
Crude fibre	5.43	5.99	5.82	5.51	5.89	6.01	6.39	5.42
ME (kcal/kg)	2966	2960	2954	2960	2954	2956	2954	2965
Cost (GH¢/ 100kg)	191.83	192.67	193.51	192.47	193.11	192.57	193.31	199.81

FFM = *Fagara zanthoxyloides* fruit meal; OLM = *Ocimum americanum* leaf meal; DCP = Dicalcium phosphate; NaCl = Sodium chloride; ME= Metabolisable energy. Toxin binder (Biomin®) was added to all the diets at the manufacturer's recommended rate. +Broiler premix composition is shown in Appendix B.

3.4 Growth Performance Determination

The birds were weighed at the start of the growth trial and subsequently at weekly intervals. Also, the amount of feed offered each time was recorded and that refused weighed weekly throughout the experiment. These values were used to estimate average daily feed intake (ADFI), average daily weight gain (ADG), and feed conversion ratio (FCR) using the formulas listed below.

$$\text{ADFI (g)} = \frac{\text{Feed offered – feed refused}}{\text{Number of days}}$$

$$\text{ADG (g)} = \frac{\text{Difference in body weights between two successive weeks}}{\text{Number of days}}$$

$$\text{FCR} = \frac{\text{Average daily feed intake (ADFI)}}{\text{Average daily weight gain (ADG)}}$$

Mortalities were accounted for in determining ADFI.

3.5 Mortalities

Mortalities were recorded each time they occurred throughout the experiment. Mortality percentage was estimated as the ratio of the number of dead birds to the total number of birds per treatment multiplied by 100, as represented below.

$$\text{Mortality (\%)} = \frac{\text{Number of dead birds}}{\text{Total number of birds per treatment}} \times 100$$

3.6 Serum Lipid Profile Test

After the feeding trial, five birds per treatment (a bird from each treatment replicate) were randomly selected, wing tagged, and starved overnight (about 9 hours). Thereafter, 2ml of blood was collected from their medial wing (brachial) veins by venipuncture into serum separator tubes (SSTs) containing gel and clot activator. The blood samples were allowed to stand for about 30 minutes to clot and then centrifuged at 3000 revolutions per minute (rpm) for 3 minutes. The sera were extracted and analysed for the concentrations of triglycerides, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using the fully automatic veterinary chemistry analyzer URIT-8021AVet (URIT Medical Electronic Co., Ltd.).

3.7 Carcass Analysis

The birds from which blood samples were taken for the serum lipid profile test were slaughtered and examined for weights of carcass parts, internal organs, and abdominal fat. Live weight before slaughter as well as de-feathered and dressed (carcass) weights were taken. The carcasses were cut into commercial parts consisting of necks, thighs, drumsticks, breasts, and wings. These parts as well as internal organs including the proventriculus, gizzard, liver, heart, and intestine were also weighed.

3.8 Digestibility Study

Three birds from each treatment were randomly selected and transferred into newly-prepared pens for a digestibility trial that lasted four days. The procedure followed that indicated by Aditya *et al.* (2017) however, slight modifications were made. The floors of the pens were covered with polythene sheets for easy collection of excreta. The birds were housed individually and allowed two days of acclimatisation to the new pens before data collection began. Afterwards, they were fasted overnight for about 11 hours to get rid of almost every digesta from their digestive tracts. The birds were then fed 120g of their respective finisher diets daily for three days. On the fourth day, feeds were withdrawn and leftover feeds weighed to determine total feed intake per bird. Excreta from the birds were collected regularly each day into containers. At the end of each day, the hoarded excreta from each bird was transferred from the collection container into a plastic bag and frozen at $-18^{o}C$. Excreta were collected also on the fourth day for 24 hours even though the birds were not fed this day to obtain the remaining faeces from the feeds they consumed. The birds had access to water throughout the digestibility trial.

Later, the excreta were removed from the freezer and allowed to thaw. The excreta collected from each bird over the four days were bulked and oven-dried until constant weight at $55^{o}C$. The oven-dried excreta were weighed, ground in a one-mm-sieve Retsch mill, and analysed for dry matter, ash, crude fibre, crude protein, and fat. Also, samples of the finisher diets served to the birds during the digestibility trial were analysed for the same nutrients. The results were used to estimate the apparent total tract digestibility for each nutrient using the formulae below stated by Liu *et al.* (2020).

$$\text{Apparent total tract nutrient digestibility (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient voided}}{\text{Nutrient intake}} \text{ X } 100$$

Nutrient intake = Nutrient in diet x Feed intake

Nutrient voided = Nutrient in faeces x Amount of faeces voided

3.9 Nitrogen Excretion Determination

The nitrogen (N) contents of the finisher diets and excreta of the birds used for the digestibility trial were used to estimate the relative amount of N excreted from each bird as 100% minus N digestibility.

3.10 Faecal Microbial Analysis

Eight new pens were prepared and their floors were covered with polythene sheets. Each day for five days, four birds from each treatment replicate were randomly selected from their original pens and transferred into the new pens. Faecal samples were collected into sterile falcon tubes and stored at 4°C for analysis. Faecal collection was done immediately after they were voided to avoid contamination. The faecal samples were analysed for total plate count (microbial load), and counts of coliforms, *Escherichia coli*, *Salmonella* and *Shigella*, as well as yeasts and moulds.

Faecal microbial analysis followed the procedure indicated by Murugesan *et al.* (2015) but slight modifications were made. 1g of each sample was taken, and 1000µl of sterile saline solution was added to it and thoroughly mixed. An aliquot of the resulting homogenous solution was taken and serially diluted in sterile saline solution from 10^{-2} to 10^{-12}, cultured with the appropriate agar media (see appendix C), and incubated. The agar plates for microbial load, coliforms, and *Salmonella* and *Shigella* were incubated at 37°C for 24 hours, *Escherichia coli* at 44°C for 24 hours, and yeasts and moulds at 37°C for 72 hours. Thereafter, visible microbial colonies that appeared on the culture plates were counted, and the results were expressed as $\log_{10}$ CFU (colony-forming units) per gram of the samples before statistical analysis.

3.11 Chemical Analyses

Samples of the experimental diets, FFM, OLM, and excreta were analysed for dry matter (DM), ash, crude fibre (CF), crude protein (CP), and ether extract (EE) following the methods described by the Association of Official Analytical Chemists (AOAC, 2000). The results were all expressed on percentage dry matter basis.

3.12 Statistical Analysis

The data collected were all subjected to analysis of variance (ANOVA) and analysed as a completely randomised design (CRD) using the Genstat statistical software (12th edition, 2009). Mean values with probability less than 5% ($p<0.05$) were considered significant and separated using the Student Newman-Keuls (SNK) test. The weight of birds at the onset of the trial (d 8) were not statistically different ($p>0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Nutrient Composition of FFM and OLM

The results of the proximate analysis performed on samples of FFM and OLM to ascertain their nutritive value are presented in Table 4.1.

Table 4. 1: Nutrient composition of FFM and OLM

Nutrient (% DM)	**FFM**	**OLM**
Dry matter	89.40	92.22
Crude protein	16.13	15.03
Ash	6.95	8.75
Fat	23.76	6.25
Crude fibre	25.23	31.98
ME (Kcal/kg)	2924	1306

FFM = *Fagara zanthoxyloides* fruit meal; OLM = *Ocimum americanum* leaf meal; ME= Metabolisable Energy

4.2 Effects of Dietary Treatments on Growth Performance

4.2.1 Effects of Dietary Treatments on Body Weight

Shown in Table 4.2 are the effects of the dietary treatments on body weight. Compared with the negative control group (BD), there were no significant differences in body weight throughout the experiment. However, at the end of the first week, birds fed 0.2FFM were heavier ($p<0.05$) than that fed PEN. Also, in week 3, the weight of birds fed 0.2FFM was higher than those fed 0.2OLM and 0.1FFM+0.1OLM. Birds in the latter groups however recorded similar ($p>0.05$) weight.

Table 4. 2: Body weight of broilers fed diets supplemented with FFM, OLM and penicillin

Experimental week	Dietary treatment means								SEM	p-value
	BD	0.2FFM	0.4FFM	0.2OLM	0.4OLM	0.1FFM+ 0.1OLM	0.2FFM+ 0.2OLM	PEN		
0	156.7	162.3	155.7	153.3	154.8	155.9	156.4	152.2	4.25	0.448
1	409.2^{ab}	427.8^{a}	397.8^{ab}	394.2^{ab}	409.8^{ab}	391.4^{ab}	393.2^{ab}	382.5^{b}	11.99	0.024
2	838.6	864.6	814.5	801.1	826.3	795.4	828.2	811.9	21.45	0.072
3	1272.4^{ab}	1316.4^{a}	1243.3^{ab}	1193.8^{b}	1245.8^{ab}	1194.6^{b}	1265.8^{ab}	1252.8^{ab}	37.09	0.049
4	1722	1772	1680	1662	1693	1649	1709	1705	53.0	0.426
5	2220	2219	2152	2079	2203	2136	2181	2130	71.3	0.472
6	2597	2516	2543	2500	2633	2510	2553	2434	100.1	0.633

^{a,b} Means on the same row bearing similar superscripts are not significantly different ($p>0.05$)
SEM = Standard error of means

4.2.2 Effects of Dietary Treatments on Average Daily Weight Gain

Dietary supplementation with FFM, OLM, and penicillin did not affect (p>0.05) average daily weight gain (ADG) throughout the trial in comparison with birds fed the basal diet (BD). However, only in week 1, birds fed 0.2FFM gained more weight on daily basis than those fed 0.1FFM+0.1OLM, 0.2FFM+0.2OLM and PEN. Thereafter, ADG remained uniform (p>0.05) among the eight dietary treatment groups, as depicted in Table 4.3.

Table 4. 3: Average daily weight gain (g) of broilers fed diets supplemented with FFM, OLM and penicillin

Experimental week	Dietary treatment means								SEM	p-value
	BD	0.2FFM	0.4FFM	0.2OLM	0.4OLM	0.1FFM+ 0.1OLM	0.2FFM+ 0.2OLM	PEN		
1	36.07[ab]	37.93[a]	34.59[ab]	34.41[ab]	36.43[ab]	33.64[b]	33.83[b]	32.90[b]	1.320	0.012
2	61.34	62.40	59.52	58.13	59.50	57.71	62.14	61.34	1.927	0.140
3	61.97	64.54	61.26	56.09	59.93	57.03	62.51	62.99	3.082	0.123
4	64.3	65.1	62.3	66.9	64.0	64.9	63.3	64.6	4.01	0.976
5	65.7	63.9	67.5	59.6	72.8	69.5	67.4	60.8	5.07	0.207
6	53.8	42.4	55.8	60.1	61.5	53.5	53.1	46.5	7.69	0.252

[a,b] Means on the same row bearing similar superscripts are not significantly different (p>0.05).
SEM = Standard error of means

4.2.3 Effects of Dietary Treatments on Average Daily Feed Intake

The effects of dietary treatments on average daily feed intake are presented in Table 4.4. In comparison with the negative control group (BD), there were no differences (p>0.05) in average daily feed intake among the treatment groups. However, birds in the 0.2FFM group consumed more (p<0.05) feed than those in PEN in week 1.

Table 4. 4: Average daily feed intake (g) of broilers fed diets supplemented with FFM, OLM and penicillin

Experimental week	Dietary treatment means								SEM	p-value
	BD	0.2FFM	0.4FFM	0.2OLM	0.4OLM	0.1FFM+ 0.1OLM	0.2FFM+ 0.2OLM	PEN		
1	60.63^{ab}	63.37^{a}	61.60^{ab}	60.17^{ab}	59.66^{ab}	56.94^{ab}	56.11^{ab}	54.86^{b}	2.384	0.017
2	123.5	131.6	125.3	124.9	122.6	120.2	119.4	124.3	4.57	0.271
3	133.0	133.1	137.5	128.2	128.4	135.4	128.3	132.9	5.72	0.632
4	148.4	153.8	150.2	146.4	145.5	144.1	145.3	144.3	7.35	0.882
5	160.5	169.6	159.4	160.2	167.0	155.3	167.3	156.3	7.34	0.410
6	148.3	160.5	160.4	159.6	162.0	158.3	162.9	152.8	9.92	0.819

^{a,b} Means on the same row bearing similar superscripts are not significantly different (p>0.05).
SEM = Standard error of means

4.2.4 Effects of Dietary Treatments on Feed Conversion Ratio

Throughout the six-week experimental period, feed conversion ratio was unaffected (p>0.05) by treatment diets, as displayed in Table 4.5.

Table 4. 5: Feed conversion ratio of broilers fed diets supplemented with FFM, OLM and penicillin

Experimental week	Dietary treatment means								SEM	p-value
	BD	0.2FFM	0.4FFM	0.2OLM	0.4OLM	0.1FFM+0.1OLM	0.2FFM+0.2OLM	PEN		
1	1.68	1.68	1.78	1.75	1.64	1.70	1.66	1.67	0.069	0.468
2	2.02	2.11	2.11	2.16	2.06	2.09	1.92	2.04	0.106	0.492
3	2.16	2.06	2.25	2.31	2.15	2.38	2.07	2.11	0.143	0.293
4	2.32	2.41	2.42	2.22	2.28	2.23	2.31	2.24	0.171	0.883
5	2.31	2.75	2.38	2.74	2.31	2.26	2.51	2.58	0.249	0.314
6	2.83	4.7	2.98	2.70	2.64	3.11	3.13	3.68	0.708	0.128

All the means in this table are not significantly different (p>0.05).

SEM = Standard error of means

4.3 Effects of Dietary Treatments on Mortality

As presented in Table 4.6, dietary treatments did not affect (p<0.05) mortality throughout the study.

Table 4. 6: Mortality (%) of broilers fed diets supplemented with FFM, OLM and penicillin

Experimental week	Dietary treatment means								SEM	p-value
	BD	0.2FFM	0.4FFM	0.2OLM	0.4OLM	0.1FFM+0.1OLM	0.2FFM+0.2OLM	PEN		
1-6	2.00	4.00	10.00	8.00	2.00	4.00	2.00	8.00	3.761	0.224

The means in this table are not significantly different (p>0.05).

SEM = Standard error of means

4.4 Effects of Dietary Treatments on Carcass and Organ Characteristics

There were no significant differences (p>0.05) among the eight dietary treatment groups in terms of live weight before slaughter, as well as de-feathered, dressed, carcass part, abdominal fat (Table 4.7), and internal organ (Tables 4.8) weights.

Table 4. 7: Carcass characteristics of broilers fed diets supplemented with FFM, OLM and penicillin

	Dietary treatment means									
Parameter (g)	**BD**	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**	**SEM**	**p-value**
Live w.	2652	2798	2616	2541	2768	2606	3012	2662	162.2	0.150
Defeathered w.	2470	2591	2439	2378	2547	2419	2778	2473	146.4	0.203
Dressed w.	2056	2127	2019	1960	2112	2002	2328	2081	135.8	0.252
Neck	99.4	98.4	92.6	94.6	99.0	110.6	113.0	103.4	7.33	0.100
Breast	791	863	775	758	804	703	881	749	73.7	0.296
Drumsticks	280.6	298.2	267.8	265.8	270.6	264.8	291.0	274.0	23.66	0.794
Thighs	307.0	312.0	299.2	308.0	314.6	316.0	344.2	328.0	24.07	0.686
Wings	225.0	215.6	242.0	199.6	221.4	237.8	247.0	216.6	16.07	0.101
Abdominal fat	11.6	17.8	20.0	17.6	14.2	14.2	18.8	17.8	6.49	0.903

All the means in this table are not significantly different (p>0.05).

SEM = Standard error of means; w. = weight

Table 4. 8: Internal organ characteristics of broilers fed diets supplemented with FFM, OLM and penicillin

	Dietary treatment means									
Parameter (g)	**BD**	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**	**SEM**	**p-value**
Proventriculus	8.40	10.00	9.40	9.40	10.40	10.20	11.40	9.40	1.24	0.428
F. gizzard	64.0	63.6	55.6	55.8	67.0	63.0	62.6	53.6	6.20	0.310
E. gizzard	43.0	45.8	40.4	40.6	42.2	43.2	44.4	36.8	4.31	0.568
Liver	36.0	39.6	36.8	34.0	41.8	36.4	42.2	35.8	3.35	0.173
Heart	8.80	9.00	8.40	8.60	10.80	9.80	9.20	9.40	1.02	0.362
F. intestine	75.4	89.6	75.6	76.8	85.2	89.0	91.8	69.2	8.48	0.101
E. intestine	53.8	66.2	55.6	54.4	60.4	65.0	66.8	52.2	5.91	0.078

All the means in this table are not statistically different ($p>0.05$)

E. = Empty; F. = Full; SEM = Standard error of means

4.5 Effects of Dietary Treatments on Apparent Total Tract Nutrient Digestibility and Nitrogen Excretion

The results for apparent total tract nutrient digestibility and nitrogen excretion are both presented in Table 4.9. Dry matter, crude protein, ash, and crude fibre digestibility, as well as nitrogen excretion, were not influenced (p>0.05) by the treatment diets. However, in terms of fat digestibility, there was a significant difference between the treatment groups and the negative control group.

Table 4. 9: Apparent total tract nutrient digestibility and excreted nitrogen in broilers fed diets supplemented with FFM, OLM and penicillin

	Dietary treatment means									
Nutrient (%)	**BD**	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**	**SEM**	**p-value**
Dry matter	74.40	71.08	74.38	73.69	74.96	72.54	75.72	75.98	2.218	0.418
Crude protein	68.6	59.9	70.1	67.0	69.5	65.5	72.1	67.8	4.99	0.420
Fat	97.75^a	62.59^b	70.62^b	67.99^b	66.74^b	64.12^b	64.50^b	72.25^b	3.376	<.001
Ash	28.4	25.7	38.7	24.4	24.2	25.7	30.4	37.1	8.93	0.592
Crude fibre	38.3	47.8	49.8	43.4	48.0	39.2	50.2	51.3	5.10	0.133
Excreted N	31.4	40.1	29.9	33.0	30.5	34.5	27.9	32.2	4.99	0.420

[a,b] Means on the same row bearing different superscripts are significantly different (p<0.05).
N = Nitrogen; SEM = Standard error of means

The supplements (FFM, OLM and penicillin) caused a significant decrease ($p<0.05$) in fat digestibility to the same extent as shown in Figure 4.1.

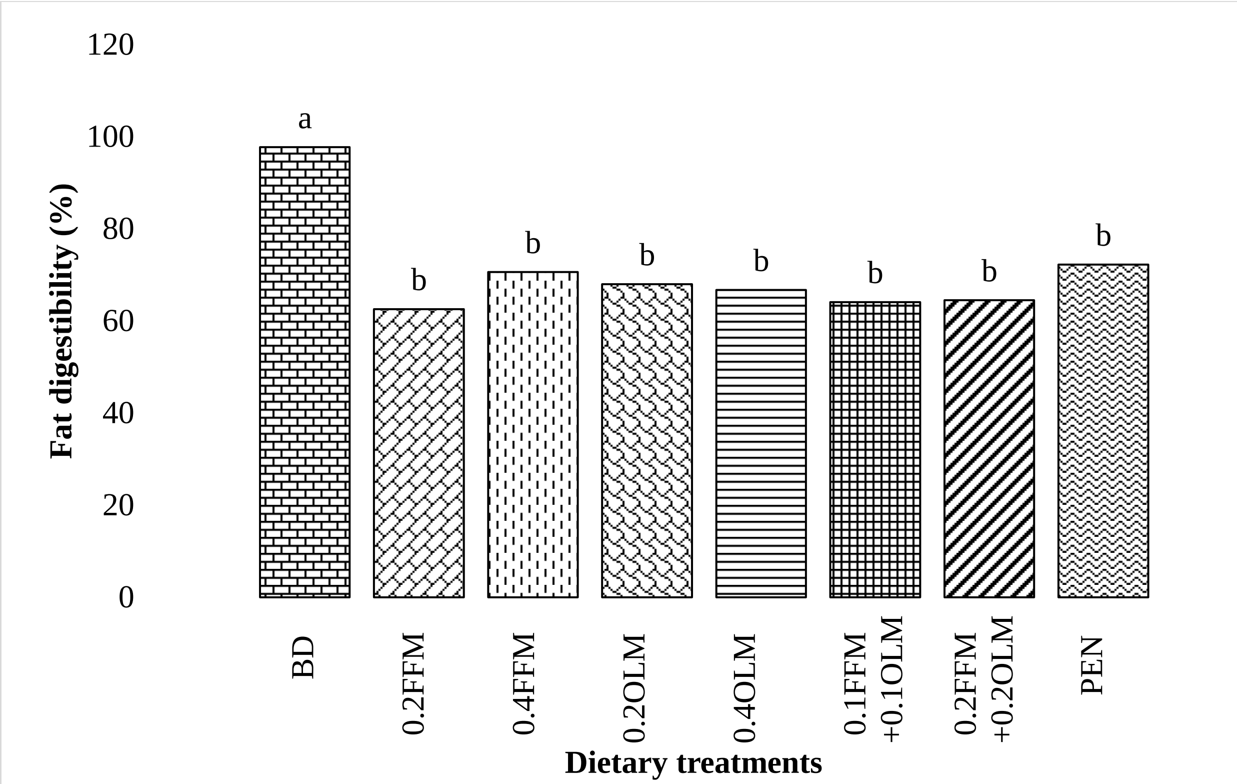

Figure 4. 1: Apparent total tract fat digestibility

[a,b] Bars with similar letters are not statistically different ($p>0.05$)

4.6 Effects of Dietary Treatments on Serum Lipid Profile

The results of the serum lipid analysis in Table 4.10 reveal no effects (p>0.05) of the dietary treatments on the concentrations of cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides.

Table 4. 10: Serum lipid profile of broilers fed diets supplemented with FFM, OLM and penicillin

	Dietary treatment means									
Variable (mmol/L)	**BD**	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**	**SEM**	**p-value**
Cholesterol	3.82	4.13	2.47	3.75	3.75	3.75	3.65	3.97	0.500	0.086
HDL	1.102	1.170	0.652	1.206	1.104	1.114	1.042	1.156	0.1737	0.085
LDL	2.456	2.692	1.632	2.224	2.384	2.308	2.348	2.504	0.3174	0.103
Triglycerides	0.568	0.600	0.418	0.708	0.580	0.728	0.580	0.684	0.1613	0.622

All the means in this table are not significantly different (p>0.05).

SEM = Standard error of means

4.7 Effects of Dietary Treatments on Faecal Microbial Composition

The results for the faecal microbial analysis are presented in Table 4.11. Except for the counts of total microbes (microbial load) and yeasts and moulds, the counts of coliforms, *Escherichia coli*, as well as *Salmonella* and *Shigella* in faecal samples were not affected ($p>0.05$) by the dietary treatments.

Table 4. 11: Faecal microbial composition of broilers fed diets supplemented with FFM, OLM and penicillin

Parameter ($\log_{10}$ CFU/g)	**Dietary treatment means**									
	BD	**0.2FFM**	**0.4FFM**	**0.2OLM**	**0.4OLM**	**0.1FFM+ 0.1OLM**	**0.2FFM+ 0.2OLM**	**PEN**	**SEM**	**P-value**
Microbial load	13.23[a]	10.65[b]	9.46[b]	10.24[b]	10.08[b]	9.46[b]	9.53[b]	9.11[b]	0.605	<.001
Coliform	5.01	5.21	4.51	2.39	5.11	3.99	4.65	4.16	0.875	0.066
E. coli	5.07	1.59	1.35	1.74	1.34	1.70	1.64	2.12	1.221	0.083
SS	4.73	5.48	4.91	1.85	4.90	4.29	3.92	4.72	1.071	0.071
Y and M	8.28[a]	6.91[a]	4.79[ab]	7.10[a]	4.91[ab]	6.31[a]	5.86[a]	2.36[b]	1.184	0.001

[a,b] Means on the same row bearing different superscripts are significantly different ($p<0.05$).
CFU = Colony-forming units; *E. coli* = *Escherichia coli*; SEM = Standard error of means; SS = *Salmonella* and *Shigella*; Y and M = Yeasts and Moulds

FFM, OLM, and penicillin reduced (p<0.05) faecal microbial load to the same extent when compared with birds that received the basal diet (BD), as depicted in Figure 4.2.

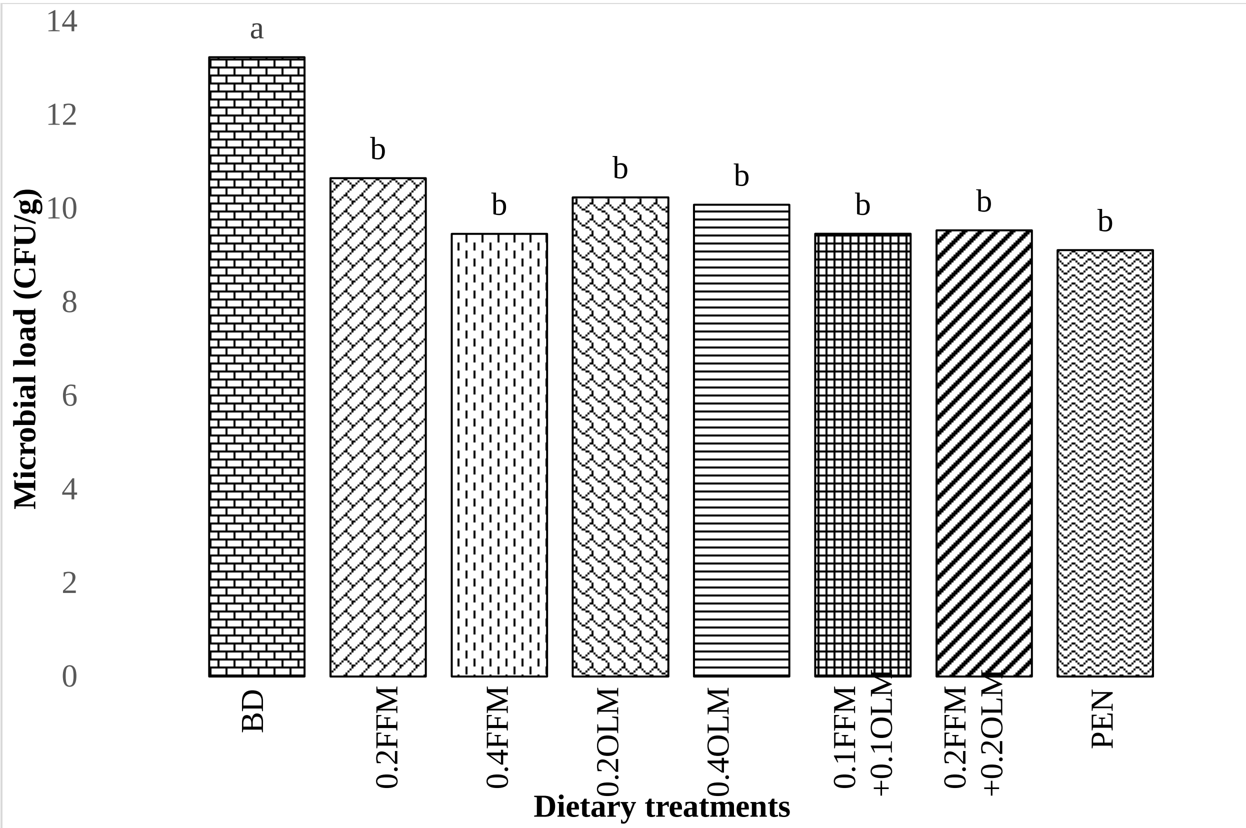

Figure 4. 2: Faecal microbial load

[a,b] Bars with similar letters are not statistically different (*p*>0.05)

Furthermore, birds fed the diet supplemented with penicillin (PEN) recorded the least faecal count of yeasts and moulds which was not different (p>0.05) from that of birds fed 0.4FFM and 0.4OLM. However, the counts of yeasts and moulds in faecal samples of the birds fed the remaining diets (BD, 0.2FFM, 0.2OLM, 0.1FFM+0.1OLM, and 0.2FFM+0.2OLM) was similar (p>0.05), as shown in Figure 4.3.

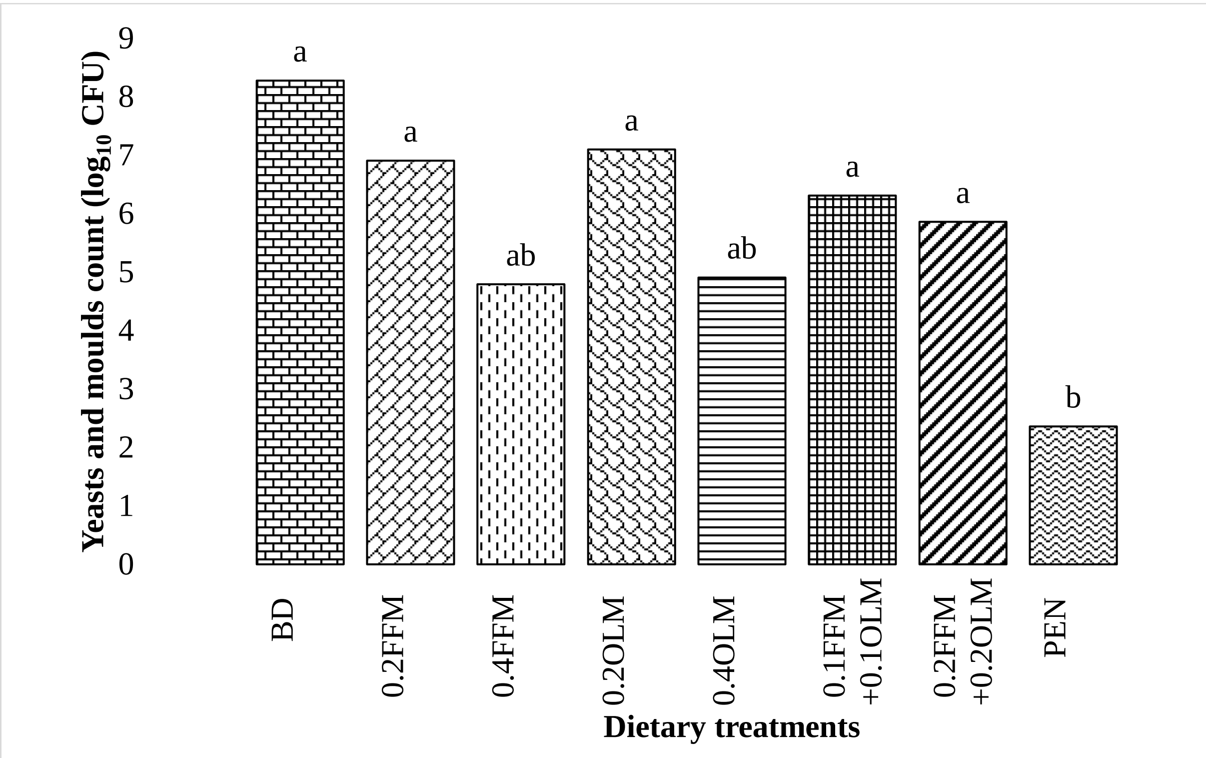

Figure 4. 3: Faecal count of yeasts and moulds

[a,b] Bars with similar letters are not statistically different (*p*>0.05)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of FFM, OLM and Penicillin Supplementation on Growth Performance

5.1.1 Effects of penicillin supplementation on growth performance

In the present study, supplementing diet with penicillin did not affect feed intake, body weight gain, and feed conversion ratio compared with the negative control group. This result is consistent with those of other researchers who supplemented broiler diets with lincomycin (Proudfoot *et al.,* 1990) and avilamycin (Riyazi *et al.,* 2015). Similarly, Baurhoo *et al.* (2009) reported no growth-promoting effect of bacitracin or virginiamycin in feed on broilers. In contrast, Onu *et al.* (2004) fed broilers a diet containing penicillin at the same inclusion rate tested in this study (i.e. 100ppm) and observed significant increases in feed intake, body weight gain, and feed conversion efficiency. Other scientists equally found significant improvement effects of antibiotic supplementation on broiler growth performance (Onifade, 1997; Guban *et al.,* 2006).

The absence of growth-promoting effects of penicillin noted in this study could be due to the low stocking density, high biosecurity, and good hygienic conditions under which the birds were raised. It is well established that birds that are well-nourished and reared in clean environments and at moderate stocking densities do not respond positively to antibiotic growth promoters (Prescott and Baggot, 1993; Anderson *et al.,* 1999) and that, the growth-promoting effects of antibiotics are realized only when animals are raised under unhygienic conditions (Sahu and Saxena, 2014; Biswas *et al.,* 2017). This implies that under hygienic rearing conditions, farmers do not need to add antibiotics to diets.

Furthermore, antibiotics are well known to promote growth through their antibacterial effects on pathogenic gut microbes (Butaye *et al.*, 2003). However, penicillin in this study did not affect any of the tested pathogenic microbes (coliforms, *Escherichia coli*, and *Salmonella* and *Shigella*). This can further explain why the birds fed the penicillin-supplemented diet did not show any improvement in growth.

5.1.2 Effects of FFM and OLM supplementation on growth performance

FFM and OLM at all the inclusion rates tested in this experiment did not affect growth performance in comparison with birds fed the basal diet. This finding concurs with those of other researchers who admixed diets with PFAs (Hernandez *et al.*, 2004; Mohammed and Yusuf, 2011; Issa and Omar, 2012; Ojelade *et al.*, 2012; Riyazi *et al.*, 2015). For example, Ojelade *et al.* (2012) supplemented broiler diets with *Hibiscus sabdariffa* (roselle) calyx meal and/or *Ocimum gratissimum* (African basil) leaf meal and found no effects on feed intake, body weight gain, and feed conversion ratio. Similar observations were made when broiler rations were supplemented with ginger powder (Mohammed and Yusuf, 2011), garlic powder (Issa and Omar, 2012), and sweet basil essential oil (Riyazi *et al.*, 2015). Conversely, inclusion of Shatavari root powder (Rekhate *et al.*, 2004), thyme or cinnamon essential oil (Al-Kassie, 2009), marjoram leaf meal (Ali, 2014), aridan fruit powder (Kana *et al.*, 2017), and Ashanti pepper leaf or seed meal (Effiong and Ochagu, 2019) in broiler diets significantly increased growth performance.

The inclusion of FFM and OLM in diets was expected to improve growth performance. The absence of growth-promoting effects could be due to the low dietary inclusion rates tested in this study. Yang *et al.* (2009) reported that the inclusion rates of phytogenic products determine the concentrations of their active compounds in diets and ultimately their effects on farm animals. Higher inclusion rates of FFM and OLM beyond 0.4% could have increased growth performance. Parallel to this assertion, Al-Kassie *et al.* (2011a) added a mixture of cumin and turmeric to broiler diet at 0.25% and observed no effect ($p>0.05$) on body weight gain. However, higher inclusion rates of the mixture (0.5, 0.75, and 1%) increased body weight gain significantly (Al-Kassie *et al.*, 2011a). Similarly, increasing the supplementation rate of garlic powder in ration from 1 to 3% increased ($p<0.05$) body weight gain and final body weight of broilers (Mulugeta *et al.*, 2019).

Phytogenic feed additives also promote growth through their antimicrobial effects on pathogenic gut microbes (Al-Kassie, 2009; Goodarzi *et al.*, 2014; Murugesan *et al.*, 2015). As such, in clean rearing environments where pathogens are absent or at minimal levels, phytogenic feed additives do not influence growth, similar to antibiotics. In this research work, FFM and OLM did not affect the counts of the tested pathogenic microbes and also, the rearing environment was good. This may further explain the non-effect of FFM and OLM on growth performance.

Furthermore, the failure of FFM and OLM to improve protein digestibility or reduce nitrogen excretion in this study may also explain their inability to promote growth. This is because phytogenic supplements that promote growth do so by increasing protein digestibility and decreasing nitrogen excretion (Abbas, 2010; Jahejo *et al.*, 2019).

Even though FFM and OLM did not promote growth, they had no negative effects on growth performance.

Since FFM and OLM did not promote the growth performance of birds, there is no need to include them in diets at the levels tested in this study, especially when the rearing conditions are good.

5.1.3 Effects of penicillin, FFM and OLM supplementation on feed intake

In this study, there was no influence of the antibiotic or herbal products on feed intake compared to the control group. Similar to this finding, other researchers observed no effects of dietary supplementation with avilamycin (Hernandez *et al.*, 2004), bacitracin or virginiamycin (Baurhoo *et al.*, 2009) on feed intake by broilers. These results contradict the findings of Onifade (1997) and Guban *et al.* (2006) who observed increased feed intake among birds fed diets supplemented with antibiotics. Similar to the finding of the present study, Avila-Ramos *et al.* (2012) and Goliomytis *et al.* (2014) found no effects of phytogenic feed additives on broiler feed intake. In contrast, some scientists observed increased feed intake among birds fed diets containing phytogenic products (Ragab *et al.*, 2013; EL-Shoukary *et al.*, 2014; Wan *et al.*, 2017).

Possibly, FFM and OLM at the tested dietary inclusion rates did not impact the taste, flavour, and palatability of the feeds to influence feed intake. Borneol is a compound present in some medicinal plants that stimulate appetite of farm animals (Mirzaei-Aghsaghali, 2012). It is present in aniseed (Al-Kassie, 2008), hot red pepper (Al-Kassie *et al.*, 2011b), coriander (EL-Shoukary *et al.*, 2014), as well as *Ocimum americanum* (Nascimento *et al.*, 2011). Consequently, addition of aniseed (Al-Kassie, 2008), coriander seeds (EL-Shoukary *et al.*, 2014), and hot red pepper (Al-Kassie *et al.*, 2011b) to broiler diets increased feed consumption. However, in this experiment, OLM did not affect feed intake. It could be that due to the low dietary inclusion rates, the concentration of borneol in the feeds were not sufficient to improve feed intake.

5.2 Effects of Penicillin, FFM and OLM Supplementation on Carcass Parameters

Dietary supplementation with penicillin did not affect any of the carcass variables measured in this study, in agreement with the findings of Baurhoo *et al.* (2009) who observed no effect of bacitracin or virginiamycin in broiler diet on the relative weights of drumsticks, thighs, whole breast, and wings. Still parallel with these observations, Bozkurt *et al.* (2008) supplemented broiler diet with avilamycin, and Attia *et al.* (2011) with flavomycin and found no impacts ($p>0.05$) on carcass variables. Contrarily, Onifade (1997) reported significant increases in some carcass parameters of broilers fed diets containing antibiotics. Also, antibiotics in poultry diets reduce intestine weight by shortening the gut and thinning the wall of the intestine (Gaskins *et al.*, 2002). This report does not concur with the result of the present research since no difference (p>0.05) in intestine weight was observed between birds fed the penicillin-supplemented diet and the negative control group.

The non-effect (p>0.05) of FFM and OLM on the weights of carcass cuts, internal organs, and abdominal fat conforms to the finding of Riyazi *et al.* (2015) who observed no significant effects of dietary supplementation with sweet basil essential oil on carcass, breast, thigh, liver, gizzard, heart, and abdominal fat weights of broilers. Hernandez *et al.* (2004) also found no effects of plant extracts in broiler rations on the relative weights of the liver, pancreas, proventriculus, ventriculus, and small and large intestines. Also, the inclusion of fenugreek, parsley, and sweet basil seeds in feeds for broilers did not affect any of the carcass variables measured by Abbas (2010). Still consistent with these findings, other researchers supplemented broiler diets with ginger root powder, ginger essential oil (Habibi *et al.*, 2014), and onion extract (Aditya *et al.*, 2017) and noticed no effects on slaughter characteristics. In contrast, Wan *et al.* (2017) supplemented diets of male broiler chickens with enzymatically-treated *Artemisia annua* whole plant and observed significant increases in carcass parameters. Similarly, Eltazi (2014) reported significant increases in the percentages of dressed carcass cuts and a decrease in abdominal fat of broilers fed diets containing a mixture of garlic and ginger powder. Likewise, dietary supplementation with 200ppm essential oil extract of either thyme or cinnamon decreased abdominal fat and increased dressing percentage as well as liver and gizzard weights of broilers (Al-Kassie, 2009).

The similarity in carcass variables among the birds used in this study may be due to the similarity in their growth performance. As explained by Ferket and Gernat (2006), factors such as feed intake, feed conversion efficiency, and body weight gain of meat birds affect their carcass characteristics.

5.3 Effects of Penicillin, FFM and OLM Supplementation on Nutrient Digestibility

In this experiment, dry matter, crude protein, ash, and crude fibre digestibility were not altered ($p>0.05$) by either the antibiotic or herbal products. This finding agrees with that of Jamroz *et al.* (2003) who observed no effects of avilamycin or a blend of carvacrol, capsaicin, and cinnamaldehyde in broiler diets on apparent ileal dry matter, crude ash, and nitrogen digestibility. Equally, dietary supplementation with flavomycin had no impacts on apparent total tract ash retention, and dry matter, crude protein, and crude fibre digestibility in broilers (Attia *et al.*, 2011). Contrarily, Hernandez *et al.* (2004) observed improvements ($p<0.05$) in apparent whole tract dry matter and crude protein digestibility in finisher broiler chickens fed diets supplemented with avilamycin or plant extracts. Similarly, other researchers who supplemented broiler diets with bacitracin methylene disalicylate (Murugesan *et al.*, 2015) and garlic powder (Issa and Omar, 2012) observed significant increases in total tract dry matter and crude protein digestibility.

Likely, penicillin, FFM, and OLM did not affect the secretion of gastric and pancreatic juices or activity of the protein-digesting enzymes; chymotrypsin, elastase, pepsin, and trypsin. This could explain the similarity in protein digestibility between birds fed the supplemented and non-supplemented diet. The similarity in protein digestibility may partially explain the similarity in body weight. Also, the non-effect of the dietary treatments on fibre digestibility imply a similarity in intestinal transit time. An increase in fibre digestibility leads to a corresponding increase in intestinal transit time which makes animals consume more feed (Burrows *et al.*, 1982). The similarity in fibre digestibility among the experimental birds may therefore explain the similarity in feed intake.

The decline in fat digestibility observed among the birds fed the antibiotic and herb supplemented diets contradicts the findings of Guban *et al.* (2006) and Issa and Omar (2012) who observed better ($p<0.05$) fat digestibility among broilers fed diets supplemented with monensin and garlic powder respectively. Still contrary to the results of this study, other researchers who supplemented broiler diets with flavomycin (Attia *et al.*, 2011) and plant extracts (Jamroz *et al.*, 2003; Hernandez *et al.*, 2004) found no effects on fat digestibility. The depression in fat digestibility caused by penicillin, FFM, and OLM may be due to inhibitory effects on the activity of pancreatic lipase and/or the synthesis, deconjugation, and release of bile salts to emulsify fats for lipid digestion and absorption. The tannins and saponins in FFM and OLM may be responsible for these effects, as inferred from the report of Serrano *et al.* (2009) who indicated that antinutritional factors can inhibit the activity of digestive enzymes and reduce nutrient availability for the host animal. This assertion contradicts the report of other scientists who indicated that some herbal feed additives improve bile flow, likewise the synthesis, secretion, and activity of pancreatic enzymes (Jang *et al.*, 2007; Hashemipour *et al.*, 2014).

5.4 Effects of Penicillin, FFM and OLM Supplementation on Nitrogen Excretion

Some researchers indicated that antibiotics included in feed at growth-promoting levels increase nitrogen retention and utilization to form amino acids for growth enhancement and thus, reduce nitrogen excretion from animals (Wierup, 2001; Gaskins *et al.*, 2002). Also, Jahejo *et al.* (2019) found that supplementing broiler diets with sweet basil seed powder significantly reduced ($p<0.05$) nitrogen excretion. Contrary to these reports, the relative amount of nitrogen excreted from the broilers used for this trial was not affected ($p>0.05$) by treatment diets. This could be due to the similarity in crude protein digestibility. Similar to the result of the current study, Jamroz *et al.* (2003) supplemented broiler diet with avilamycin or a blend of carvacrol, capsaicin, and cinnamaldehyde and observed no effects on nitrogen excretion.

5.5 Effects of Penicillin, FFM and OLM Supplementation on Serum Lipid Profile

In the present investigation, neither did the antibiotic nor herbal products affect serum cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. Riyazi *et al.* (2015) reported a similar observation when broiler diet was supplemented with avilamycin or sweet basil essential oil. Likewise, Eslami *et al.* (2010) found no effects of antibiotic supplementation on the serum lipid profile of broilers. In agreement with these results, dietary inclusion of onion extract at the rates of 5, 7.5, and 10g/kg did not affect total cholesterol, high-density lipoprotein, and triglycerides in broiler chickens (Aditya *et al.*, 2017).

Contrarily, some researchers reported decreases in serum cholesterol, triglyceride, and/or LDL or an increase in HDL in birds fed diets containing phytogenic products (Kim *et al.*, 2009; Prasad *et al.*, 2009; Torki *et al.*, 2018; Daramola, 2019). For example, Prasad *et al.* (2009) found that garlic supplementation significantly increased HDL and decreased cholesterol, triglyceride, LDL, and very-low-density lipoprotein (VLDL) in broilers. Similarly, supplementing diets of broilers with garlic bulb or garlic husk decreased serum cholesterol and LDL (Kim *et al.*, 2009). Also, dietary supplementation with African basil leaf meal (Olumide *et al.*, 2018), bitter leaf meal and/or *moringa* leaf meal (Daramola, 2019), and fenugreek, parsley, and sweet basil seeds (Abbas, 2010) significantly reduced blood cholesterol levels in broilers. The inconsistency in results may be due to differences in breed and age of birds, as well as dietary compositions. According to Toghyani *et al.* (2010), these factors influence the cholesterolemic effects of dietary components on farm animals.

Fagara zanthoxyloides and *Ocimum americanum* extracts contain alkaloids, flavonoids, phenols, saponins, and tannins (Adesina, 2005; Anuradha, 2014). These compounds have hypocholesterolemic properties (Santoso and Fenita, 2017). Moringa leaves also contain alkaloids, flavonoids, and phenolic compounds (Moyo *et al.*, 2012). Consequently, the addition of moringa leaf powder to broiler diets at 1.5 and 2% decreased ($p<0.05$) serum cholesterol and triglyceride levels (Mandal *et al.*, 2014). However, FFM and OLM did not affect serum cholesterol and triglycerides. The low supplementation rates of both additives might have limited their ability to cause the lipid-lowering effect. Possibly, higher supplementation rates beyond 0.4% may have imparted the serum lipid profile positively. In line with this assertion, Nobakht and Moghaddam (2013) supplemented diets of laying chickens with 0.5, 1, 1.5, and 2% dried aerial parts powder of *Tanacetum balsamita* (costmary). In that study, only the highest inclusion rate of the herb lowered blood cholesterol. Likewise, *moringa* leaf powder at lower inclusion rates of 0.5 and 1% did not affect cholesterol and triglycerides but higher inclusion rates of 1.5 and 2%, significantly reduced the concentrations of both lipids (Mandal *et al.*, 2014). Still in line with these results, hot red pepper at 0.25% in feed, did not affect blood cholesterol concentration in broilers but higher doses of 0.5, 0.75, and 1% reduced cholesterol significantly ($p<0.05$) (Al-Kassie *et al.*, 2011b). Likewise, dietary supplementation with 0.5% *Ocimum sanctum* leaf powder did not affect serum cholesterol level but 1% lowered it (Lanjewar *et al.*, 2009).

The non-effect of FFM and OLM on serum cholesterol suggests no effect on meat cholesterol content, as inferred from the finding of Lanjewar *et al.* (2009) who observed a positive relationship between meat (breast and thigh muscles) and serum cholesterol level in broilers fed a diet supplemented with 1% *Ocimum sanctum* leaf powder.

5.6 Effects of FFM, OLM and Penicillin Supplementation on Faecal Microbial Composition

5.6.1 Effects of penicillin supplementation on faecal counts of the tested pathogenic microbes

Antibiotic growth promoters decrease the populations of pathogenic bacteria in the gastrointestinal tract (Thomke and Elwinger, 1998; Gauer, 2004; Daramola, 2019). Concurrently, Jamroz *et al.* (2003) found that supplementing ration with avilamycin significantly reduced *Escherichia coli* and *Clostridium perfringens* counts in the recta of broilers. Similarly, Wati *et al.* (2015) observed that dietary supplementation with bacitracin methylene disalicylate effectively controlled *Escherichia coli*, *Salmonella,* and *Clostridium* in the caeca of broiler chickens. Likewise, virginiamycin in feed decreased ileal count of *Escherichia coli* in broilers (Darabighane *et al.*, 2012).

However, in this work, penicillin in diet did not affect the counts of the tested pathogenic bacteria strains (coliforms, *Escherichia coli,* and *Salmonella* and *Shigella*). This result concurs with the finding of Diarra *et al.* (2007) who supplemented broiler diet with bacitracin, bambermycin, penicillin, or salinomycin and observed no significant effects on *Escherichia coli, Enterococcus*, and *Clostridium perfringens* counts in litter, caecal and cloacal samples. Baurhoo *et al.* (2009) also found no effect of dietary supplementation with bacitracin or virginiamycin on caecal counts of *Campylobacter* and *Escherichia coli* in broilers on days 14 and 24 of the experiment.
Possibly, the pathogens tested in the current study gained resistance and hence, were not eliminated. This assertion is not strange since, in some research trials, antibiotic-resistant bacteria have been isolated from poultry (Kolář *et al.*, 2002; Diarra *et al.*, 2007).

5.6.2 Effects of FFM and OLM supplementation on faecal counts of the tested pathogenic microbes

The results of the faecal microbial analysis also reveal no effects of FFM and OLM on the counts of coliforms, *Escherichia coli*, as well as *Salmonella* and *Shigella*. This result agrees with the findings of Ahsan *et al.* (2018) who supplemented broiler diets with a commercial product containing extracts of anise, cinnamon, cumin, fennel (*Foeniculum vulgare*), garlic, and peppermint, and observed no effects on caecal counts of coliforms and *Escherichia coli*. Similarly, Murugesan *et al.* (2015) reported no significant effects of a blend of plant extracts in broiler diet on caecal counts of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Also, Mandal *et al.* (2014) found that dietary supplementation with moringa leaf powder at levels ranging from 0.5 to 1.5% did not affect coliform count in caeca content of broilers.

Notwithstanding, some researchers reported significant decreases in counts of pathogenic gut microbes in birds fed diets containing phytogenic products (Khalaji *et al.*, 2011; Goodarzi *et al.*, 2014; Wati *et al.*, 2015). For example, Wati *et al.* (2015) supplemented broiler ration with a phytogenic product containing extracts of anise, clove, melissa balm, oak, peppermint, and thyme, and found this product to be as effective as bacitracin methylene disalicylate in reducing caecal counts of *Escherichia coli*, *Salmonella enteritidis*, and *Clostridium perfringens*. Likewise, dietary supplementation with *Artemisia sieberi* leaves significantly reduced the number of coliforms and *Escherichia coli* in digesta samples collected from the caeca of broilers (Khalaji *et al.*, 2011). In agreement with these findings, Goodarzi *et al.* (2014) observed a significant decrease in *Escherichia coli* population in the ilea of broilers fed diets supplemented with fresh onion bulb.

The antibacterial properties of *Fagara zanthoxyloides* and *Ocimum americanum* extracts have been reported. For example, in some in-vitro studies, extracts of the fruits of *Fagara zanthoxyloides* inhibited the growth of *Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes* (Gardini *et al.*, 2009), *Bacillus subtilis, Salmonella typhimurium, Streptococcus mutans, Micrococcus luteus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* (Misra *et al.*, 2013). Ngassoum *et al.* (2003) also found the essential oil of dried *Fagara zanthoxyloides* fruits to be effective against *Bacillus subtilis, Bacillus cereus, Corynebacterium glutamicum, Enterococcus faecalis, Escherichia coli, Streptococcus faecalis,* and *Staphylococcus aureus*. These antibacterial effects are attributable to the geraniol and 3,4,5,7-tetrahydroxy-1-methoxy-10-methyl-9-acridone contents of the fruits (Gardini *et al.*, 2009; Wouatsa *et al.*, 2013; Sado Kamdem *et al.*, 2015). On the other hand, Anuradha (2014) observed excellent bactericidal effects of silver nanoparticles synthesized from aqueous *Ocimum americanum* leaf extract on *Escherichia coli* and *Staphylococci aureus*, which according to Gberikon *et al.* (2018) is caused by the cardiac glycosides and tannins contents of the leaves. The failure of FFM and OLM to lower the counts of the tested pathogens in this research work may be due to the low inclusion rates used in the feeds such that the concentrations of the antibacterial compounds were not sufficient to eliminate the bacteria from the gut. Perhaps, higher inclusion rates of FFM and OLM beyond 0.4% could have decreased the counts of the pathogens. To support this statement, Jamroz *et al.* (2003) observed that, at the inclusion rate of 150ppm of a mixture of capsaicin, carvacrol, and cinnamaldehyde in the ration of broilers, *Clostridium perfringens* population in rectal digesta samples was not affected in comparison with the control group. However, at a higher inclusion rate of 300ppm, the count of the pathogen was significantly reduced. Similarly, moringa leaf powder at inclusion rates of 0.5, 1.0, and 1.5% in broiler feeds did not affect caecal count of coliforms but at 2%, coliform count was significantly reduced (Mandal *et al.*, 2014).

Unlike the case of penicillin, it is not likely that the pathogens gained resistance to FFM and OLM. As explained in the literature, herbs are composed of a complex blend of phytochemicals with each having a different antimicrobial mode of action which makes it very difficult for bacteria to gain resistance to them (Suresh *et al.*, 2018). Other authors equally reported that using herbs as feed additives results in little to no cases of bacteria resistance (Hashemi and Davoodi, 2011; Sethiya, 2016).

5.6.3 Effects of penicillin, FFM and OLM supplementation on faecal microbial load

The decrease in faecal microbial load observed among the birds fed the penicillin, FFM and OLM supplemented diets in this study agrees with the finding of Samarasinghe *et al.* (2003) who reported a significant reduction in total viable microbes in the ilea of broilers fed diet supplemented with virginiamycin or turmeric root powder. Similar to this observation, Murugesan *et al.* (2015) recorded lower total caecal count of anaerobic bacteria in broilers fed diet containing a blend of plant extracts. These observations contradict the results of Mandal *et al.* (2014) who found no impact of dietary supplementation with moringa leaf powder (0.5, 1.0, and 1.5%) or bacitracin methylene disalicylate (0.02%) on caecal microbial population of broilers.

Microbial load consists of pathogenic and non-pathogenic microorganisms (Diaz Carrasco *et al.*, 2019). From the results, penicillin, FFM, and OLM did not affect faecal counts of the tested pathogenic microbes. The reduction in microbial load caused by penicillin, FFM, and OLM, therefore, implies that these supplements possibly inhibited beneficial bacteria in the gut. This validates the report of Hernandez *et al.* (2004) who indicated that plant extracts and antibiotics, in addition to inhibiting the growth and colonization of pathogenic gut bacteria, may inhibit the beneficial ones. In line with this report, Guban *et al.* (2006) fed broilers diets containing bacitracin methylene disalicylate and monensin alone or in combination and observed a lower ileal count of

Lactobacillus salivarius. It has been explained that most antibiotics do not differentiate between beneficial and pathogenic bacteria and hence, may destroy beneficial ones in the gut (Henry Ford, 2020). Likewise, Anugom and Ofongo (2019) administered aqueous African basil leaf extract to broiler chickens and recorded significantly lower ($p<0.05$) counts of *Lactobacillus* in digesta samples collected from the crop, proventriculus, caecum, and ileum on day 35 of the experiment. These researchers concluded that the active compounds in the herb possibly have bactericidal effects also on beneficial microbes. In agreement with these results, Adefisoye *et al.* (2012) found that *Fagara zanthoxyloides* stem extracts displayed antibacterial activity against both pathogenic (*Escherichia coli* and *Proteus vulgaris*) and beneficial (*Lactobacillus plantarum* and *Lactobacillus brevis*) bacteria strains.

However, other researchers observed higher counts of beneficial bacteria in chickens fed diets containing either phytogenic products or antibiotics. For instance, supplementing diet with fresh onion bulb or virginiamycin increased ileal *Lactobacillus* population in broilers (Goodarzi *et al.*, 2014). Also, Darabighane *et al.* (2012) observed a higher ileal count of *Lactobacillus* in broiler chickens fed diets supplemented with *Aloe vera* gel. Similarly, amla fruit powder incorporated into broiler feed increased ileal *Lactobacillus* population (Dalal *et al.*, 2018b). The increase in populations of beneficial gut bacteria in birds fed diets containing phytogenic products is due to the ability of some phytoconstituents to promote beneficial bacteria growth and proliferation (Windisch *et al.*, 2008; Diaz-Sanchez *et al.*, 2015; Suganya *et al.*, 2016). Also, the bactericidal effects of antibiotics on pathogenic gut bacteria allow the beneficial ones to proliferate (Mehdi *et al.*, 2018). This explains the increase in counts of beneficial gut bacteria in birds fed diets supplemented with antibiotics.

5.6.4 Effects of penicillin, FFM and OLM supplementation on faecal yeasts and moulds count

The results of the faecal microbial examination reveal no effects of FFM and OLM on the count of yeasts and moulds in comparison with the negative control group (BD). However, *Fagara zanthoxyloides* and *Ocimum americanum* extracts have been shown to have antifungal properties. For example, Osho and Adelani (2012) demonstrated that aqueous and ethanolic extracts of chewing sticks made from *Fagara zanthoxyloides* inhibit the growth of *Candida albicans, Candida krusei*, and *Candida tropicalis*. Similarly, Gberikon *et al.* (2018) found *Ocimum americanum* leaf and flower extracts to have antifungal activity against *Trichophyton mentagrophytes*. In another study, silver nanoparticles synthesized from aqueous *Ocimum americanum* leaf extract inhibited the mycelia growth of *Aspergillus niger* (Anuradha, 2014).

Fagara zanthoxyloides extracts are rich in flavonoids, saponins, and tannins (Adesina, 2005; Guendéhou *et al.*, 2018). These compounds are toxic to fungi and inhibit their growth (Kim *et al.*, 1995; Oyewole *et al.*, 2004). In addition to flavonoids and tannins, *Ocimum americanum* leaves contain methyl chavicol also with antifungal properties (Anuradha, 2014). The inability of FFM and OLM to control yeasts and moulds implies that the concentrations of the anti-fungal compounds in them were not sufficient to inhibit the fungi possibly due to the low inclusion levels used in the diets. Higher inclusion rates of FFM and OLM beyond 0.4% could have decreased the yeasts and moulds count since at 0.2% FFM and 0.2% OLM, the fungi population was distinctly not affected ($p>0.05$) but at 0.4% FFM and 0.4% OLM, the fungi population tended to decrease ($p>0.05$).

Only penicillin distinctly reduced ($p<0.05$) faecal yeasts and moulds count in this study compared to the non-supplemented group. This concurs with work done by Samarasinghe *et al.* (2003) who observed a significantly lower count of yeasts and moulds in the caeca of broilers fed a diet supplemented with virginiamycin. These findings disagree with the report of Mehdi *et al.* (2018)

who indicated that antibiotics only control bacteria and not fungi or viruses. The ability of penicillin to lower the faecal yeasts and moulds population implies that it can control fungal diseases of poultry such as candidiasis, aspergillosis, cryptococcosis, dactlariosis, favus, histoplasmosis, mucormycoses, rhodotorulosis, and torulopsis which are known to cause economic losses to farmers (Dhama *et al.,* 2013).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of this study show that;

- Supplementing broiler diets with FFM and OLM alone or in combination up to 0.4% does not affect growth performance, carcass characteristics, total tract dry matter, protein, ash, and fibre digestibility, nitrogen excretion, serum lipid profile, and load of pathogenic gut microbes.

- Dietary supplementation with FFM, OLM, and penicillin reduces total tract fat digestibility and faecal microbial load in broilers.

- FFM and OLM each at 0.4% function as an antimicrobial agent in broiler diets similar to antibiotics.

- Feeding antimicrobial substances to birds raised in clean environments will have no impact on growth performance.

6.2 Recommendations

- Higher dietary inclusion rates of FFM and OLM beyond 0.4% must be investigated to examine their effects on broiler growth performance, nutrient digestibility, and load of pathogenic gut microbes.

- The effects of OLM and FFM on selected strains of beneficial gut microbes in poultry also need to be studied to be able to tell with certainty whether these herbs have bactericidal effects on the beneficial gut microbes.

- There is a need to examine the flavouring effects of FFM and OLM on broiler meat to ascertain the acceptability of the meat by consumers.

- The present study tested the efficacy of the fruits of *Fagara zanthoxyloides* to promote the growth of broilers. The growth-promoting effects of other parts of the plant can equally be exploited in future research trials.

- Other researchers can consider supplementing broiler diets with combinations of FFM or OLM with any other alternative to antibiotic growth promoters to determine their effects on growth performance.

REFERENCES

Abbas, R.J. 2010. Effect of using fenugreek, parsley and sweet basil seeds as feed additives on the performance of broiler chickens. Int. J. Poult. Sci, 9 (3): 278-282.

Abd El-Hack, M.E., Abdelnour, S.A., Taha, A.E., Khafaga, A.F., Arif, M., Ayasan, T., Swelum, A.A., Abukhalil, M.H., Alkahtani, S., Aleya, L. and Abdel-Daim, M.M. 2020. Herbs as thermoregulatory agents in poultry: An overview. Science of the Total Environment, 703: 134399.

Abdelqader, A., Qarallah, B., Al-Ramamneh, D. and Daş, G. 2012. Anthelmintic effects of citrus peels ethanolic extracts against *Ascaridia galli*. Veterinary Parasitology, 188(1-2): 78-84.

Adebiyi, A.O., Koekemoer, T., Adebiyi, A.P., Smith, N., Baxter, E., Naude, R.J. and Van de Venter, M. 2009. Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. Pharmaceutical Biology, 47(4): 320-327.

Adefisoye, M.A., AjibadeAko-Nai, K. and Bisi-Johnson, M.A. 2012. Phytochemical and antibacterial activity of the extracts of *Fagara zanthoxyloides* on selected cariogenic and enteric bacterial isolates. Journal of Complementary Medicine Research, 1(1): 1-6.

Adegbolagun, O.M. and Olukemi, O.O. 2010. Effect of light irradiation on the antimicrobial activity of *Zanthoxylum zanthoxyloides* Lam. methanolic extract. African Journal of Pharmacy and Pharmacology, 4(4): 145-150.

Adesina, S.K. 2005. The Nigerian Zanthoxylum; chemical and biological values. African Journal of Traditional, Complementary and Alternative Medicines, 2(3): 282-301.

Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Veterinary Medicine International, 2010.

Aditya, S., Ahammed, M., Jang, S.H. and Ohh, S.J. 2017. Effects of dietary onion (*Allium cepa*) extract supplementation on performance, apparent total tract retention of nutrients, blood profile and meat quality of broiler chicks. Asian-Australasian Journal of Animal Sciences, 30(2): 229.

AFRC, R.F. 1989. Probiotics in man and animals. Journal of Applied Bacteriology, 66(5): 365-378.

Ahsan, U., Kuter, E., Raza, I., Köksal, B.H., Cengiz, Ö., Yıldız, M., Kızanlık, P.K., Kaya, M., Tatlı, O. and Sevim, Ö. 2018. Dietary supplementation of different levels of phytogenic feed additive in broiler diets: The dynamics of growth performance, caecal microbiota, and intestinal morphometry. Brazilian Journal of Poultry Science, 20(4): 737-746.

Akbari, MR. 2014. Effects of dietary inclusion of turmeric (*Curcuma longa*) and cinnamon (*Cinnamomum verum*) powders on performance, organs relative weight and some immune system parameters in broiler chickens. Poultry Science Journal, 2(2): 153-163.

Akbarian, A., Golian, A., Kermanshahi, H., De Smet, S. and Michiels, J. 2015. Antioxidant enzyme activities, plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and *Curcuma xanthorrhiza* essential oil. Journal of Animal Physiology and Animal Nutrition, 99(1): 150-162.

Akhtar, M., Hai, A., Awais, M.M., Iqbal, Z., Muhammad, F., ul Haq, A. and Anwar, M.I. 2012. Immunostimulatory and protective effects of *Aloe vera* against coccidiosis in industrial broiler chickens. Veterinary Parasitology, 186(3-4): 170-177.

Akhtar, M.S. and Riffat, S. 1985. Evaluation of *Melia azedarach* Linn. fruit (Bakain) against *Ascaridia galli* infection in chickens. Pakistan Veterinary Journal, 5(1): 34-37.

Al-Dobaib, S.N. and Mousa, H.M. 2009. Benefits and risks of growth promoters in animal production. J Food Agric Environ, 7(2): 202-8.

Alhendi, A.B., Homeida, A.A.M. and Gaili, E.S. 2000. Drug residues in broiler chickens fed with antibiotics in ration. Veterinarski Arhiv, 70(4): 199-206.

Ali, A. 2014. Productive performance and immune response of broiler chicks as affected by dietary marjoram leaves powder. Egyptian Poultry Science Journal, 34(1): 57-70.

Alipour, F., Hassanabadi, A., Golian, A. and Nassiri-Moghaddam, H. 2015. Effect of plant extracts derived from thyme on male broiler performance. Poultry Science, 94(11): 2630-2634.

Al-Kassie, G.A. 2009. Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pakistan Veterinary Journal, 29(4): 169-173.

AL-Kassie, G.A., Mohseen, A.M. and Abd-AL-Jaleel, R.A. 2011a. Modification of productive performance and physiological aspects of broilers on the addition of a mixture of cumin and turmeric to the diet. Research Opinions in Animal and Veterinary Sciences, 1(1): 31-34.

Al-Kassie, G.A., Al-Nasrawi, M.A. and Ajeena, S.J. 2011b. The Effects of Using Hot Red Pepper as a Diet Supplement on some performance traits in broiler. Pakistan Journal of Nutrition, 10(9): 842-845.

Allen, H.K., Levine, U.Y., Looft, T., Bandrick, M. and Casey, T.A. 2013. Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. Trends in Microbiology, 21(3): 114-119.

Amevor, F.K. 2017. Moringa (Moringa Oleifera) Leaf Meal Supplementation On the Performance, Carcass Characteristics and Blood Profile of Broilers (Doctoral dissertation, University of Ghana).

Anderson, D.B., McCracken, V.J., Aminovi, R.I., Simpson, J.M., Mackie, R.I., Verstegen, M.W.A. and Gaskins, H.R. 1999. Gut microbiology and growth-promoting antibiotics in swine. Pig News and Information, 20: 115-122.

Anthony, J.P., Fyfe, L. and Smith, H. 2005. Plant active components–a resource for antiparasitic agents? Trends in Parasitology, 21(10): 462-468.

Anugom, Y.O. and Ofongo, R.T.S. 2019. Research Article Impact of Aqueous *Ocimum gratissimum* (Lyn) Leaf Extract on Growth Performance, Gut pH and Bacterial Counts in Broiler Chickens. International Journal of Poultry Science, 18 (7): 309-316.

Anuradha, G. 2014. Synthesis Characterization Antibacterial and Antifungal Activity Studies of Silver Nanoparticles from Selected Ocimum Species.

Apata, D.F. 2008. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. Journal of the Science of Food and Agriculture, 88(7): 1253-1258.

Asase, A., Oteng-Yeboah, A.A., Odamtten, G.T. and Simmonds, M.S. 2005. Ethnobotanical study of some Ghanaian anti-malarial plants. Journal of Ethnopharmacology, 99(2): 273-279.

Asuzu, I.U. and Onu, U.O. 1994. Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens. Fitoterapia (Milano), 65(4): 291-297.

Athanasiadou, S., Githiori, J. and Kyriazakis, I. 2007. Medicinal plants for helminth parasite control: facts and fiction. Animal, 1(9): 1392-1400.

Athanasiadou, S., Kyriazakis, I., Jackson, F. and Coop, R.L. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Veterinary Parasitology, 99(3): 205-219.

Atteh, J.O., Onagbesan, O.M., Tona, K., Decuypere, E., Geuns, J.M.C. and Buyse, J. 2008. Evaluation of supplementary stevia (*Stevia rebaudiana*, bertoni) leaves and stevioside in broiler diets: effects on feed intake, nutrient metabolism, blood parameters and growth performance. Journal of Animal Physiology and Animal Nutrition, 92(6): 640-649.

Attia, Y.A., Qota, E.M., Zeweil, H.S., Bovera, F., Abd Al-Hamid, A.E. and Sahledom, M.D. 2012. Effect of different dietary concentrations of inorganic and organic copper on growth performance and lipid metabolism of White Pekin male ducks. British Poultry Science, 53(1): 77-88.

Attia, Y.A., Zeweil, H.S., Alsaffar, A.A. and El-Shafy, A.S. 2011. Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. Arch. Geflügelk, 75: 40-48.

Avila-Ramos, F., Pro-Martínez, A., Sosa-Montes, E., Cuca-Garcia, J.M., Becerril-Pérez, C.M., Figueroa-Velasco, J.L. and Narciso-Gaytán, C. 2012. Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. Poultry Science, 91(2): 505-511.

Awad, W.A., Ghareeb, K., Abdel-Raheem, S. and Böhm, J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science, 88(1): 49-56.

Azando, E.V.B., Hounzangbé–Adoté, M.S., Olounladé, P.A., Brunet, S., Fabre, N., Valentin, A. and Hoste, H. 2011. Involvement of tannins and flavonoids in the *in vitro* effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloïdes* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. Veterinary Parasitology, 180(3-4): 292-297.

Azando, E.V.B., Olounlade, A.P., Hounzangbe-Adote, M.S., HA, T., Fabre, N. and Valentin, A. 2017. Control of gastrointestinal parasitosis ovines by *Zantholylum zantholyloids* (*Fagara zanthoxyloids*). Rev. Med. Vet. (Bogota), 168: 205-212.

Azeezah, A.A., Adebayo, Q. and Ismaila, A.R. 2019. Qualities of Meat of Cockerel and Egg of Pullet Fed with Varying Levels of *Moringa oleifera* and *Ocimum gratissimum* Leaf Meal. World Journal of Innovative Research, 6(2): 56-64.

Azouz, H.M.M. 2001. Effect of Hot Pepper and Fenugreek Seeds Supplementation on Broiler Diets (Doctoral dissertation, Ph.D. Thesis, Faculty of Agric., Cairo Univ. Egypt).

Babak, D. and Nahashon, S.N. 2014. A review on effects of *Aloe vera* as a feed additive in broiler chicken diets. Annals of Animal Science, 14(3): 491-500.

Banday, M.T., Adil, S., Khan, A.A. and Untoo, M. 2015. A study on efficacy of fumaric acid supplementation in diet of broiler chicken. International Journal of Poultry Science, 14(11): 589.

Barnabas, B.B., Mann, A., Ogunrinola, T.S. and Anyanwu, P.E. 2010. Screening for Anthelminthic activities from extracts of *Zanthoxylum zanthoxyloides*, *Neocarya macrophylla* and *Celosia laxa* against ascaris infection in rabbits. International Journal of Applied Research in Natural Products, 3(4): 1-4.

Barreto, M.S.R., Menten, J.F.M., Racanicci, A.M.C., Pereira, P.W.Z. and Rizzo, P.V. 2008. Plant extracts used as growth promoters in broilers. Brazilian Journal of Poultry Science, 10(2): 109-115.

Basilico, M.Z. and Basilico, J.C. 1999. Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin A production. Letters in Applied Microbiology, 29(4): 238-241.

Basmacioğlu Malayoğlu, H., Baysal, Ş., Misirlioğlu, Z., Polat, M.E.L.T.E.M., Yilmaz, H. and Turan, N. 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat–soybean meal diets. British Poultry Science, 51(1): 67-80.

Baurhoo, B., Ferket, P.R. and Zhao, X. 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poultry Science, 88(11): 2262-2272.

Beermann, D.H. 1995. Growth promotants-promise, problems, and perceptions. In Proc. Reciprocal Meat Conf, 48: 45-50.

Berhow, M.A., Affum, A.O. and Gyan, B.A. 2012. Rosmarinic acid content in antidiabetic aqueous extract of *Ocimum canum* Sims grown in Ghana. Journal of Medicinal Food, 15(7): 611-620.

Bhadauria, P., Keshava, P.M. and Murai, A. 2016. Management of Heat Stress in Poultry.

Biswas, A.K., Rahman, M.M., Hassan, M.Z., Sultana, S., Rahman, M.M. and Mostofa, M. 2017. Effect of tulsi (*Ocium sanctum*) leaves extract as a growth promoter in broiler production. Asian Journal of Medical and Biological Research, 3(2): 226-232.

Blajman, J.E., Frizzo, L.S., Zbrun, M.V., Astesana, D.M., Fusari, M.L., Soto, L.P., Rosmini, M.R. and Signorini, M.L. 2014. Probiotics and broiler growth performance: A meta-analysis of randomised controlled trials. British Poultry Science, 55(4): 483-494.

Bölükbaşı Ş.C., Erhan, M.K. and Kaynar, Ö. 2008. The effect of feeding thyme, sage and rosemary oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces. Archiv fur Geflugelkunde, 72(5): 231–237.

Boroojeni, F.G., Vahjen, W., Mader, A., Knorr, F., Ruhnke, I., Röhe, I., Hafeez, A., Villodre, C., Männer, K. and Zentek, J. 2014. The effects of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poultry Science, 93(6): 1440-1452.

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J. and Spais, A.B. 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. British Poultry Science, 43(2): 223-230.

Bozkurt, M., Küçükyilmaz, K., Çatli, A.U. and Çinar, M. 2008. Growth performance and slaughter characteristics of broiler chickens fed with antibiotic, mannan oligosaccharide and dextran oligosaccharide supplemented diets. Int. J. Poult. Sci, 7(10): 969-977.

Brainer, M.M.D.A., Menten, J.F.M., Vale, M.M.D. and Morais, S.C.D.D. 2003. Cupric citrate as growth promoter for broiler chickens in different rearing stages. Scientia Agricola, 60(3): 441-445.

Bravo, D., Pirgozliev, V. and Rose, S.P. 2014. A mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin improves energy utilization and growth performance of broiler chickens fed maize-based diet. Journal of Animal Science, 92(4): 1531-1536.

Brennan, J., Skinner, J., Barnum, D.A. and Wilson, J. 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poultry Science, 82(3): 360-363.

Brisbin, J.T., Gong, J. and Sharif, S. 2008. Interactions between commensal bacteria and the gut-associated immune system of the chicken. Animal Health Research Reviews, 9(1): 101.

Brown, M. 2011. Modes of action of probiotics: recent developments. Journal of Animal and Veterinary Advances, 10(14): 1895-1900.

Buret, A.G. 2010. Immuno-modulation and anti-inflammatory benefits of antibiotics: the example of tilmicosin. Canadian Journal of Veterinary Research, 74(1): 1-10.

Burrell, A.L., Dozier, W.A., Davis, A.J., Compton, M.M., Freeman, M.E., Vendrell, P.F. and Ward, T.L. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. British Poultry Science, 45(2): 225-263.

Burrows, C.F., Kronfeld, D.S., Banta, C.A. and Merritt, A.M. 1982. Effects of fiber on digestibility and transit time in dogs. The Journal of Nutrition, 112(9): 1726-1732.

Butaye, P., Devriese, L.A. and Haesebrouck, F. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical Microbiology Reviews, 16(2): 175-188.

Butcher, G.D. and Miles, R.D. 1995. Intestinal Parasites in Backyard Chicken Flocks. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Calik, A. and Ergün, A. 2015. Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poultry Science, 94(9): 2173-2182.

Cao, F.L., Zhang, X.H., Yu, W.W., Zhao, L.G. and Wang, T. 2012. Effect of feeding fermented Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers. Poultry Science, 91(5): 1210-1221.

Castanon, J.I.R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science, 86(11): 2466-2471.

Chacher, M.F.A., Kamran, Z., Ahsan, U., Ahmad, S., Koutoulis, K.C., Qutab Ud Din, H.G. and Cengiz, Ö. 2017. Use of mannan oligosaccharide in broiler diets: an overview of underlying mechanisms. World's Poultry Science Journal, 73(4): 831-844.

Chagonda, L.S., Makanda, C.D. and Chalchat, J.C. 2000. The essential oils of *Ocimum canum* Sims (basilic camphor) and *Ocimum urticifolia* Roth from Zimbabwe. Flavour and Fragrance Journal, 15(1): 23-26.

Chalghoumi, R., Belgacem, A., Trabelsi, I., Bouatour, Y. and Bergaoui, R. 2013. Effect of dietary supplementation with probiotic or essential oils on growth performance of broiler chickens. International Journal of Poultry Science, 12(9): 538-544.

Chang, S.T., Chen, P.F. and Chang, S.C. 2001. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. Journal of Ethnopharmacology, 77(1): 123-127.

Chattopadhyay, M.K. 2014. Use of antibiotics as feed additives: a burning question. Frontiers in Microbiology, 5: 334.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L. and Yuan, Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Frontiers in Microbiology, 5: 217.

Choct, M. 2006. Enzymes for the feed industry: past, present and future. World's Poultry Science Journal, 62(1): 5-16.

Ciorba, M.A. 2012. A gastroenterologist's guide to probiotics. Clinical Gastroenterology and Hepatology, 10(9): 960-968.

Cogliani, C., Goossens, H. and Greko, C. 2011. Restricting antimicrobial use in food animals: lessons from Europe. Microbe, 6(6): 274.

Cook, M.E. 2004. Antibodies: alternatives to antibiotics in improving growth and feed efficiency. Journal of Applied Poultry Research, 13(1): 106-119.

Dalal, R., kumar Ahlawat, P., Vinus, S. and Panwar, V.S. 2018a. Effect of amla fruit powder on haemato-biochemical parameters of broiler poultry chicken. The Pharma Inno. J. 7(4): 20-24.

Dalal, R., Ahlawat, P.K., Sonu, V., Panwar, V.S., Tewatia, B.S. and Sheoran, N. 2018b. Evaluation of Antimicrobial Effect of *Emblica officinalis* Fruit Powder on Intestinal Micro-biota in Broilers Chicken. Int. J. Curr. Microbiol. App. Sci, 7(04): 1432-1438.

Darabighane, B., Zarei, A. and Shahneh, A.Z. 2012. The effects of different levels of *Aloe vera* gel on ileum microflora population and immune response in broilers: a comparison to antibiotic effects. Journal of Applied animal research, 40(1): 31-36.

Daramola, O.T. 2019. Medicinal plants leaf meal supplementation in broiler chicken diet: effects on performance characteristics, serum metabolite and antioxidant status. Animal Research International, 16(2): 3334-3342.

Deans, S.G., Noble, R.C., Penzes, L. and Imre, S.G. 1993. Promotional effects of plant volatile oils on the polyunsaturated fatty acid status during aging. Age, 16(2): 71-74.

Demir, E., Sarica, S., Ozcan, M.A. and Suicmez, M. 2005. The use of natural feed additives as alternatives to an antibiotic growth promoter in broiler diets. Archiv Fur Geflugelkunde, 69(3): 110-116.

Denli, M. and Demirel, R. 2018. Replacement of antibiotics in poultry diets. CAB Reviews, 13(35): 1-9.

Denloye, A.A., Makanjuola, W.A., Ajelara, O., Akinlaye, O.J., Olowu, R.A. and Lawal, O.A. 2010. Toxicity of powder and extracts of *Zanthoxylum zanthoxyloides* Lam (Rutaceae) root bark from Nigeria to three storage beetles. Julius-Kühn-Archiv, (425): 833-839.

Dhama, K., Chakraborty, S., Verma, A.K., Tiwari, R., Barathidasan, R., Kumar, A. and Singh, S.D. 2013. Fungal/mycotic diseases of poultry-diagnosis, treatment and control: a review. Pakistan Journal of Biological Sciences: PJBS, 16(23): 1626-1640.

Diarra, M.S., Silversides, F.G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., Bonnet, C., Delaquis, P., Bach, S., Skura, B.J. and Topp, E. 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Applied and Environmental Microbiology, 73(20): 6566-6576

Diaz Carrasco, J.M., Casanova, N.A. and Fernández Miyakawa, M.E. 2019. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms, 7(10): 374.

Diaz-Sanchez, S., D'Souza, D., Biswas, D. and Hanning, I. 2015. Botanical alternatives to antibiotics for use in organic poultry production. Poultry Science, 94(6): 1419-1430.

Dibner, J.J. and Richards, J.D. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science, 84(4): 634-643.

Dipeolu, M.A. 2004. Problems and prospects of antibiotics residues in meat products in Nigeria Vom. Journal of Veterinary Sciences, 1(1): 63-67

Dofuor, A.K., Djameh, G.I., Ayertey, F., Bolah, P., Amoa-Bosompem, M., Kyeremeh, K., Okine, L.K., Gwira, T.M. and Ohashi, M. 2019. Antitrypanosomal Effects of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler Extracts on African Trypanosomes. Evidence-Based Complementary and Alternative Medicine, 2019.

Donoghue, D.J. 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? Poultry Science, 82(4): 618-621.

Dorman, H.D. and Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2): 308-316.

Doyle, M.E. 2006. Veterinary drug residues in processed meats potential health risk: A review of the scientific literature. Food Research Institute, University of Wisconsin, Madison,https://fri.wisc.edu/files/Brief s_File/FRIBrief_VetDrgRes.pdf:1-11. (Assesed on January 21, 2019)

Durrani, F.R., Sultan, A., Ahmed, S., Chand, N., Khattak, F.M. and Durrani, Z. 2007. Efficacy of Aniseed Extract as Immune Stimulant and Growth Promoter in Broiler Chicks. Pakistan Journal of Biological Sciences, 10(20): 3718-3721.

Effiong, O.O. and Ochagu, S.I. 2019. Chemical composition and growth promoting effect of *Piper guineense* leaf and seed meals on broiler chicks at starter phase. Animal Research International, 16(2): 3285-3294.

El-Ghamry, A.A., Azouz, H.M. and El-Yamany, A.T. 2004. Effect of hot pepper and fenugreek seeds supplementation to low energy diets on Muscovi ducklings performance. Egypt. Poult. Sci, 24: 613-627.

EL-Shoukary, R.D., Darwish, M.H. and Abdel-Rahman, M.A. 2014. Behavioral, performance, carcass traits and hormonal changes of heat stressed broilers feeding black and coriander seeds. Journal of Advanced Veterinary Research, 4(3): 97-101.

Eltazi, S. 2014. Response of Broiler Chicks to Diets Containing Different Mixture Levels of Garlic and Ginger Powder as Natural Feed Additives. International Journal of Pharmaceutical Research and Allied Sciences, 3(4).

Elujoba, A.A., Odeleye, O.M. and Ogunyemi, C.M. 2005. Traditional medicine development for medical and dental primary health care delivery system in Africa. African Journal of Traditional, Complementary and Alternative Medicines, 2(1): 46-61.

Engberg, R.M., Hedemann, M.S., Leser, T.D. and Jensen, B.B. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poultry Science, 79(9): 1311-1319.

Enechi, O.C., Amah, C.C., Okagu, I.U., Ononiwu, C.P., Azidiegwu, V.C., Ugwuoke, E.O., Onoh, A.P. and Ndukwe, E.E. 2019. Methanol extracts of *Fagara zanthoxyloides* leaves possess antimalarial effects and normalizes haematological and biochemical status of *Plasmodium berghei*-passaged mice. Pharmaceutical Biology, 57(1): 577-585.

Erener, G., Ocak, N., Altop, A., Cankaya, S., Aksoy, H.M. and Ozturk, E. 2011. Growth performance, meat quality and caecal coliform bacteria count of broiler chicks fed diet with green tea extract. Asian-Australasian Journal of Animal Sciences, 24(8): 1128-1135.

Esan, A.J., Omisakin, C.T., Titilayo, O.E. and Fasakin, K.A. 2014. Gender difference on stress induced by malaria parasite infection and effect of anti-malaria drug on stress index. American Journal of Biomedical Research, 2(3): 42-46.

Eslami, M., Baghaei, M., Mamouei, M., Chaji, M. and Ashayerizadeh, A. 2010. Effects of avilamycin, formycingold and FYTO-MIX on serum lipid concentrations of young broilers. J. Anim. Vet. Adv, 9: 108-111.

Ewing, H.P., Pesti, G.M., Bakalli, R.I. and Menten, J.F. 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate, and copper oxychloride to broiler chickens. Poultry Science, 77(3): 445-448.

Ewing, W.N. and Cole, D.J.A. 1994. The use of antibiotics. The Living Gut, 75-89.

Fallah, R., Kiani, A. and Azarfar, A. 2013. A review of the role of five kinds of alternatives to in-feed antibiotics in broiler production. Journal of Veterinary Medicine and Animal Health, 5(11): 317-321.

Fawzy, M.M., El-Sadawi, H.A., El-Dien, M.H. and Mohamed, W.A.M. 2016. Hematological and biochemical performance of poultry following zinc oxide and sodium selenite supplementation as food additives. Annals of Clinical Pathology, 4(4): 1076.

Feighner, S.D. and Dashkevicz, M.P. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Applied and Environmental Microbiology, 53(2): 331-336.

Ferket, P.R. and Gernat, A.G. 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci, 5(10): 905-911.

Frankič, T., Voljč, M., Salobir, J. and Rezar, V. 2009. Use of herbs and spices and their extracts in animal nutrition. Acta Agric Slov, 94(2): 95-102.

Franz, C., Baser, K.H.C. and Windisch, W. 2010. Essential oils and aromatic plants in animal feeding–a European perspective. A review. Flavour and Fragrance Journal, 25(5): 327-340.

Gadde, U., Kim, W.H., Oh, S.T. and Lillehoj, H.S. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Animal Health Research Reviews, 18(1): 26-45.

Gadzirayi, C.T., Masamha, B., Mupangwa, J.F. and Washaya, S. 2012. Performance of broiler chickens fed on mature *Moringa oleifera* leaf meal as a protein supplement to soyabean meal. International Journal of Poultry Science, 11(1): 5-10.

Gardini, F., Belletti, N., Ndagijimana, M., Guerzoni, M.E., Tchoumbougnang, F., Zollo, P.H.A., Micci, C., Lanciotti, R. and Kamdem, S.L.S. 2009. Composition of four essential oils obtained from plants from Cameroon, and their bactericidal and bacteriostatic activity against *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*. African Journal of Microbiology Research, 3(5): 264-271.

Gaskins, H.R., Collier, C.T. and Anderson, D.B. 2002. Antibiotics as growth promotants: mode of action. Animal Biotechnology, 13(1): 29-42.

Gauer, R.V. 2004. Drug free diet performance optimized by nutritional programs. World Poult, 20: 14-15.

Gberikon, G.M., Dabo, A.D. and Agbo, E.B. 2018. Phytochemical and Antibacterial Activities of Combined Leaves and Flower Extracts of English Camphor Basil (*Ocimum canum*) on Some Selected Bacteria Associated with Skin Infections. International Journal of Contemporary Research and Review, 9(6): 20246-20253.

Geidam, Y.A., Usman, H., Musa, H.I., Anosike, F. and Adeyemi, Y. 2009. Oxytetracycline and procaine penicillin residues in tissues of slaughtered cattle in Maiduguri, Borno State, Nigeria. Terrestrial and Aquatic Environmental Toxicology, 3(2): 68-70.

Gharaghani, H., Shariatmadari, F. and Torshizi, M.A. 2015. Effect of fennel (*Foeniculum vulgare* Mill.) used as a feed additive on the egg quality of laying hens under heat stress. Brazilian Journal of Poultry Science, 17(2): 199-207.

Ghazanfari, S., Mohammadi, Z. and Adib Moradi, M. 2015. Effects of coriander essential oil on the performance, blood characteristics, intestinal microbiota and histological of broilers. Brazilian Journal of Poultry Science, 17(4): 419-426.

Giannenas, I., Florou-Paneri, P., Papazahariadou, M., Christaki, E., Botsoglou, N.A. and Spais, A.B. 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Archives of Animal Nutrition, 57(2): 99-106.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition, 125(6): 1401-1412.

Gill, C. 1999. Herbs and plant extracts as growth enhancers. Feed Management, 50: 29-32.

Goliomytis, M., Kartsonas, N., Charismiadou, M.A., Symeon, G.K., Simitzis, P.E. and Deligeorgis, S.G. 2015. The influence of naringin or hesperidin dietary supplementation on broiler meat quality and oxidative stability. PloS One, 10(10): 0141652.

Goliomytis, M., Tsoureki, D., Simitzis, P.E., Charismiadou, M.A., Hager-Theodorides, A.L. and Deligeorgis, S.G. 2014. The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. Poultry Science, 93(8): 1957-1962.

Goodarzi, M., Nanekarani, S. and Landy, N. 2014. Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. Asian Pacific Journal of Tropical Disease, 4: S297-S301.

Grela, E.R., Klebaniuk, R., Kwiecien, M. and Pietrzak, K. 2013. Phytobiotics in animal production. Breeding Review, 3(81).

Guban, J., Korver, D.R., Allison, G.E. and Tannock, G.W. 2006. Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of *Lactobacillus salivarius*, and reduced bile salt deconjugation in the ileum of broiler chickens. Poultry Science, 85(12): 2186-2194.

Guendéhou, F., Djossa, B.A., Kènou, C. and Assogbadjo, C. 2018. Review of Studies on *Zanthoxylum zanthoxyloides* (L.): availability and ethnomedical, phytochemical, pharmacological uses. Sch. J. Res. Agric. Biol, 3: 244-254.

Guo, F.C., Savelkoul, H.F.J., Kwakkel, R.P., Williams, B.A. and Verstegen, M.W.A. 2003. Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. World's Poultry Science Journal, 59(4): 427-440.

Gupta, G. and Charan, S. 2005. Antimicrobial and immunomodulatory effects of *Ocimum sanctum* (Shyama Tulsi) against infectious bursal disease virus infection in chickens as model. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 26(2): 92-98.

Gustafson, R.H. and Bowen, R.E. 1997. Antibiotic use in animal agriculture. Journal of Applied Microbiology, 83(5): 531-541.

Habibi, R., Sadeghi, G.H. and Karimi, A. 2014. Effect of different concentrations of ginger root powder and its essential oil on growth performance, serum metabolites and antioxidant status in broiler chicks under heat stress. British Poultry Science, 55(2): 228-237.

Halliwell, B. and Gutteridge, J.M. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochemical Journal, 219(1): 1-14.

Haque, M.N., Islam, K.M., Akbar, M.A., Chowdhury, R., Khatun, M., Karim, M.R. and Kemppainen, B.W. 2010. Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. Canadian Journal of Animal Science, 90(1): 57-63.

Hashemi, S.R. and Davoodi, H. 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Veterinary Research Communications, 35(3): 169-180.

Hashemi, S.R. and Davoodi, H. 2012. Herbal plants as new immuno-stimulator in poultry industry: a review. Asian Journal of Animal and Veterinary Advances, 7(2): 105-116.

Hashemipour, H., Kermanshahi, H., Golian, A. and Veldkamp, T. 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poultry Science, 92(8): 2059-2069.

Hashemipour, H., Kermanshahi, H., Golian, A. and Khaksar, V. 2014. Effects of carboxy methyl cellulose and thymol + carvacrol on performance, digesta viscosity and some blood metabolites of broilers. Journal of Animal Physiology and Animal Nutrition, 98(4): 672-679.

Henry Ford Health System. 2020. Antibiotics And Probiotics: How Medications Affect Your Gut. https://www.henryford.com/blog/2020/06/antibiotics-and-probiotics-how-medications-affect-your-gut. (Accessed on October 22, 2020)

Herago, T. and Agonafir, A. 2017. Growth Promoters in Cattle. Advances in Biological Research, 11(1): 24-34.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83(2): 169-174.

Hernandez, F., Garcia, V., Madrid, J., Orengo, J., Catalá, P. and Megias, M.D. 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. British Poultry Science, 47(1): 50-56.

Higuchi, O., Tateshita, K. and Nishimura, H. 2003. Antioxidative activity of sulfur-containing compounds in Allium species for human low-density lipoprotein (LDL) oxidation *in vitro*. Journal of Agricultural and Food Chemistry, 51(24): 7208-7214.

Hoda, A.A. and Maha, M.H. 1995. Potency of copper as growth promoter in broiler chickens. Veterinary Medical Journal, 43: 77-85.

Hooge, D.M. and Connolly, A. 2011. Meta-analysis summary of broiler chicken trials with dietary Actigen® (2009–2011). Int. J. Poult. Sci, 10: 819-824.

Hooge, D.M., Pierce, J.L., McBride, K.W. and Rigolin, P.J. 2010. Meta-analysis of broiler chicken trials using diets with or without Allzyme® SSF enzyme complex. International Journal of Poultry Science, 9(9): 819-823.

Hounzangbe-Adote, M.S., Zinsou, F.E., Hounpke, V., Moutairou, K. and Hoste, H. 2005. *In vivo* effects of Fagara leaves on sheep infected with gastrointestinal nematodes. Tropical Animal Health and Production, 37(3): 205-214.

Hughes, P. and Heritage, J. 2002. Antibiotic Growth-Promoters in Food Animals. FAO Corporate Document Repository, 160: 129-152.

Humphrey, B.D. and Klasing, K.C. 2004. Modulation of nutrient metabolism and homeostasis by the immune system. World's Poultry Science Journal, 60(1): 90-100.

Huyghebaert, G., Ducatelle, R. and Van Immerseel, F. 2011. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal, 187(2): 182-188.

Islam, M.R., Oomah, D.B. and Diarra, M.S. 2017. Potential immunomodulatory effects of non-dialyzable materials of cranberry extract in poultry production. Poultry Science, 96(2): 341-350.

Issa, K.J. and Omar, J.A. 2012. Effect of garlic powder on performance and lipid profile of broilers. Open Journal of Animal Sciences, 2(2): 62-68.

Jackson, M.E. and Hanford, K. 2014. Statistical meta-analysis of pen trials conducted testing heat-sensitive β-mannanase (Hemicell) feed enzyme in male broilers grown to market age. Poultry Science, 93: 66.

Jacobsen, C., Let, M.B., Nielsen, N.S. and Meyer, A.S. 2008. Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation. Trends in Food Science and Technology, 19(2): 76-93.

Jahejo, A.R., Rajput, N., Wen-xia, T., Naeem, M., Kalhoro, D.H., Kaka, A., Niu, S. and Jia, F.J. 2019. Immunomodulatory and growth promoting effects of basil (*Ocimum basilicum*) and ascorbic acid in heat stressed broiler chickens. Pakistan Journal of Zoology, 51(3): 801.

Jamroz, D., Orda, J., Kamel, C., Wiliczkiewicz, A., Wertelecki, T. and Skorupinska, J. 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. Journal of Animal and Feed Sciences, 12(3): 583-596.

Jang, I.S., Ko, Y.H., Kang, S.Y. and Lee, C.Y. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Animal Feed Science and Technology, 134(3-4): 304-315.

Javed, I., Akhtar, M.S., Rahman, Z.U., Khaliq, T. and Ahmad, M. 1994. Comparative anthelminthic efficacy and safety of *Caesalpinia crista* seed and piperazine adipate in chickens with artificially induced *Ascaridia galli* infection. Acta Veterinaria Hungarica, 42(1): 103-109.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poultry Science, 77(9): 1259-1265.

Jing, T., Gao, X.D., Wang, P., Wang, Y., Lin, Y.F., Hu, X.Z., Hao, Q.L., Zhou, Y.K. and Mei, S.R. 2009. Determination of trace tetracycline antibiotics in foodstuffs by liquid chromatography–

tandem mass spectrometry coupled with selective molecular-imprinted solid-phase extraction. Analytical and Bioanalytical Chemistry, 393(8).

Jirovetz, L., Buchbauer, G. and Ngassoum, M. 1997. GC/MS-analysis of essential oils from Cameroon plants used as spices in local foodstuff. Recent Research and Development In Agricultural and Food Chemistry, 241-255.

Joshua, E., Ifeanyichukwu, I., Chika, E., Okoro, N., Carissa, D., Emmanuel, N. and Chukwuka, A. 2016. *In vitro* evaluation of antibacterial activity of *Parkia biglobosa*, *Hymenocardia acida* and *Zanthoxylum zanthoxyloides* extracts on pathogenic *Staphylococcus aureus* Isolates. International Journal of Life Sciences, 5(2): 72-77.

Junaid, S.A., Olabode, A.O., Onwuliri, F.C., Okwori, A.E.J. and Agina, S.E. 2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. African Journal of Biotechnology, 5(22).

Jung, S.J., Houde, R., Baurhoo, B., Zhao, X. and Lee, B.H. 2008. Effects of galacto-oligosaccharides and a *Bifidobacteria lactis*-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poultry Science, 87(9): 1694-1699.

Juskiewicz, J., Gruzauskas, R., Zdunczyk, Z., Semaskaite, A., Jankowski, J., Totilas, Z., Jarule, V., Sasyte, V., Zdunczyk, P., Raceviciute-Stupeliene, A. and Svirmickas, G. 2011. Effects of dietary addition of *Macleaya cordata* alkaloid extract on growth performance, caecal indices and breast meat fatty acids profile in male broilers. Journal of Animal Physiology and Animal Nutrition, 95(2): 171-178.

Kabir, S.L., Rahman, M.M., Rahman, M.B., Rahman, M.M. and Ahmed, S.U. 2004. The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci, 3(5): 361-364.

Kabir, S.M. 2009. The role of probiotics in the poultry industry. International Journal of Molecular Sciences, 10(8): 3531-3546.

Kana, J.R., Mube, K.H., Ngouana, T.R., Komguep, R., Yangoue, A., Tsafong, F. and Teguia, A. 2017. Growth Performances and Serum Biochemical Response of Broiler Chickens Fed on Diet Supplemented with *Tetrapleura tetrapleura* Fruit Powder as Substitute to Antibiotic Growth Promoters. International Journal of Innovation and Applied Studies, 21(1): 68-76.

Kapica, M., Kwiecień, M., Puzio, I., Bieńko, M., Radzki, R.P. and Pawłowska, M. 2006. Influence of some herbs on the activity of digestive enzymes in chickens. Medycyna Weterynaryjna, 62(9): 1048-1050.

Karaoglu, M. and Durdag, H. 2005. The influence of dietary probiotic (*Saccharomyces cerevisiae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. International Journal of Poultry Science, 4(5): 309-316.

Karimi Torshizi, M.A., Moghaddam, A.R., Rahimi, S.H. and Mojgani, N. 2010. Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. British Poultry Science, 51(2): 178-184.

Karuppusamy, S., Rajasekaran, K.M. and Karmegam, N. 2002. Evaluation of phytomedicines from street herbal vendors in Tamil Nadu, South India. Indian Journal of Traditional Knowledge, 1(1): 26-39.

Khalaji, S., Zaghari, M., Hatami, K.H., Hedari-Dastjerdi, S., Lotfi, L. and Nazarian, H. 2011. Black cumin seeds, Artemisia leaves (*Artemisia sieberi*), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poultry Science, 90(11): 2500-2510.

Khokon, J.U., Sharif, U.Z., Emran, H.S., Mohammad, A.R., James, J.K. and Mahbub, M. 2014. Efficacy of neem leaf extract against ascariasis in indigenous chicken. International Journal of Natural and Social Sciences, 1: 25-30.

Kiarie, E., Romero, L.F. and Nyachoti, C.M. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutrition Research Reviews, 26(1): 71-88.

Kim, J., Marshall, M.R. and Wei, C.I. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. Journal of Agricultural and Food Chemistry, 43(11): 2839-2845.

Kim, S.J., Lee, K.W., Kang, C.W. and An, B.K. 2016. Growth performance, relative meat and organ weights, cecal microflora, and blood characteristics in broiler chickens fed diets containing different nutrient density with or without essential oils. Asian-Australasian Journal of Animal Sciences, 29(4): 549.

Kim, Y.J., Jin, S.K. and Yang, H.S. 2009. Effect of dietary garlic bulb and husk on the physicochemical properties of chicken meat. Poultry Science, 88(2): 398-405.

Klaenhammer, T.R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Reviews, 12(1-3): 39-85.

Klotins, K. 2005. Antibiotic Use for Growth Improvement: Controversy and Resolution. Ontario, Ministry of Agriculture, Food and Rural Affairs.

Knarreborg, A., Simon, M.A., Engberg, R.M., Jensen, B.B. and Tannock, G.W. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Applied and Environmental Microbiology, 68(12): 5918-5924.

Knarreborg, A., Lauridsen, C., Engberg, R.M. and Jensen, S.K. 2004. Dietary antibiotic growth promoters enhance the bioavailability of α-tocopheryl acetate in broilers by altering lipid absorption. The Journal of nutrition, 134(6): 1487-1492.

Kolář, M., Pantůček, R., Bardoň, J., Vágnerová, I., Typovska, H., Válka, I. and Doškař, J. 2002. Occurrence of antibiotic-resistant bacterial strains isolated in poultry. Vet Med Czech, 47: 52-59.

Kollanoor-Johny, A., Mattson, T., Baskaran, S.A., Amalaradjou, M.A., Babapoor, S., March, B., Valipe, S., Darre, M., Hoagland, T., Schreiber, D. and Khan, M.I. 2012. Reduction of *Salmonella enterica* serovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. Applied and Environmental Microbiology, 78(8): 2981-2987.

Kopřiva, V., Suchý, P., Straková, E., Žďárský, M. and Dvořák, P. 2014. Colour and viscosity of egg yolk after addition of beetroot to feed for laying hens. Acta Veterinaria Brno, 83(1): 39-44.

Lanjewar, R.D., Zanzad, A.A., Ramteke, B.N., Lalmuanpuii, P.E., Taksande, P.E. and Patankar, R.B. 2009. Incorporation of Tulsi (*Ocimum sanctum*) Leaf Powder in diet of broilers for quality meat production. Veterinary World, 2(9).

Lara, L.J. and Rostagno, M.H. 2013. Impact of heat stress on poultry production. Animals, 3(2): 356-369.

Lawal, J.R., Jajere, S.M., Geidam, Y.A., Bello, A.M., Wakil, Y. and Mustapha, M. 2015. Antibiotic residues in edible poultry tissues and products in Nigeria: A potential public health hazard. International Journal of Animal and Veterinary Advances, 7(3): 55-61.

Lee, K.W., Li, G., Lillehoj, H.S., Lee, S.H., Jang, S.I., Babu, U.S., Lillehoj, E.P., Neumann, A.P. and Siragusa, G.R. 2011a. *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. Research in Veterinary Science, 91(3): 87-91.

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Park, M.S., Bravo, D. and Lillehoj, E.P. 2011b. Cinnamaldehyde enhances *in vitro* parameters of immunity and reduces *in vivo* infection against avian coccidiosis. British Journal of Nutrition, 106(6): 862-869.

Lee, S.H., Lillehoj, H.S., Chun, H.K., Tuo, W., Park, H.J., Cho, S.M., Lee, Y.M. and Lillehoj, E.P. 2007. *In vitro* treatment of chicken peripheral blood lymphocytes, macrophages, and tumor cells with extracts of Korean medicinal plants. Nutrition Research, 27(6): 362-366.

Lei, K., Li, Y.L., Yu, D.Y., Rajput, I.R. and Li, W.F. 2013. Influence of dietary inclusion of *Bacillus licheniformis* on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. Poultry Science, 92(9): 2389-2395.

Liu, X., Yan, H., Le Lv, Q.X., Yin, C., Zhang, K., Wang, P. and Hu, J. 2012. Growth performance and meat quality of broiler chickens supplemented with *Bacillus licheniformis* in drinking water. Asian-Australasian Journal of Animal Sciences, 25(5): 682.

Liu, Y., Yan, G., Chen, G. and Zhang, J. 2009. Efficacy trials of crude extraction from *Artemisia annul* L. against Newcastle disease virus *in vivo* in Xinjiang. Modern Applied Science, 3(5): 176-178.

Liu, Z.L., Xue, J.J., Huang, X.F., Luo, Y., Liang, M.R., Li, C.J., Wang, Q.G. and Wang, C. 2020. Effect of feeding frequency on the growth performance, carcass traits, and apparent nutrient digestibility in geese. Poultry Science, 99 (10): 4818-4823.

Lopes-Lutz, D., Alviano, D.S., Alviano, C.S. and Kolodziejczyk, P.P. 2008. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry, 69(8): 1732-1738.

Lu, Q., Wen, J. and Zhang, H. 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poultry Science, 86(6): 1059-1064.

Mahdavi, A.H., Rahmani, H.R., Nili, N., Samie, A.H., Soleimanian-Zad, S. and Jahanian, R. 2010. Effects of dietary egg yolk antibody powder on growth performance, intestinal *Escherichia coli* colonization, and immunocompetence of challenged broiler chicks. Poultry Science, 89(3): 484-494.

Mandal, A.B., Biswas, A., Yadav, A.S. and Biswas, A.K. 2014. Effect of dietary *Moringa oleifera* leaves powder on growth performance, blood chemistry, meat quality and gut microflora of broiler chicks. Animal Nutrition and Feed Technology, 14(2): 349-357.

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Côté, C., Ramirez, A.A. and Godbout, S. 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, 4(2): 170-178.

Meineri, G., Cornale, P., Tassone, S. and Peiretti, P.G. 2016. Effects of Chia (*Salvia hispanica* L.) seed supplementation on rabbit meat quality, oxidative stability and sensory traits. Italian Journal of Animal Science, 9(1): 45-49.

Mine, Y. and Kovacs-Nolan, J. 2002. Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. Journal of Medicinal Food, 5(3): 159-169.

Mirzaei-Aghsaghali, A. 2012. Importance of medical herbs in animal feeding: A review. Ann. Biol. Res, 3(9): 3-933.

Misra, L.N., Wouatsa, N.V., Kumar, S., Kumar, R.V. and Tchoumbougnang, F. 2013. Antibacterial, cytotoxic activities and chemical composition of fruits of two Cameroonian Zanthoxylum species. Journal of Ethnopharmacology, 148(1): 74-80.

Mohammed, M.A., Hassan, H.M.A. and El-Barkouky, E.M.A. 2008. Effect of mannan oligosaccharide on performance and carcass characteristics of broiler chicks. Journal of Agriculture and Social Sciences, 4(1): 13-17.

Mohammed, A.A. and Yusuf, M. 2011. Evaluation of ginger (*Zingiber officinale*) as a feed additive in broiler diets. Livest. Res. Rural Dev, 23, 202.

Mohnl, M., Acosta Aragon, Y., Acosta Ojeda, A., Rodriguez Sanchez, B. and Pasteiner, S. 2007, January. Effect of synbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. In Journal of Dairy Science, 90: 217-217.

Mokhtari, R., Yazdani, A. and Kashfi, H. 2015. The effects of different growth promoters on performance and carcass characteristics of broiler chickens. Journal of Veterinary Medicine and Animal Health, 7(8): 271-277.

Monroe, S. and Polk, R. 2000. Antimicrobial use and bacterial resistance. Current Opinion in Microbiology, 3(5): 496-501.

Montes-Belmont, R. and Carvajal, M. 1998. Control of *Aspergillus flavus* in maize with plant essential oils and their components. Journal of Food Protection, 61(5): 616-619.

Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N. and Ho, Y.W. 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. Journal of the Science of Food and Agriculture, 94(2): 341-348.

Moore, P.R., Evenson, A., Luckey, T.D., McCoy, E., Elvehjem, C.A. and Hart, E.B. 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J. biol. Chem, 165(2): 437-441.

Moyo, B., Oyedemi, S., Masika, P.J. and Muchenje, V. 2012. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. Meat Science, 91(4): 441-447.

Muanda, F., Koné, D., Dicko, A., Soulimani, R. and Younos, C. 2011. Phytochemical composition and antioxidant capacity of three Malian medicinal plant parts. Evidence-Based Complementary and Alternative Medicine, 2011.

Mulugeta, M., Worku, Z., Seid, A. and Debela, L. 2019. Effect of garlic powder (*Allium sativum*) on performance of broiler chicken. Livestock Research for Rural Development, 31: 4.

Murugesan, G.R., Syed, B., Haldar, S. and Pender, C. 2015. Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Frontiers in Veterinary Science, 2: 21.

Musa, A.K. 2012. Suppression of Seed Beetle (*Callosobruchus maculatus*) Population with Root Bark Powder of *Zanthoxylum Zanthoxyloides* (Lam.) Waterm. (Rutaceae) on Cowpea (*Vigna unguiculata* L.) Walp. Agrosearch, 12(2): 196-204.

Mustafa, A.A. and El-kamali, H.H. 2019. Chemical Composition of *Ocimum americanum* In Sudan. Research in Pharmacy and Health Sciences, 5(3): 172-178.

Nakamura, C.V., Ueda-Nakamura, T., Bando, E., Melo, A.F.N., Cortez, D.A.G. and Dias Filho, B.P. 1999. Antibacterial activity of *Ocimum gratissimum* L. essential oil. Memórias do Instituto Oswaldo Cruz, 94(5): 675-678.

Nakatani, N. 1997. Antioxidants from spices and herbs. Natural antioxidants: chemistry, health effects, and applications, 64-75.

Nascimento, J.C., Barbosa, L.C., Paula, V.F., David, J.M., Fontana, R., Silva, L.A. and França, R.S. 2011. Chemical composition and antimicrobial activity of essential oils of *Ocimum canum* Sims and *Ocimum selloi* Benth. Anais da Academia Brasileira de Ciências, 83(3): 787-800.

Nezhad, Y.E., Gale-Kandi, J.G., Farahvash, T. and Yeganeh, A.R. 2011. Effect of combination of citric acid and microbial phytase on digestibility of calcium, phosphorous and mineralization parameters of tibia bone in broilers. African Journal of Biotechnology, 10(66): 15089-15093.

Ngassoum, M.B., Essia-Ngang, J.J., Tatsadjieu, L.N., Jirovetz, L., Buchbauer, G. and Adjoudji, O. 2003. Antimicrobial study of essential oils of *Ocimum gratissimum* leaves and *Zanthoxylum xanthoxyloides* fruits from Cameroon. Fitoterapia, 74(3): 284-287.

Ngassoum, M.B., Ousmaila, H., Ngamo, L.T., Maponmetsem, P.M., Jirovetz, L. and Buchbauer, G. 2004. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. Journal of Food Composition and Analysis, 17(2): 197-204.

Nieto, G. 2017. Biological activities of three essential oils of the Lamiaceae family. Medicines, 4(3): 63.

Niewold, T.A. 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poultry Science, 86(4): 605-609.

Nisha, A.R. 2008. Antibiotic residues-a global health hazard. Veterinary World, 1(12): 375.

Nobakht, A. and Moghaddam, M. 2013. The effects of different levels of costmary (*Tanacetum balsamita*) medicinal plant on performance, egg traits and blood biochemical parameters of laying hens. Iranian Journal of Applied Animal Science, 3(2): 307-312.

Nortey, T.N., Ewusi, I., Kpogo, L.A., Oddoye, E.O.K. and Naazie, A. 2015. Cocoa pod husk with enzyme supplementation is a potential feed ingredient in broiler diets. Livestock Research for Rural Development, 27(5): 10-15.

Nyarko, A.K., Asare-Anane, H., Ofosuhene, M. and Addy, M.E. 2002. Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β-islet cells. Phytomedicine, 9(4): 346-351.

Odebiyi, O.O. and Sofowora, E.A. 1979. Antimicrobial alkaloids from a Nigerian chewing stick (*Fagara zanthoxyloides*). Planta Medica, 36(07): 204-207.

Odoemelam, V.U., Etuk, I.F., Ndelekwute, E.K. and Ekwe, T.C.I.C.C. 2013. Herbs and spices: Options for sustainable animal production. Journal of Biology, Agriculture and Healthcare, 3(7): 116-124.

Odoemelam, V. U., Unamba-oparah, I. C., Irondi, J. I. and Anyanwu, N. J. 2017. Performance and carcass quality of broilers fed dietary scent leaf, garlic and antibiotic Nigerian Journal of Agriculture, Food and Environment. 13(1): 188-193

Ogunleye, A.J. 2019. Performance of Broilers fed graded levels of Basil leaf (*Ocimum gratissimum*) as supplement in finisher diet. The International Journal of Organic Agriculture Research and Development, 16.

Ogwal-Okeng, J.W. 1990. The *in vitro* Study of the Anhelmintic Activity of Crude Extracts of Two Ugandan Medicinal Plants: *Cissus adenocaulis* and *Fagara zanthoxyloides* (Doctoral dissertation, M. Sc. Thesis, Makerere University).

Ogwal-Okeng, J.W., Obua, C. and Anokbonggo, W.W. 2003. Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (Lam.) root-bark. African Health Sciences, 3(3): 124-126.

Ojelade, A.Y.P., Lamidi, A.W., Umoru, J.I., Ajbade, A.S. and Agbaye, P. 2012. Utilization of *Ocimum gratissimum* and the calyx of *Hibiscus sabdariffa* leaf meal in the diet of broiler chicks. Journal of Agriculture, Forestry and the Social Sciences, 10(2): 257-264.

Olatoye, I.O. and Ehinmowo, A.A. 2010. Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. Nigerian Veterinary Journal, 31(2).

Olfati, A. And Mojtahedin, A. 2018. Dietary inclusion of thyme essential oil alleviative effects of heat stress on growth performance and immune system of broiler chicks. J. Biotechnol., 1: 60-71.

Olounladé, P.A., Azando, E.V.B., Hounzangbé-Adoté, M.S., Ha, T.T., Leroy, E., Moulis, C., Fabre, N., Magnaval, J.F., Hoste, H. and Valentin, A. 2012. *In vitro* anthelmintic activity of the essential oils of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* against *Strongyloides ratti*. Parasitology Research, 110(4): 1427-1433.

Olumide Martha, D., Gibson, C., Ajayi Opeyemi, A. and Akinboye Olufunso, E. 2018. Performance, haematological and serum biochemical profile of broilers chicken fed diets supplemented with *Ocimum gratissimum* meal. International Journal of Modern Biological Research, 6: 27-34.

Onifade, A.A. 1997. Growth performance, carcass characteristics, organs measurement and haematology of broiler chickens fed a high fibre diet supplemented with antibiotics or dried yeast. Food/Nahrung, 41(6): 370-374.

Onu, P.N., Ude, F.E. and Okpaniezeani, P.E. 2004. Effect of graded levels of dietary penicillin on the growth rate and feed conversion of broiler chicks. Journal of Agriculture and Social Research (JASR), 4(2): 25-32.

Osho, A. and Adelani, O.A. 2012. The antimicrobial effect of some selected Nigerian chewing sticks on clinical isolates of Candida species. J Microbiol Res, 2(1): 1-5.

Osman, M., Yakout, H.M., Motawe, H.F. and El-Arab, W.E. 2010. Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. Egyptian Poultry Science Journal, 30(1): 25-53.

Owens, B., Tucker, L.C.M.A., Collins, M.A. and McCracken, K.J. 2008. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. British Poultry Science, 49(2): 202-212.

Oyewole, A.O., Audu, O.T. and Amupitan, J.O. 2004. A survey of chemical constituents and biological activities of some medicinal plants. Journal of Chemical Society of Nigeria, 4: 162-165.

Park, I.J., Cha, S.Y., Kang, M., So, Y.S., Go, H.G., Mun, S.P., Ryu, K.S. and Jang, H.K. 2011. Effect of proanthocyanidin-rich extract from *Pinus radiata* bark on immune response of specific-pathogen-free White Leghorn chickens. Poultry Science, 90(5): 977-982.

Paskudska, A., Kołodziejczyk, D. and Socha, S. 2018. The use of herbs in animal nutrition. Acta Sci. Pol. Zootechnica, 17(2): 3-14.

Patterson, J.A. and Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry production. Poultry Science, 82(4): 627-631.

Pesti, G.M. and Bakalli, R.I. 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poultry Science, 75(9): 1086-1091.

Pourmahmoud, B., Aghazadeh, A.M. and Sis, N.M. 2013. The effect of thyme extract on growth performance, digestive organ weights and serum lipoproteins of broilers fed wheat-based diets. Italian Journal of Animal Science, 12(3): 53.

Prajwal, S., Vasudevan, V.N., Sathu, T., Irshad, A., Nayankumar, S.R. and Pame, K. 2017. Antibiotic residues in food animals: Causes and health effects. Pharma Innov J, 6: 1-4.

Prasad, R., Rose, M.K., Virmani, M., Garg, S.L. and Puri, J.P. 2009. Lipid profile of chicken (*Gallus domesticus*) in response to dietary supplementation of garlic (*Allium sativum*). International Journal of Poultry Science, 8(3): 270-276.

Prempeh, A.B.A. 2008. Analgesic activity of crude aqueous extract of the root bark of *Zanthoxylum xanthoxyloides*. Ghana Medical Journal, 42(2).

Prescott, J.F., and Baggot, J.D. 1993. Antimicrobial Therapy in Veterinary Medicine. Iowa State University Press, 2: 564-565.

Proudfoot, F.G., Hulan, H.W. and Jackson, E.D. 1988. The response of male broiler chicks to the consumption of low levels of chlortetracycline as a growth promoter. Canadian Journal of Animal Science, 68(4): 1285-1290.

Proudfoot, F.G., Hulan, H.W., Jackson, E.D. and Salisbury, C.D.C. 1990. Effect of lincomycin as a growth promoter for broiler chicks. British Poultry Science, 31(1): 181-187.

Qureshi, A.A., Din, Z.Z., Abuirmeileh, N., Burger, W.C., Ahmad, Y. and Elson, C.E. 1983. Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: impact on serum lipids. The Journal of Nutrition, 113(9): 1746-1755.

Qureshi, A.A., Crenshaw, T.D., Abuirmeileh, N., Peterson, D.M. and Elson, C.E. 1987. Influence of minor plant constituents on porcine hepatic lipid metabolism: impact on serum lipids. Atherosclerosis, 64(2): 109-115.

Rady, M.R. and Nazif, N.M. 2005. Rosmarinic acid content and RAPD analysis of *in vitro* regenerated basil (*Ocimum americanum*) plants. Fitoterapia, 76(6): 525-533.

Ragab, M.S., Namra, M.M.M., Aly, M.M.M. and Fathi, M.A. 2013. Impact of inclusion fennel seeds and thyme dried leaves in broiler diets on some productive and physiological performance during summer season. Egyptian Poultry Science Journal, 33: 197-219.

Ranjan, A., Sinha, R., Devi, I., Rahim, A. and Tiwari, S. 2019. Effect of heat stress on poultry production and their managemental approaches. Int. J. Curr. Microbiol. A Sci, 8(2): 1548-1555.

Reddy, L.V.P., Thangavel, A., Leela, V. and Raju, K.N. 2009. Antioxidant enzyme status in broilers: role of dietary supplementation of Tulasi (*Ocimum sanctum*) and selenium. Tamilnadu Journal of Veterinary and Animal Sciences, 5(6): 251-256.

Rekhate, D.H., Ukey, S. and Dhok, A.P. 2004. Performance and haemobiochemical profile of broilers fed on supplementation of shatavari (*Asparagus racemosus* willd.) root powder. Indian Journal of Poultry Science, 39(2): 182-184.

Richards, J.D., Zhao, J., Harrell, R.J., Atwell, C.A. and Dibner, J.J. 2010. Trace mineral nutrition in poultry and swine. Asian-Australasian Journal of Animal Sciences, 23(11): 1527-1534.

Riyazi, S.R., Ebrahimnezhad, Y., Hosseini, S.A., Meimandipour, A. and Ghorbani, A. 2015. Comparison of the effects of basil (*Ocimum basilicum*) essential oil, avilamycin and protexin on broiler performance, blood biochemistry and carcass characteristics. Archiv Fuer Tierzucht, 58(2): 425.

Robertsson, J.A. and Lundeheim, N. 1994. Prohibited use of antibiotics as a feed additive for growth promotion effects on piglet health and production parameters. In 13. International Pig Veterinary Society Congress, Bangkok: 26-30.

Sado Kamdem, S.L., Belletti, N., Tchoumbougnang, F., Essia-Ngang, J.J., Montanari, C., Tabanelli, G., Lanciotti, R. and Gardini, F. 2015. Effect of mild heat treatments on the antimicrobial activity of essential oils of *Curcuma longa*, *Xylopia aethiopica*, *Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* against *Salmonella enteritidis*. Journal of Essential Oil Research, 27(1): 52-60.

Sahu, R. and Saxena, P. 2014. Antibiotics in Chicken Meat. Centre for Science and Environment, 41.

Samanta, S., Haldar, S. and Ghosh, T.K. 2010. Comparative efficacy of an organic acid blend and bacitracin methylene disalicylate as growth promoters in broiler chickens: effects on performance, gut histology, and small intestinal milieu. Veterinary Medicine International, 2010.

Samarasinghe, K., Wenk, C., Silva, K.F.S.T. and Gunasekera, J.M.D.M. 2003. Turmeric (*Curcuma longa*) root powder and mannanoligosaccharides as alternatives to antibiotics in broiler chicken diets. Asian-Australasian Journal of Animal Sciences, 16(10): 1495-1500.

Sanjyal, S. and Sapkota, S. 2011. Supplementation of broilers diet with different sources of growth promoters. Nepal Journal of Science and Technology, 12: 41-50.

Santoso, U. and Fenita, Y.K. 2017. The effect of medicinal herb inclusion on the hematologic status and blood lipid profiles in broiler chickens. Int. J. Poult. Sci, 16: 415-423.

Sarica, S., Ciftci, A., Demir, E., Kilinc, K. and Yildirim, Y. 2005. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science, 35(1): 61-72.

Schade, R., Calzado, E.G., Sarmiento, R., Chacana, P.A., Porankiewicz-Asplund, J. and Terzolo, H.R. 2005. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. Alternatives to Laboratory Animals, 33(2): 129-154.

Schjørring, S. and Krogfelt, K.A. 2011. Assessment of bacterial antibiotic resistance transfer in the gut. International Journal of Microbiology, 2011.

Scott, H.M. 2012. Heavy Metals as Alternatives to Antibiotics: Panacea or Pandora's box. In International Symposium on Alternatives to Antibiotics, Paris, France.

Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A.M. and Saura-Calixto, F. 2009. Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Molecular Nutrition and Food Research, 53(S2): S310-S329.

Sethiya, N.K. 2016. Review on natural growth promoters available for improving gut health of poultry: an alternative to antibiotic growth promoters. Asian J Poult Sci, 10: 1-29.

Shareef, A.M., Jamel, Z.T. and Yonis, K.M. 2009. Detection of antibiotic residues in stored poultry products. Iraqi Journal of Veterinary Sciences, 23(3).

Shifa, U.I.H. 2014. Anthelmintic plants and herbs https://www.slideshare.net/ShifaUlHaq/anthelmintic-plants-and-herbs (Accessed on August 2, 2020)

Shokryazdan, P., Jahromi, M.F., MD Saadand, S., Ebrahimi, M., Idrus, Z., Zhou, H., Diao, X.P. and Liang, J.B. 2017. Chinese herbal medicines as potential agents for alleviation of heat stress in poultry. Scientifica, 2017.

Shoukary, R.D.E., Sayed, R.K. and Hassan, R.I. 2018. Behavioral, hepato-morphological, and biochemical studies on the possible protective effect of black seed and water bath against change-mediated heat stress on pigeon. The Journal of Basic and Applied Zoology, 79(1): 23.

Siamba, D.N., Okitoi, L.O., Watai, M.K., Wachira, A.M., Lukibisi, F.B. and Mukisira, E.A. 2007. Efficacy of *Tephrosia vogelli* and *Vernonia amygdalina* as anthelmintics against *Ascaridia galli* in indigenous chicken. Livestock Research for Rural Development, 19(12).

Simon, O., Jadamus, A. and Vahjen, W. 2001. Probiotic feed additives-effectiveness and expected modes of action. Journal of Animal and Feed Sciences, 10: 51-68.

Sirri, F., Iaffaldano, N., Minelli, G., Meluzzi, A., Rosato, M.P. and Franchini, A. 2007. Comparative pigmentation efficiency of high dietary levels of apo-ester and marigold extract on quality traits of whole liquid egg of two strains of laying hens. Journal of Applied Poultry Research, 16(3): 429-437.

Skomorucha, I. and Sosnówka-Czajka, E. 2012. Effects of dietary herbal supplements on poultry productivity and health. Wiadomości Zootechniczne, 50(4): 51-55.

Snyder, D.L. and Wostmann, B.S. 1987. Growth rate of male germfree Wistar rats fed ad libitum or restricted natural ingredient diet. Laboratory Animal Science, 37(3): 320-325.

Sood, R., Swarup, D., Bhatia, S., Kulkarni, D.D., Dey, S., Saini, M. and Dubey, S.C. 2012. Antiviral activity of crude extracts of *Eugenia jambolana* Lam. against highly pathogenic avian influenza (H5N1) virus. Indian Journal of Experimental Biology, 50(03): 179-186.

Spring, P., Wenk, C., Dawson, K.A. and Newman, K.E. 2000. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poultry Science, 79(2): 205-211.

Stef, L., Dumitrescu, G., Drinceanu, D., Stef, D., Mot, D., Julean, C., Tatileanu, R. and Corcionivoschi, N. 2009. The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile. Romanian Biotechnological Letters, 14(4): 4606-4614.

Steiner, T. 2006. Managing Gut Health: Natural Growth Promoters as a Key to Animal Performance. Nottingham University Press.

Steiner, T. and Syed, B. 2015. Phytogenic feed additives in animal nutrition. In Medicinal and Aromatic Plants of the World, 403-423.

Suganya, T., Senthilkumar, S., Deepa, K., Muralidharan, J., Gomathi, G. and Gobiraju, S. 2016. Herbal feed additives in poultry. Int. J. Sci. Environ. Technol, 5(3): 1137-45.

Suresh, G., Das, R.K., Kaur Brar, S., Rouissi, T., Avalos Ramirez, A., Chorfi, Y. and Godbout, S. 2018. Alternatives to antibiotics in poultry feed: molecular perspectives. Critical reviews in Microbiology, 44(3): 318-335.

Swiatkiewicz, S. and Koreleski, J. 2007. Feed additives with immunomodulatory acting in poultry feeding. Vet Med, 63: 1291-1295.

Swiątkiewicz, S., Arczewska, A. and Koreleski, J. 2009. Effect of chosen feed additives on coccidiosis in poultry. Medycyna Weterynaryjna, 65(11): 758-761.

Symeonidou, I., Bonos, E., Moustakidis, K., Florou-Paneri, P., Christaki, E. and Papazahariadou, M. 2018. Botanicals: a natural approach to control ascaridiosis in poultry. Journal of the Hellenic Veterinary Medical Society, 69(1): 711-722.

Tabak, M., Armon, R. and Neeman, I. 1999. Cinnamon extracts' inhibitory effect on *Helicobacter pylori*. Journal of Ethnopharmacology, 67(3): 269-277.

Tamilzarasan, K.B., Dinakaran, A.M., Selvaraju, G. and Dorairajan, N. 2009. Efficacy of egg yolk immunoglobulins (IGY) against enteric pathogens in poultry. Tamilnadu Journal of Veterinary and Animal Sciences, 5(6): 264-268.

Tavárez, M.A., Boler, D.D., Bess, K.N., Zhao, J., Yan, F., Dilger, A.C., McKeith, F.K. and Killefer, J. 2011. Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poultry Science, 90(4): 922-930.

Thacker, P.A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. Journal of Animal Science and Biotechnology, 4(1): 35.

Thomke, S. and Elwinger, K. 1998. Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. In Annales de Zootechnie, 47(3): 153-167.

Tiihonen, K., Kettunen, H., Bento, M.H.L., Saarinen, M., Lahtinen, S., Ouwehand, A.C., Schulze, H. and Rautonen, N. 2010. The effect of feeding essential oils on broiler performance and gut microbiota. British Poultry Science, 51(3): 381-392.

Toghyani, M., Toghyani, M., Gheisari, A., Ghalamkari, G. and Mohammadrezaei, M. 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Livestock Science, 129(1-3): 173-178.

Torki, M., Sedgh-Gooya, S. and Mohammadi, H. 2018. Effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and some blood parameters of laying hens subjected to heat stress. Journal of Applied Animal Research, 46(1): 1118-1126.

Torres-Rodriguez, A., Donoghue, A.M., Donoghue, D.J., Barton, J.T., Tellez, G. and Hargis, B.M. 2007. Performance and condemnation rate analysis of commercial turkey flocks treated with a Lactobacillus-based probiotic. Poultry Science, 86(3): 444-446.

Tropical Plants Database (TPD) 2020a. https://tropical.theferns.info/ viewtropical.php?id=*Zanthoxylum+zanthoxyloides* (Accessed on May 31, 2020).

Tropical Plants Database (TPD) 2020b. https://tropical.theferns.info/ viewtropical.php?id=*Ocimum+americanum* (Accessed on August 1, 2020)

Tucker, L. 2002. Botanical broilers: Plant extracts to maintain poultry performance. Feed Int, 23: 26-29.

Upadhayay, U.P.P.D.D. and Vishwa, P.C.V. 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances—A review. Int. J. Pharmacol, 10(3): 129-159.

Utami, M.M.D., Pantaya, D. and Agus, A. 2018, January. Addition of Garlic Extract in Ration to Reduce Cholesterol Level of Broiler. In Journal of Physics: Conference Series, 953(1): 012124.

Vasanth S, Dipu MT, Mercy AD and Shyama K. 2015. Efficiency of copper sulphate versus flavomycin as a growth promoter in broiler chickens. International Journal of Current Research, 7: 16536–16539.

Vasanthakumar, P., Sasikumar, P., Pangayarselvi, B., Chandrasekaran, D., Doraisamy, K.A., Senthilkumar, S. and Purushothaman, M.R. 2013. Performance of broiler chicken fed tulsi leaf powder and leaf extract supplemented diets during summer to alleviate heat stress. Indian Journal of Animal Sciences, 83(9): 930-931.

Verma, A.R., Vijayakumar, M., Mathela, C.S. and Rao, C.V. 2009. *In vitro* and *in vivo* antioxidant properties of different fractions of Moringa oleifera leaves. Food and Chemical Toxicology, 47(9): 2196-2201.

Vinus, R.D., Sheoran, N., Maan, N. and Tewatia, B. 2018. Potential benefits of herbal supplements in poultry feed: A Review. Pharma Innov, 7(6): 651-6.

Waldenstedt, L. 2003. Effect of vaccination against coccidiosis in combination with an antibacterial oregano (*Origanum vulgare*) compound in organic broiler production. Acta Agriculturae Scandinavica, Section A-Animal Science, 53(2): 101-109.

Wan, X., Jiang, L., Zhong, H., Lu, Y., Zhang, L. and Wang, T. 2017. Effects of enzymatically treated *Artemisia annua L.* on growth performance and some blood parameters of broilers exposed to heat stress. Animal Science Journal, 88(8): 1239-1246.

Wang, L., Piao, X.L., Kim, S.W., Piao, X.S., Shen, Y.B. and Lee, H.S. 2008. Effects of Forsythia suspensa extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. Poultry Science, 87(7): 1287-1294.

Wang, Y. and Gu, Q. 2010. Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. Research in Veterinary Science, 89(2): 163-167.

Wati, T., Ghosh, T.K., Syed, B. and Haldar, S. 2015. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. Animal Nutrition, 1(3): 213-219.

Wenk, C. 2000. Why all discussion about herbs? Biotechn in the feed industry. In Proc. of Alltechs 16th Annu. Symp. Alltech Technical Publications, Nottginham Univ. Press. Nicholasvile, KY, 79-96

Whitehead, C.C. 1998. Vitamin E can alleviate the depressed egg production of heat-stressed laying hens. British Poultry Science, 39(S1): 44-46.

Wierup, M. 2001. The experience of reducing antibiotics used in animal production in the Nordic countries. International Journal of Antimicrobial Agents, 18(3): 287-290.

Williams, A.R., Soelberg, J. and Jäger, A.K. 2016. Anthelmintic properties of traditional African and Caribbean medicinal plants: identification of extracts with potent activity against Ascaris suum *in vitro*. Parasite, 23.

Wilson, J., Tice, G., Brash, M.L. and Hilaire, S.S. 2005. Manifestations of *Clostridium perfringens* and related bacterial enteritides in broiler chickens. World's Poultry Science Journal, 61(3): 435-449.

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86(S14): 140-148.

World Health Organization (WHO) 2020. Malaria. https://www.who.int/news-room/fact-sheets/detail/malaria (Accessed on October 24, 2020).

Wouatsa, V.N., Misra, L., Kumar, S., Prakash, O., Khan, F., Tchoumbougnang, F. and Venkatesh, R.K. 2013. Aromatase and glycosyl transferase inhibiting acridone alkaloids from fruits of Cameroonian Zanthoxylum species. Chemistry Central Journal, 7(1): 125.

Xu, Y., Li, X., Jin, L., Zhen, Y., Lu, Y., Li, S., You, J. and Wang, L. 2011. Application of chicken egg yolk immunoglobulins in the control of terrestrial and aquatic animal diseases: a review. Biotechnology advances, 29(6): 860-868.

Xu, Q.Q., Yan, H., Liu, X.L., Lv, L., Yin, C.H. and Wang, P. 2014. Growth performance and meat quality of broiler chickens supplemented with *Rhodopseudomonas palustris* in drinking water. British Poultry Science, 55(3): 360-366.

Yang, Y., Iji, P.A. and Choct, M. 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poultry Science Journal, 65(1): 97-114.

Yarru, L.P., Settivari, R.S., Gowda, N.K.S., Antoniou, E., Ledoux, D.R. and Rottinghaus, G.E. 2009. Effects of turmeric (*Curcuma longa*) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poultry Science, 88(12): 2620-2627.

Yazdankhah, S., Rudi, K. and Bernhoft, A. 2014. Zinc and copper in animal feed–development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microbial Ecology in Health and Disease, 25(1): 25862.

Yegani, M. and Korver, D.R. 2010. Application of egg yolk antibodies as replacement for antibiotics in poultry. World's Poultry Science Journal, 66(1): 27-38.

Yeo, J. and Kim, K.I. 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Science, 76(2): 381-385.

Yıldırım, A., Şekeroğlu, A., Eleroğlu, H., Şen, M.I. and Duman, M. 2013. Effects of Korean ginseng (*Panax ginseng CA Meyer*) root extract on egg production performance and egg quality of laying hens. South African Journal of Animal Science, 43(2): 194-207.

Yim, D., Kang, S.S., Kim, D.W., Kim, S.H., Lillehoj, H.S. and Min, W. 2011. Protective effects of *Aloe vera*-based diets in *Eimeria maxima*-infected broiler chickens. Experimental Parasitology, 127(1): 322-325.

Yitbarek, A., Echeverry, H., Munyaka, P. and Rodriguez-Lecompte, J.C. 2015. Innate immune response of pullets fed diets supplemented with prebiotics and synbiotics. Poultry Science, 94(8): 1802-1811.

Ynalvez, R.A., Cardenas, C., Addo, J.K., Adukpo, G.E., Dadson, B.A. and Addo-Mensah, A. 2012. Evaluation of the antimicrobial activity of *Zanthoxylum zanthoxyloides* root bark extracts. Res J Med Pl, 6: 149-159.

Zaunschirm, M. 2018. Can antibiotic growth promoters be replaced with phytogenic feed additives? https://www2.biomin.net/pt/artigos/can-antibiotic-growth-promoters-be-replaced-with-phytogenic-feed-additives (Accessed on September 12, 2020).

Zeferino, C.P., Komiyama, C.M., Pelícia, V.C., Fascina, V.B., Aoyagi, M.M., Coutinho, L.L., Sartori, J.R. and Moura, A.S.A.M.T. 2016. Carcass and meat quality traits of chickens fed diets concurrently supplemented with vitamins C and E under constant heat stress. Animal, 10(1): 163-171.

Zhao, X.H., He, X., Yang, X.F. and Zhong, X.H. 2013. Effect of *Portulaca oleracea* extracts on growth performance and microbial populations in ceca of broilers. Poultry Science, 92(5): 1343-1347.

APPENDICES

Appendix A: Composition of vitamins

Constituent	Amount	Unit
Vitamin B_1	7.20	mg
Vitamin B_2	2.74	mg
Vitamin B_3	14.40	mg
Vitamin B_5	7.20	mg
Vitamin B_6	3.60	mg
Vitamin B_7	0.57	µg
Vitamin B_9	0.02	mg
Vitamin B_{12}	7.20	µg
Vitamin K_3	10.00	mg
Methionine	7.20	mg
Choline chloride	11.00	mg

Appendix B: Composition of broiler premix

Constituent	**Amount**	**Unit**
Vitamin A	12000000	IU
Vitamin D_3	2000000	IU
Vitamin E	10000	mg
Vitamin K_3	1500	mg
Vitamin B_1	1500	mg
Vitamin B_2	4000	mg
Vitamin B_6	1500	mg
Vitamin B_{12}	15	mg
Pantothenic acid	8000	mg
Nicotinic acid	20000	mg
Folic acid	500	mg
Biotin	150	mg
Choline chloride	120000	mg
Iron	40000	mg
Mn	60000	mg
Cu	6000	mg
Zn	50000	mg
Iodate	2000	mg
Selenium	150	mg
Anti-oxidant (PHT)	25000	mg

Appendix C: Agar media used for faecal microbial analysis

Parameter	**Agar media used**	**Product code**
Microbial load	Plate Count (Tryptone Glucose Yeast Agar)	Oxoid CMO325
Coliforms	Violet Red Bile Agar	(Not indicated)
Escherichia coli	Eosin Methylene Blue Agar	M317-500G
Salmonella and *Shigella*	Salmonella and Shigella (SS) Agar	Modified 610042
Yeast and molds	Potato Dextrose Agar	Oxoid CMO139

Appendix D: Analysis of variance (ANOVA) tables

1. GROWTH PARAMETERS

a. Variate: Body weight (Week 0)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	317.74	45.39	1.01	0.448
REP	4	551.29	137.82	3.05	0.033
Residual	28	1263.91	45.14		
Total	39	2132.94			

b. Variate: Body weight (Week 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	7067.8	1009.7	2.81	0.024
REP	4	4537.5	1134.4	3.16	0.029
Residual	28	10065.3	359.5		
Total	39	21670.7			

c. Variate: Body weight (Week 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	17238.	2463.	2.14	0.072
REP	4	5886.	1471.	1.28	0.302
Residual	28	32213.	1150.		
Total	39	55337.			

d. Variate: Body weight (Week 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	57177.	8168.	2.38	0.049
REP	4	13542.	3385.	0.98	0.432
Residual	28	96288.	3439.		
Total	39	167006.			

e. Variate: Body weight (Week 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	51126.	7304.	1.04	0.426
REP	4	5452.	1363.	0.19	0.939
Residual	28	196497.	7018.		
Total	39	253075.			

f. Variate: Body weight (Week 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	86244.	12321.	0.97	0.472
REP	4	10270.	2567.	0.20	0.935
Residual	28	355771.	12706.		
Total	39	452284.			

g. Variate: Body weight (Week 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	131297.	18757.	0.75	0.633
REP	4	39853.	9963.	0.40	0.808
Residual	28	700963.	25034.		
Total	39	872113.			

h. Variate: ADG (Week 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	99.450	14.207	3.26	0.012
REP	4	42.714	10.679	2.45	0.069
Residual	28	122.014	4.358		
Total	39	264.178			

i. **Variate: ADG (Week 2)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	113.142	16.163	1.74	0.140
REP	4	57.262	14.316	1.54	0.217
Residual	28	259.814	9.279		
Total	39	430.218			

j. Variate: ADG (Week 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	302.29	43.18	1.82	0.123
REP	4	50.13	12.53	0.53	0.716
Residual	28	664.82	23.74		
Total	39	1017.24			

k. Variate: ADG (Week 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	62.89	8.98	0.22	0.976
REP	4	170.67	42.67	1.06	0.393
Residual	28	1122.99	40.11		
Total	39	1356.55			

l. Variate: ADG (Week 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	675.88	96.55	1.50	0.207
REP	4	280.00	70.00	1.09	0.380
Residual	28	1797.60	64.20		
Total	39	2753.49			

m. Variate: ADG (Week 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1427.5	203.9	1.38	0.252
REP	4	339.2	84.8	0.57	0.684
Residual	28	4134.6	147.7		
Total	39	5901.3			

n. Variate: ADFI (Week 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	299.11	42.73	3.01	0.017
REP	4	106.78	26.70	1.88	0.142
Residual	28	397.77	14.21		
Total	39	803.67			

o. Variate: ADFI (Week 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	487.95	69.71	1.33	0.271
REP	4	458.34	114.58	2.19	0.095
Residual	28	1462.39	52.23		
Total	39	2408.68			

p. Variate: ADFI (Week 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	429.77	61.40	0.75	0.632
REP	4	766.31	191.58	2.34	0.079
Residual	28	2290.65	81.81		
Total	39	3486.73			

q. Variate: ADFI (Week 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	396.3	56.6	0.42	0.882
REP	4	829.9	207.5	1.53	0.219
Residual	28	3786.0	135.2		
Total	39	5012.1			

r. Variate: ADFI (Week 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1006.3	143.8	1.07	0.410
REP	4	577.8	144.4	1.07	0.389
Residual	28	3774.6	134.8		
Total	39	5358.7			

s. Variate: ADFI (Week 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	879.3	125.6	0.51	0.819
REP	4	669.0	167.3	0.68	0.612
Residual	28	6894.0	246.2		
Total	39	8442.4			

t. Variate: FCR (Week 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	0.08105	0.01158	0.98	0.468
REP	4	0.00183	0.00046	0.04	0.997
Residual	28	0.33241	0.01187		
Total	39	0.41529			

u. Variate: FCR (Week 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	0.18376	0.02625	0.94	0.492
REP	4	0.13843	0.03461	1.24	0.317
Residual	28	0.78117	0.02790		
Total	39	1.10337			

v. Variate: FCR (Week 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	0.45730	0.06533	1.29	0.293
REP	4	0.08063	0.02016	0.40	0.809
Residual	28	1.42281	0.05081		
Total	39	1.96074			

w. Variate: FCR (Week 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	0.21264	0.03038	0.42	0.883
REP	4	0.58731	0.14683	2.02	0.119
Residual	28	2.03945	0.07284		
Total	39	2.83941			

x. Variate: FCR (Week 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1.3473	0.1925	1.24	0.314
REP	4	1.1209	0.2802	1.81	0.156
Residual	28	4.3421	0.1551		
Total	39	6.8103			

y. Variate: FCR (Week 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	15.726	2.247	1.79	0.128
REP	4	3.553	0.888	0.71	0.592
Residual	28	35.049	1.252		
Total	39	54.328			

z. Mortality % (d 1-42)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	360.00	51.43	1.45	0.224
REP	4	250.00	62.50	1.77	0.163
Residual	28	990.00	35.36		
Total	39	1600.00			

2. CARCASS PARAMETERS

a. Abdominal fat weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	283.6	40.5	0.38	0.903
REP	4	728.2	182.1	1.73	0.172
Residual	28	2948.1	105.3		
Total	39	3960.0			

b. Breast weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	121532.	17362.	1.28	0.296
REP	4	20892.	5223.	0.38	0.818
Residual	28	379924.	13569.		
Total	39	522348.			

c. Defeathered weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	568215.	81174.	1.52	0.203
REP	4	321980.	80495.	1.50	0.228
Residual	28	1499612.	53558.		
Total	39	2389807.			

d. Dressed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	445832.	63690.	1.38	0.252
REP	4	178217.	44554.	0.97	0.441
Residual	28	1290739.	46098.		
Total	39	1914788.			

e. Drumsticks weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	5330.	761.	0.54	0.794
REP	4	7868.	1967.	1.41	0.258
Residual	28	39178.	1399.		
Total	39	52376.			

f. Empty gizzard (ventriculus) weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	271.10	38.73	0.84	0.568
REP	4	244.15	61.04	1.32	0.288
Residual	28	1298.65	46.38		
Total	39	1813.90			

g. Full gizzard (ventriculus) weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	841.50	120.21	1.25	0.310
REP	4	551.35	137.84	1.43	0.249
Residual	28	2692.25	96.15		
Total	39	4085.10			

h. Heart weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	20.700	2.957	1.15	0.362
REP	4	4.750	1.188	0.46	0.763
Residual	28	72.050	2.573		
Total	39	97.500			

i. Empty intestine weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1279.60	182.80	2.09	0.078
REP	4	71.65	17.91	0.20	0.934
Residual	28	2447.15	87.40		
Total	39	3798.40			

j. Full intestine weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	2435.0	347.9	1.93	0.101
REP	4	297.7	74.4	0.41	0.797
Residual	28	5035.2	179.8		
Total	39	7767.8			

k. Legs weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1359.6	194.2	0.68	0.689
REP	4	1329.8	332.5	1.16	0.349
Residual	28	8012.5	286.2		
Total	39	10702.0			

l. Live weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	781239	111606.	1.70	0.150
REP	4	379605	94901.	1.44	0.246
Residual	28	1841302	65761.		
Total	39	3002146			

m. Liver weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	316.18	45.17	1.61	0.173
REP	4	31.15	7.79	0.28	0.890
Residual	28	784.45	28.02		
Total	39	1131.78			

n. Neck weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1828.2	261.2	1.94	0.100
REP	4	748.2	187.1	1.39	0.262
Residual	28	3760.9	134.3		
Total	39	6337.4			

o. Stomach (Proventriculus) weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	27.775	3.968	1.04	0.428
REP	4	10.900	2.725	0.71	0.590
Residual	28	107.100	3.825		
Total	39	145.775			

p. Thighs weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	6922	989.	0.68	0.686
REP	4	7338	1834.	1.27	0.307
Residual	28	40569	1449.		
Total	39	54828			

q. Wings weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	8753.8	1250.5	1.94	0.101
REP	4	4470.0	1117.5	1.73	0.171
Residual	28	18079.6	645.7		
Total	39	31303.4			

3. APPARENT TOTAL TRACT NUTRIENT DIGESTIBILITY

a. Ash digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	680.6	97.2	0.81	0.592
REP	2	306.2	153.1	1.28	0.309
Residual	14	1676.1	119.7		
Total	23	2663.0			

b. Crude protein digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	285.10	40.73	1.09	0.420
REP	2	10.09	5.05	0.13	0.875
Residual	14	523.76	37.41		
Total	23	818.95			

c. Fat digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	2713.85	387.69	22.67	<.001
REP	2	10.16	5.08	0.30	0.748
Residual	14	239.40	17.10		
Total	23	2963.41			

d. Dry matter digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	56.443	8.063	1.09	0.418
REP	2	16.033	8.017	1.09	0.364
Residual	14	103.337	7.381		
Total	23	175.813			

e. Fibre digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	539.39	77.06	1.97	0.133
REP	2	134.20	67.10	1.72	0.215
Residual	14	547.20	39.09		
Total	23	1220.80			

4. EXCRETED NITROGEN

Excreted nitrogen

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	285.10	40.73	1.09	0.420
REP	2	10.09	5.05	0.13	0.875
Residual	14	523.76	37.41		
Total	23	818.95			

5. SERUM LIPID PROFILE

a. Cholesterol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	8.8818	1.2688	2.03	0.086
REP	4	0.6522	0.1631	0.26	0.900
Residual	28	17.4967	0.6249		
Total	39	27.0308			

b. High-density lipoprotein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	1.07746	0.15392	2.04	0.085
REP	4	0.02797	0.00699	0.09	0.984
Residual	28	2.11175	0.07542		
Total	39	3.21718			

c. Low-density lipoprotein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	3.3915	0.4845	1.92	0.103
REP	4	0.5394	0.1349	0.54	0.711
Residual	28	7.0508	0.2518		
Total	39	10.9817			

d. Triglycerides

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	0.34754	0.04965	0.76	0.622
REP	4	0.09207	0.02302	0.35	0.839
Residual	28	1.82178	0.06506		
Total	39	2.26138			

6. FAECAL MICROBIAL COMPOSITION

a. Coliform count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	29.400	4.200	2.19	0.066
REP	4	7.300	1.825	0.95	0.449
Residual	28	53.637	1.916		
Total	39	90.338			

b. *Escherichia coli* count

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
TRT	7	53.557	7.651	2.05	0.083
REP	4	46.938	11.734	3.15	0.029
Residual	28	104.289	3.725		
Total	39	204.783			

c. Microbial load

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	7	60.5070	8.6439	9.44	<.001
REP	4	18.0628	4.5157	4.93	0.004
Residual	28	25.6516	0.9161		
Total	39	104.2213			

d. *Salmonella* and *Shigella* count

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
TRT	7	43.034	6.148	2.14	0.071
REP	4	8.180	2.045	0.71	0.590
Residual	28	80.252	2.866		
Total	39	131.466			

e. Yeasts and moulds count

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
TRT	7	114.705	16.386	4.68	0.001
REP	4	26.427	6.607	1.89	0.141
Residual	28	98.073	3.503		
Total	39	239.205			