COMPARATIVE EVALUATION OF SCENT LEAF (*Ocimum gratissimum*) AND CURRY LEAF (*Ocimum canum*) MEALS AS PHYTOBIOTICSOURCES ON THE PERFORMANCE OF BROILER CHICKENS

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ZARIA, NIGERIA

MARCH, 2021

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,
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ZARIA, NIGERIA

MARCH, 2021

DECLARATION

I declare that the work in this dissertation entitled “Comparative Evaluation ofScent Leaf(*Ocimum gratissimum*) andCurry Leaf (*Ocimum canum*) Meals as Phytobiotic Sourceson the Performance of Broiler Chickens” was carried out by me in the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria under the supervision of Prof. P.A. Onimisi and Prof. J.J. Omage. All information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another Degree or Diploma at this or any other Institution.

SALIHU, Ezekiel Ametuo _____ _____

Name of student Signature Date

CERTIFICATION

This dissertation entitled "Comparative Evaluation of Scent Leaf (*Ocimum gratissimum*) and Curry Leaf (*Ocimum canum*) Meals as Phytobiotic Sources on the Performance of Broiler Chickens" by Ezekiel Ametuo, SALIHU meets the regulation governing the award of Master of Science degree (Animal Science), Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God Almighty, whose love, plan, grace and sustenance has been an anchor for my life; to Christ the first born of every creature who gave Himself for mankind and the Holy Spirit for His direction and guidance. Also to my beloved mother, Mrs. Christiana Onyeneku Salihu, who has been a source of support, encouragement and hope amidst many difficulties.

ACKNOWLEDGEMENTS

"Anything that works, is made to work". This work may never have been a reality without the immense contribution, inspiration and timely support of a number of individuals. I am highly indebted to everyone that made this work a great success. May you be favoured in all you do. Amen.I am most grateful to God Almighty, the giver of life. Indeed, He makes everything beautiful in its time. I am grateful for all You have done and will still do; to Christ, who gives strength and grace and the Holy Spirit, the best and suitable teacher.

To my Head of Department, Prof. M. Kabir; thank you for your timely counsel and valuable contributions. I cannot thank you enough, Prof. P.A. Onimisi, the chairman of my supervisory committee. Words may not suffice to appreciate your kind heart, assistance, support and encouragement I received in the course of this work both from you and your entire household. May God support and stand for you in your every hour of need.My second supervisor, Prof. J.J. Omage; I cannot thank you enough for making this work a great success. I am indeed grateful for your support, encouragement and judicious input in bringing out the best in this work. God bless and reward you all bountifully. Yours is a team anyone would love and always desire to have.

I am indeed very grateful to my lecturers, Prof. T.S. Olugbemi for his great, thoughtful and valuable input in improving the quality of this work, Dr. S. M. Yashim for his advice and input, Prof. G. S. Bawa, Prof. S. B. Abdul, Prof. F. Abeke, Prof. (Mrs.) A. A. Sekoni, Dr. M. Abdulrasheed, Dr. (Mrs.) I. I. Adedibu, Dr. M. R. Hassan, Dr. H. Y. Adamu, Mr. A. O. Lukman, Mr. T. Ibrahim, Dr. (Mrs.) I. Yahaya and Prof. D. Zahraddeen. Thank youfor the knowledge you imparted in me, your valuable counsel and timely contributions.To my Mummy in the Department, Dr. (Mrs.) O.M. Daudu, thankyou for your motherly role in

encouraging me and seeing that I am timely in all I need to do. God bless, keep and increase you. My dear Aunty, Dr. (Mrs.) M. Afolayan, I am grateful for your valuable input Ma. Thank you for your counsel and encouragement. God bless and increase you.To the Farm manager, Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Zaria, Mr. A.N. Oseni, Mr. Reuben Yakubu and other staff who contributed their quota towards making this work a success, thank you for your assistance. Special thanks to the departmental secretaries: Mrs.Wemimo Ajibade, Mrs. HajaraA. Otu and Mal. Musa Adam for their help and assistance in the course of this work and my stay in the department.

I am much grateful to Mrs. B. C. Ogbuefi and other staff members of the Biochemistry laboratory, to Mal. I. Aliu, and other staff of the Animal Product and Processing laboratory, Department of Animal Science, Ahmadu Bello University, Zaria. Thank you for your support and selfless assistance.I am sincerely grateful to Mr. Peter Akpulu of the Department of Human Anatomy, Faculty of Medicine, Mr. Yahuza Suleiman of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine and Mal. A. Kabir of the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria for their assistance in the laboratory analyses during the course of this work.

I cannot but acknowledge my father, late Mr. Obaro Salihu Uye, who first taught me that “no one can ever value you in life above yourself”. To my lovely mum, Mrs. Christiana Onyeneku Salihu, thanks for believing in and encouraging me at every point of my life. Indeed you laid a solid foundationfor me even when there was nothing to build with. God bless and keep you to enjoy all your labour of love. To my dear siblings: Bosede Adebayo, Oricha Salihu, Janet Sunday, David Salihu, Otohinoyi Salihu, Mercy Salihu and many

others, words cannot express how grateful I am for your support and immense contribution to make life and indeed this work a success.God bless you all for the love you showed despite many odds.I cannot thank you enough for the so much love, support and encouragement I received from Pastor and Mrs. Stephen Yusuf, and my little baby, Evidence Yusuf, God bless you all. Thank you so much the Otori's and the Obabu's; I may not be able to quantify in words all you have done both sacrificially in making Zaria a habitable place. God bless and reward you greatly.

Thank you for availing yourselves when strength seemed to fail; Alheri Taruwa, Victoria George, Adedayo Adewumi, Philip Elijah, Adebare Aderigbibe, Deborah Ajibade, Racheal Reuben and her family. You all are indeed a true definition of friendship. God bless and stand by you in your every hour of need.To my ever loving friends; Charles Jeremiah, Patience and Prudence Guyah, Haruna Abdulsalami, Joy Ibrahim and family, Isah Sunday, Folasade Aanuoluwapo, Gideon Usman, Iyanuoluwapo Olowoloba, Thomas Gambo, Oluomachi Moses, Vicky Zamdayu, Mary Oladipo, Jacob Dawodu, Ilebaiye David, Gladys Osagie, Grace Momoh, Elizabeth Ismail, Ruth Oladele, Rahab Monday, Toyosi Kolawole, Davina Oluwa, Celestina Kahuwai, Adeola Talabi, Eunice Moses, Mal. Waheed Idowu, Adamu Suleiman, Abiola Olakunrin, Muyiwa Johnson and many others that I may not be able to mention for time and space. I love you and appreciate your support. God bless and increase you all.To my wonderful colleagues and course mates who have been of immense support and assistance, Fadila Shehu, Abdulsalam Mohammed, Omolade Alonge, Fatimah Jumah, Isah Inuwa, Mummy Fibi Yette, Hajia Mariam, Mariam Umar (Mrs.), Ramatullah Yaqoob, Danjuma Yola, Mr. and Mrs. Olushola Odegbile, Lini Yunana; God bless and reward you mightily.

I cannot but acknowledge the leadership and entire members of the Deeper Life Campus Fellowship DAC and Zaria Region at large. Thank you all for making Zaria a home. God bless and keep you all both in His love. My “wonderful children” were not left out, thank you for always putting smile on my face in your own little but unique and resuscitating way. God bless, protect and increase you in sound wisdom and understanding. I love you all.

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LIST OF ABBREVIATIONS

ABUCAUC:	Ahmadu Bello University Committee on Animal Use and Care
ALB:	Albumin
AGP:	Antibiotic growth promoter
ALT:	Alanine-amino transferase
ALP:	Alkaline phosphatase
AST:	Aspartate-amino transferase
CAT:	Catalase
EO:	Essential oil
GLB:	Globulin
GPx:	Glutathione peroxidase
Hb:	Haemoglobin concentration
HDL:	High density lipoprotein
IARMS:	Institute for Agricultural Research Metrological Station
LDL:	Low density lipoprotein
MCH:	Mean corpuscular haemoglobin
MCHC:	Mean corpuscular haemoglobin Concentration
MCV:	Mean corpuscular volume
NGP:	Natural growth promoters
Oxytet:	Oxytetracycline
PFAs:	Phytogenic feed additives
ROS:	Reactive oxygen specie
SEM:	Standard Error of Means
SOD:	Superoxide dismutase

TP: Total protein

PCV: Packed Cell Volume

RBC: Red Blood Cell

WBC: White Blood cell.

ABSTRACT

Two feeding trials were conducted to evaluate the efficacy of *Ocimum gratissimum* and *Ocimum canum* and their combinations as alternatives to antibiotic growth promoter (AGP) in broiler chickens.In the first experiment, a total of 396 day-old Cobb 500 broiler chicks were allotted to six dietary treatments each replicated thrice, with 22 chicks in a completely randomized design. Experimental materials were included at 0g for the control, *Ocimum gratissimum* and *Ocimum canum* each at 400 g and 800 g respectively (T1-T5) while oxytetracycline was used as positive control (T6). In the second experiment, a total of 405 day-old Cobb 500 broiler chicks were allotted randomly to five dietary treatments each replicated thrice, with 27 chicks. Mixture of *Ocimum gratissimum* and *Ocimum canum* were included at 0g, 75:25, 50:50 and 25:75 (T1-T4) while oxytetracycline was used as positive control (T5).Data were collected on growth parameters, carcass characteristics, haematological parameters, liver function indices, lipid profile, antioxidant capacity, villi morphometric, gut microbial contents and organoleptic properties. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using Tukey procedures.In experiment 1, the growth performance results showed that final weight, weight gain, feed conversion ratio and feed cost per kg gain were significantly ($p<0.05$) higher in birds fed diets containing *O. gratissimum* and *O. canum* at 800 g per 100kg diets at both the starter and finisher phases. Haematological parameters, liver function parameters and serum lipid profile were significantly ($p<0.05$) improved and were within normal range reported for healthy chickens. Antioxidant capacity was significantly ($p<0.05$) improved by supplementation of *O. gratissimum* and *O. canum*; with an increase ($p<0.05$) in superoxide dismutase (SOD) activity (17.94-26.91 IU/mlas against control with 15.45 IU/ml and 14.43 IU/ml for AGP and reduction in malondialdehyde (MDA) content. The population of *Bacillus* and *Lactobacillus* spp. were significantly ($p<0.05$) increased while the concentration of *Escherichia coli*, *Clostridium spp., Salmonella spp., Enterobacteriaeae* and *Pseudomonas spp* were significantly ($p<0.05$) reduced by the inclusion of *Ocimum spp.* Villi area, villi perimeter, height, width, crypt depth and villi height/crypt depth ratio were significantly ($p<0.05$) higher in birds fed dietary supplement of 400 g of *O. gratissimum*,400 g and 800 g of *O. canum* and AGP compared to the control. Carcass characteristics of cut parts showed that birds on 400 g of *O. gratissimum*, 800 g of

O. canum and AGPs had higher ($p<0.05$) drumstick and breast cut percentages. Liver, kidney and heart weights were significantly ($p<0.05$) higher in birds fed diets containing *Ocimum spp.* at 800 g/100kg diets. Abdominal fat were lower inbirds fed diets containing *O. gratissimum* and *O. canum* at 800 g/100kg diets. The result of sensory analysis showed that meat of the broiler chickens in the treatment groups supplemented with *O. gratissimum* and *O. canum* had higher eating and sensory attributes.In experiment 2, the growth performance results showed that final weight, weight gain, feed conversion ratio and feed cost per kg gain were significantly ($p<0.05$) higher in birds fed diets containing combinations of*O. gratissimum* and *O. canum* at 600:200, 400:400 and 200:600 g per 100kg diets at both the starter and finisher phases.Haematological parameters, liver function parameters,serum lipid profile and antioxidant activities were significantly ($p<0.05$) improved.Ilea and caecal microbial population of *Bacillus* and *Lactobacillus* spp. were increased while the concentration of pathogenic bacteria were reduced by the inclusion of *Ocimum spp.*Villi area, villi perimeter, height, width, crypt depth and villi height/crypt depth ratio were higher ($p<0.05$) in birds fed dietary combinations of *O. gratissimum* and *O. canum* and AGP compared to the control.Cut parts were similar statistically but organs weights were significantly ($p<0.05$)increased while abdominal fat contents were reducedin birds fed combinations of the PFAs ($p<0.05$). Eating quality of the meat were ranked higher in birds fed higher levels of *O. gratissimum.*Inclusion of *O. gratissimum* and *O. canum* in broiler chickens diets singly or in combinations significantly improved performance with no adverse effect to the health status of the chickens. Farmers and feed millers can use*O. gratissimum* and *O. canum* each at800 g/100kg diets or their combinations at200:600 g/100kg diets at both the starter and finisher phases for improved performance.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Broiler production has grown dramatically in the past two decades and feed is the key constituent of overall costs of production accounting for about 80% (Ademola *et al*., 2009). For effective feed utilization, reduced production cost and higher economic gain, feed additives are used. These additives have established a great consideration for numerous purposes in poultry production (Akbarian *et al.*, 2012). Antibiotic growth promoters (AGPs) have been used for about 50 years to enhance growth performance (Dibner and Richards, 2004). They have been used widely not only to prevent and treat poultry diseases, but also for the improvement of meat and egg production (Ogle, 2013a). Currently, use of antibiotics is not only restricted but also their practice in livestock and poultry industry have been prohibited in many countries due to modification of natural gut microbiota and drug resistance in animals and humans (Broz and Paulus, 2015).

Natural growth promoters (NGPs) such as prebiotics, probiotics, synbiotics, enzymes and plant extracts can be incorporated into broiler chickensdiets without any adverse effect on their performance (Dhama *et al*., 2011). Beneficial properties of bioactive plant constituents in animal nutrition includes the stimulation of appetite and feed intake, the enhancement of endogenous digestive enzyme secretion, stimulation of immune responses and antibacterial, antiviral and antioxidant action (Al-Amin *et al.*, 2006; Al-Mashhadani, 2015).

Several types of herbal products and their oil extracts have enhanced broiler chickens performance and resulted in growth-promoting effects. These may serve as

environmentallyfriendly alternatives to the antibiotic growth promoters commonly used in animal and poultry feed. The proposed mode of action of herbal products is attributed to their antimicrobial properties (Lambert *et al.*, 2001; Burt, 2004); oxidative-resistant activity, enhancement of the immune system and consequently, improvement in poultry performance (Dorhoi*et al.*, 2006). Scent leaf (*Ocimum gratissimum)* and curry leaf (*Ocimum canum*) essential oils possess all the attributes of NGPs and may be used as an alternative to common artificial growth promoters like antibiotics.

Scent leaf (*O. gratissimum*), the African variety of basil (*Ocimum sp.*), is widely grown as a perennial herb in tropical Africa, South East Asia, India and Hawaii. It is highly recognized worldwide due to its versatile nutritional, anesthetic, and medicinal uses (Gill, 1992). Ijeh *et al.* (2004) noted that scent leaf is rich in alkaloids, tannins, phytates, flavonoids, oligosaccharides, terpenoids, thymol and saponin, with tolerable cyanogenic glycoside content. The proximate composition shows a crude protein of 4.70 %, 10.80 % crude fibre, and ash content of 12.24% (Mensah *et al*., 2008). The essential oil (eugenol) present in scent leaf possesses antimicrobial activities against pathogenic strains of gram negative and gram positive bacteria and pathogenic fungus (Matasyoh *et al*., 2007).

Curry leaf (*O. canum*) is a small strong flavoured perennial shrub commonly found in forests as undergrowth (Sinha *et al.*, 2012; Verma *et al.*, 2012; Priya *et al.*, 2014). This plant is known to be the richest source of carbazole alkaloids which has been reported by several authors to display various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities (Muthumani *et al*., 2010; Priya *et al.*, 2014). These leaves with high aromatic compound contents have been reported to retain their flavour and other qualities even after drying (Mandal *et al*., 2010). The essential oils from

these plants have been reported to have anti-microbial and antioxidant roles (Rao *et al*., 2011; Satish and Gopu, 2015). This study was therefore undertaken to evaluate the essential oils of *Ocimum gratissimum* and *Ocimum canum* leaves as potential sources of natural antibiotic growth promoters in broiler chickens production as replacement for synthetic antibiotic growth promoters.

1.2 Justification

The growing interest in the use of natural herbs and medicinal plants as alternative feed additives to antibiotics growth promoters (AGPs) in poultry diets following their ban by many countries of the world, has led to the evaluation and use of bio-active botanicals. Such alternatives to antibiotics that are currently being promoted are prebiotics, probiotics, organic acids, essential oils, and other medicinal plants products (Broz and Paulus, 2015). Phytochemical analyses of these botanicals have shown the presence of essential oil and phytochemicals that improve performance and reduces bacterial load in non-ruminant livestock (Hume, 2011).

Essential oil compounds are group of feed additives showing potential for the replacement of AGPs. They are active ingredients present in various plants and spices (such as thymol, carvacrol and eugenol). Due to their antibacterial activity they might be able to modify the composition of intestinal microbiota and to exert beneficial effects on performance of poultry (Mitsch *et al*., 2004; Hume, 2011; Broz and Paulus, 2015). Scent and curry leaves possess all the potentials of an effective natural growth promoter which can safely replace antibiotic growth promoters; and these phytogenic leaves meals from enhancing growth and productive performances, to influencing carcass traits and improving blood profile of the birds will undoubtedly contribute to the growth of the poultry industry. This work therefore

evaluated the performance of broiler chickens fed diets containing scent and curry leaves as phytobiotics.

1.3 Aim and objectives

The aim of this work was to evaluate scent and curry leaves feed additives as alternatives to the conventional antibiotics used in poultry production.

The specific objectives were to:

1. evaluate scent leaf (*Ocimum gratissimum*) and curry leaf (*Ocimum canum*) meals as feed additives in broiler chicken diets and their effects on growth performance, haematological parameters, liver function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

2. evaluate combinations of scent leaf (*Ocimum gratissimum*) and curry leaf (*Ocimum canum*) meals as feed additives in broiler chicken diets and their effects on growth performance, haematological parameters, liver function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

1.4 Research Hypotheses

The following null and alternate hypotheses were formulated to guide the research work and were tested at $p \leq 0.05$ level of significance.

1. **H_0**: Scent and curry leaves feed additives inclusion in the diets of broiler chickens does not have effect on growth performance, haematological parameters, liver

function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

H_a: Scent and curry leaves feed additives inclusion in the diets of broiler chickens have effect on growth performance, haematological parameters, liver function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

2. **H_0**: Combination of scent and curry leaves feed additives in the diets of broiler chickens does not have effect on growth performance, haematological parameters, liver function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

   H_a: Combination of scent and curry leaves feed additives in the diets of broiler chickens have effect on growth performance, haematological parameters, liver function indices, lipid profile, antioxidant activities, carcass characteristics, gut microbial count, villi morphometric, organoleptic properties and economic analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview and Prospect of Poultry Production

Poultry is used for any kind of domesticated bird including domestic fowls, chickens, turkeys, geese and ducks, guinea fowl, waterfowl, swans, ostriches, quails and squabs (young pigeons) raised for the production of meat or eggs and the word is also used for the flesh of these birds used as food (Encyclopaedia Britannica, 2016). Poultry production is clearly the most dynamic livestock species in terms of gaining a market share, that is, highly demanded; adapting technology for breeding, feeding, production, processing, and marketing; and being in a position to benefit from major consumer food trends as elaborated by William (1999). Due to increase in its demand, they are reported to have a world population of 19 billion in 2011 and are well domesticated in the world than any other species of bird(The Economist, 2011).

2.1.1 Chicken production

Chicken rearing is one of the most suitable activities to improve the livelihoods of the poor due to the advantages it has in terms of the small amount of capital required and the relative ease to set-up such a production system in the rural communities (Ogunlade and Adebayo, 2009; Ja'afa-Furo and Gabdo, 2010). Amos (2006) also indicated that a small-scale poultry enterprise has a quick monetary turnover. The earliest undisputed domestic chicken bones come from the Hebei province of China radiocarbon dated to approximately 5,400 BC. This northeastern region of China does not support wild *Gallus* species and the birds were therefore either living domestically in the area or shipped in from another location (Condon,

2012). Chickens are often the most common and wide spread domestic animals and with a population of 19 billion in 2011, there are more chickens in the world than any other species of birds (Okpala *et al*., 2016).

2.1.2 Broiler chicken production

Modern varieties of chicken such as the Cornish Cross are bred specifically for meat production, with an emphasis placed on the ratio of feed to meat produced by the animal. Chickens raised intensively for their meat are known as "broilers". Breeds have been developed that can grow to an acceptable carcass size (2 kg (4.4 lb)) in six weeks or less. Broilers grow so fast, their legs may not always be able to support their weight and their hearts and respiratory systems may not be able to supply enough oxygen to their developing muscles (Browne, 2002). They are hybrid of the egg-laying chicken, both being a subspecies of the red jungle fowl (*Gallus gallus*) (Ahmed *et al*., 2018). Typical broiler chickens have white feathers and yellowish skin (Wang *et al*., 2016). Most commercial broilers reach slaughter weight between five to seven weeks of age, although slower growing breeds reach slaughter-weight at approximately fourteen weeks of age (Zheng *et al*., 2016).

Broiler production in Nigeria involves the keeping of chickens of heavy meat breeds for the purpose of getting good-quality meat products, usually sold live or processed from six weeks of age and more (Aluwong *et al*., 2017). Broiler production is carried out in all parts of the country with no known religious, social or cultural inhibitions associated with their consumption (Egbuniwe *et al.*, 2017). Global broiler meat production rose to 84.6 million tones in 2013, the largest producers were the United States (20.00 %), China (16.60 %), Brazil (15.10 %) and the European Union (11.30 %) (Maduka *et al*., 2016). Specifically,

investment in broiler enterprise is attractive because the production cost per unit is low relative to other types of livestock. Poultry meat is very tender and broiler enterprises have short production cycles (Wondmeneh *et al*., 2016). The high demand for poultry products, the success of exotic breeds and the ease of mastering the techniques of poultry production among other factors have made it developed to the status of agribusiness in Nigeria as distinct from subsistence production (Sanni, 2015).

2.2 Nutrition in Poultry

Poultry nutrition is more than just giving any available feed to your birds (Salihu, 2014). They require proper nutrition to grow from start to finishing in all species of birds; chickens, quails and turkeys. Poultry require the correct balance of five classes of nutrients (proteins, carbohydrates, fats and oils, vitamins, minerals, and water) for optimum growth, maintenance, finishing, work, reproduction, and production (Leeson and Summers, 2005). They convert the nutrients into useful forms via the digestive system while blood then carries the nutrients throughout the body for normal physiological functions. Nutrients pass through capillary walls and enter body cells to provide nourishment and energy for life processes (Gillespie, 1987).

Feed formulation for livestock requires a clear understanding of the nutrient requirements of different classes of livestock in relation to age, production objectives and physiological state. In addition, the nutrient compositions of the available raw material resources need to be known (Ranjhan, 2000). The low level or absence of specific nutrients in the diet may lead to low production rate, nutritional deficiency symptoms and easy susceptibility to diseases.

2.2.1 Broiler chicken nutrition

Poultry nutrition is vital for efficient livestock production. Nutrition plays a vital role in broiler production and according to many authors, it accounts for 70% and above of the total cost of production (Afolayan and Afolayan, 2008). Poultry require the correct balance of five classes of nutrients (proteins, carbohydrates, fats and oils, vitamins, minerals, and water) for optimum growth, maintenance, finishing, work, reproduction, and production (Leeson and Summers, 2005).

2.2.2 Nutrient requirements of broiler chickens

Nutrient requirement shows the amount of energy, protein, amino acid, minerals and vitamins needed for the optimum growth and production of animals. According to the reports of Atteh (2004), sustainable poultry production can only be attained with better understanding of the bird's nutrient requirement. The need to meet the nutrient requirement of poultry birds is of paramount importance in production. Poultry convert feed into food products quickly, efficiently, and with relatively low environmental impact compared with other livestock. The high rate of productivity of poultry results in relatively high nutrient needs. Poultry require the presence of at least 38 dietary nutrients in appropriate concentrations and balance. The nutrient requirement figures published in Nutrient Requirements of Poultryby National Research Council (NRC, 1994) are the most recent available and should be viewed as minimal nutrient needs for poultry as presented in Table 2.1.

Table 2.1: Nutrient requirements of broiler chicken

	Weeks		
Nutrients	**0-3**	**4-6**	**7-9**
Energy (MEKcal/kg)	3200	3200	3200
Crude protein	23.00	20.00	18.00
Arginine	1.25	1.10	1.00
Glycine + serine	1.25	1.14	0.97
Histidine	0.35	0.32	0.27
Isoleucine	0.80	0.73	0.62
Leucine	1.20	1.09	0.93
Lysine	1.10	1.00	0.85
Methionine	0.50	0.38	0.32
Methionine + cysteine	0.90	0.72	0.60
Phenylalanine	0.72	0.65	0.56
Phenylalanine + tyrosine	1.34	1.22	1.04
Proline	0.60	0.55	0.46
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Valine	0.90	0.82	0.70
Calcium	1.00	0.90	0.80
Phosphorus	0.45	0.35	0.30

NRC, 1994.

2.2.2.1 *Protein*

Protein is an array of amino acids linked by peptide bonds (Onimisi and Omage, 2019). It's usage in poultry ration is usually based on the crude protein content (Oladipo, 2015). Hence, various authors like Fetuga (1984) recommended 23-24% CP for broiler starter while Ensminger *et al*. (1990) opined that broiler starter ration should contain from 21-24% crude protein. Aduku (1992) also reported 23% and 20% for broiler starter and finisher, respectively. NRC (1994) recommended 23% CP for broiler chicks of 0-3 weeks old, 20% CP for birds 3-6 weeks of age and 18% CP for birds 6-8 weeks of age.

There are variations in the reported requirements, however the NRC report has been accepted by many nutritionists (Lesson and Summers, 1997). The overall cost of protein sources in livestock diet and its importance in growth and meat yield while meeting the amino acid (AA) requirements of the bird is of great importance. Hence, dietary protein level has major effects on growth, carcass composition and overall cost of the finished products as production of lean meat has become more important (Collin *et al*., 2003).

2.2.2.2 Energy requirements

Energy, a property of energy yielding nutrients (carbohydrates, lipids, proteins) upon oxidation is essentially needed by broilers for growth, maintenance of body tissues, egg production, regulation of body temperature and activity. Studies have shown that dietary energy levels affect broiler chickens' feed intake and performance. Nahashon *et al*. (2006) reported that as dietary energy level increases, birds satisfy their energy needs by decreasing feed intake. Hence, there is a decreases in feed intake with high energy levels in the diets of broiler chickens (Leeson and Summers, 2005; Veldkamp *et al*., 2005). Most of the dietary

energy comes from plant sources in the form of carbohydrates from cereal grains and cereal by-products such as maize, sorghum, millet, maize bran or offal and starchy roots and tubers such as cassava flour, cassava chips and sweet potatoes and their by-products. Olukosi *et al.* (2008) associated performance of chickens with feed nutrients and energy utilization, which is primarily related to availability of more nutrients and energy from the feed ingredients. Lower energy level (2,800 kcal/kg ME) during the starting period and higher energy level (3,000 kcal/kg ME) during finishing was advocated by Olomu (2011).

2.2.2.3 Lipids

Fats and oils are required in poultry diet formulations to enhance the palatability of the diet, the absorption of fat-soluble vitamins, and to regulate the passage rate of the digesta in the gastrointestinal tract (Fouad *et al*., 2013; Fouad and El-Senousey, 2014). They are also the most concentrated sources of energy (Baião and Lara, 2005). Fats are insoluble in water but soluble in organic solvents such as chloroform. Fats provide the body with energy approximately twice the amount of that of proteins and carbohydrates. Poultry birds require 5-6% of fat (Olomu, 1995). The following foods are rich in fat and oil: groundnut oil, palm oil, oil sludge, and full fat soyabeans. A small amount of fat in poultry ration helps to make feed less dusty, more palatable and of high energy (Kekeocha, 1985). Aduku (2004) reported that fat is a major storage and most efficient form of metabolic fuel which carries fat soluble vitamins (A, D, E and K). It is also a structural component of membranes that provides insulation to keep the body warm, form protective coat under the skin and improve egg size and feathering.

Broilers have a dietary requirement for essential fatty acids (EFA). The main essential fatty acid is linoleic acid which acts as a precursor for other members of the n-6 series such as arachidonic acid and derived prostaglandins (Scott and Kenneth, 2000). Specific lesions, particularly in the skin, can result from essential fatty acid deficiency if the dietary content of linoleic acid falls below the requirement of about 10g/kg (NRC, 1994). Fat has twice and a quarter (2¼) times more energy than carbohydrates on an equal-weight basis (Chiba, 2007). It is important in broiler nutrition as it performs certain physiological functions such as insulation against temperature extremes, protection of vital organs etc. Addition of fat to broiler diet during hot weather should be accompanied with the use of an antioxidant as it will help to prevent rancidity of the fat (Chiba, 2007).

2.2.2.4 Vitamins

Vitamins are organic substances required by animals in very small amounts which according to Oluyemi and Roberts (2000) helps in regulating various body processes to maintain normal health. They are very essential in a good nutrition program (Scott and Kenneth, 2000). Vitamins are primarily divided into two classes: fat soluble and water soluble vitamins. The fat soluble vitamins include vitamin A, D, E, and K while the water-soluble vitamins include vitamin C and the B-complex vitamins (vitamin B12, biotin, choline, folacin, niacin, pantothenic acid, pyridoxine, riboflavin, and thiamine). They are required in small amounts and they represent about 0.05% of the total weight of the feed and about 1.5% of the feed cost (Coelho and McNaughton, 1995). Vitamin requirement of poultry is usually affected by several factors such as age, breed/strain, diet composition, disease, minerals –vitamin bioavailability, physiological condition and stress (McNaughton, 1990). Acamovic (2001) reported that optimum vitamin nutrition in broiler chickens diets have

shown increased growth rate, improved feed efficiency and increased oxidative stability of meat and better resistance to high stocking density stress conditions. Vitamins, A, B2 and D3 are called “critical vitamins” for poultry, as any deficiency in their availability severely impairs the growth and egg production (Oluyemi and Roberts, 2000).

2.2.2.5 Minerals

Minerals are involved in the formation of structural components in the skeleton. There are two types based on the levels of requirements namely macro and trace minerals. Levels of major minerals (macro minerals) are critical, especially for young growing poultry. Calcium and phosphorus are two macro minerals of great importance in poultry production (Underwood and Suttle, 1999). The skeleton accounts for about 99% calcium and 80% phosphorus in the body (Theerachai, 2006). Most cereal grains and their by-products are deficient in calcium though they contain notable quantities of iron ranging between 60-210ppm (Drinceanu *et al.,* 2009). Young chicks (meat and egg types) require calcium and phosphorus in relatively large amounts for bone growth. A calcium to phosphorus ratio of 2:1 was best for adequate mineral absorption and metabolism (Chiba, 2007). Poultry requirement for calcium and phosphorus were influenced by the amount of vitamin D in the diet (Smith, 1990). Excess supply of calcium in poultry diets may lead to serious problems of mineral interaction as it would interfere with the availability of other minerals such as phosphorus, magnesium, manganese, and zinc (NRC, 1994). In general, the need for calcium and phosphorus increases as the level of vitamin D decreases and vice versa (Smith, 1990).

Cereal grains are poor sources of manganese with concentrations ranging between 0.13-0.23percent (Drinceanu *et al.*, 2009). Consequently, nutritionists use trace mineral premixes when formulating diets to supply the required amount of micro minerals needed for maintenance and production (Scott and Kenneth, 2000). Other important minerals in broiler nutrition include chlorine which is necessary in digestion as a component of hydrochloric acid, sodium and potassium serve as electrolytes important for metabolic, muscle, and nerve functions. Magnesium is needed for metabolic and muscle functions (Scott and Kenneth, 2000). Others are manganese which is essential in egg shell production, iodine necessary for the production of thyroid hormones, zinc is involved in enzymatic processes and iron for oxygen transportation within the body.

2.2.2.6 Water

Water is an essential nutrient for life. Water consumption can be limited if the water is too hot or is contaminated with excess minerals (Poultry CRC, 2020). Water and food consumption rates are interdependent, so reduced water intake can also lead to reduced food intake. There are other factors that affect water intake, with temperature being the most obvious one. For example, chickens drink between 30-50% more water when the environmental temperature is above 32^{o}C compared with when it is 21^{o}C. Water intake is also affected by the type of drinkers used. The rule of thumb for water intake is that water intake is usually 1.5 to 2 times feed intake.Water is the most important nutrient for poultry. In addition to being a nutrient, water also softens food and carries it through the body, aids in digestion and absorption, and cools the body as it evaporates through the bird's lungs and air sacs (Carter and Sneed, 1987). Water helps remove waste, lubricates joints, is a major component of blood, and a necessary medium for many chemical

reactions that help form meat and eggs. Although water is regarded as the most essential nutrient, it is impossible to state its exact requirement. Under normal conditions, chickens will consume by weight twice as much water as food. During periods of extreme heat stress, water requirements may easily quadruple. The recommended daily water consumption for broiler chickens is presented in Table 2.2.

Ad libitum water intake of broilers can be highly variable and depends on diet composition and feed form, on production performance, on intestinal health, stress and on environmental conditions. High *ad libitum* water consumption under moderate climate conditions will generally result in high water excretion, wet litter problems, and increased ammonia production (Manning *et al.*, 2007).Water intake of broilers is increased during periods of physiological stress (Virden *et al.*, 2009) which might be an important cause for variation in the *ad libitum* water intake. Based on the relationship between stress and water intake, Manning *et al.* (2007) indicated that water consumption in L/bird/day can be used as a management tool, since a change in water consumption might indicate health problems.

Dietary factors that increase feed intake like pelleting and nutrient concentration will generally also impact water intake, whereas other factors like dietary fibre content and its characteristics primarily affect water intake. Dietary nutrient contents in excess to the animal's requirements will also have a higher impact on water intake than on feed intake as water is the vehicle for excretion of excess nutrients via the kidney. It is therefore important to balance nutrient supply to the animal's requirements to minimize *ad libitum* water intake.

Table 2.2: Daily water consumption for broilers chickens at 20^{o}C (litres per 1000 mixed sex birds)

Weeks	Water intake (Litres)
1	65
2	120
3	180
4	245
5	290
6	330
7	355
8	370

Poultry CRC (2020).

2.3 Use of Feed Additives in Poultry Diet

Feed additives are used in only small quantities, and it is particularly important that they are mixed carefully with the main ingredients so that they are evenly distributed (Ravindran, 2016). The nutritional quality of a feed also depends on a variety of other factors, including feed presentation, microbial contamination, and content of antinutritional factors, digestibility, palatability, and intestinal healthiness. A variety of feed additives are available to deal with these issues (Jacob, 2015, Ravindran, 2016, PoultryHub, 2016). Two non-nutritive feed additives have gained much interest in poultry nutrition, which are enzymes and antibiotics (FAO, 2014).

2.3.1 Antibiotics usage in poultry

There are many different kinds of antibiotics, and they destroy bacteria in different ways. The antibiotics within a class generally have similar effectiveness and mechanisms of action and resistance and they tend to attack the same types of bacteria. Some antibiotics, referred to as broad-spectrum antibiotics, treat a wide range of infections while others, called narrow-spectrum antibiotics, are effective against only a few types of bacteria (Adzitey, 2015; Jacob, 2015).

Antibiotics are added to the animal feed or drinking water of cattle, hogs, poultry and other food-producing animals to help them gain weight faster or use less food to gain weight (FDA, 2015). The use of antibiotics as growth promoter intended not only as therapeutic but also as feed additive of continuous use in animal started appropriately five decades ago. Usually, antimicrobial growth promoters (AGPs) are administered at low doses, absorbed minimally from the gut and when incorporated into the feed, they act by specifically

reducing the number of pathogenic bacteria (Dafwang *et al*., 1987; Dibner and Richards, 2005;Segura and De Bloos, 2008; Ravindran, 2016).

The use of antibiotics as growth promoters in poultry feed has been practiced worldwide during the last 50 years (Yegani and Korver, 2008). This application has been acknowledged to improve feed conversion and growth, and reduce morbidity and mortality due to clinical and subclinical diseases. The mechanisms through which antibiotics promote the growth of chickens are still not exactly known, but a study with germ-free chicken indicates that the growth promoters are mediated by their antimicrobial effect (Yang *et al*., 2009). Antibiotics may reduce the microbial load in the gut leading to more nutrient availability for the host (Brisbin *et al*., 2008). Beyond the beneficial features, the risk concerning the development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota led the European Union to ban the application of antibiotics as growth promoters since 1st January 2006, which was followed by the other parts of the world including North America (Yegani and Korver, 2008). The removal of AGP from poultry diets has led to animal performance problems and a rise in the incidence of certain poultry diseases, such as subclinical necrotic enteritis and dysbacteriosis (Huyghebaert *et al*., 2011).

2.3.2 Threats to use of antibiotics

The main reasons for the use of antibiotics in meat-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals (Castanon, 2007; Matthew *et al*., 2009). Antimicrobial use in animal production dates as far back as 1910 when due to shortage of meat products, workers carried out protests and riots across America (Ogle, 2013b). Scientists at that time started looking for means of producing more meat at relatively cheaper costs; resulting in the use of

antibiotics and other antimicrobial agents (Dibner and Richards, 2005).With the global threat of antibiotic resistance and increasing treatment failures, the non-therapeutic use of antibiotics in animal production has been banned in some countries (Castanon, 2007).

Bacteria counteract the actions of antibiotics by four well-known mechanisms, namely; enzyme modification, alteration in target binding sites, efflux activity and decreased permeability of bacterial membrane (Bassetti *et al*., 2013). This expression of resistance towards antibiotics by bacteria could either be intrinsic or acquired. Intrinsic resistance is due to inherent properties within the bacteria chromosome such as mutations in genes and chromosomally inducible enzyme production (Davies, 2007), whereas acquired resistance could be due to the transmission of resistance genes from the environment and/or horizontally transfer from other bacteria (McDermott *et al*., 2003; Randall *et al*., 2004).

The use of antimicrobial drugs, in both humans and animals, contribute to the development of antimicrobial resistance which have become a threat to public health (Kaufman, 2000). Illnesses caused by drug-resistant strains of bacteria are more likely to be potentially fatal when the medicines used in treating them are rendered less effective (Kaufman, 2000; FDA, 2015). With continual use of antibiotics, a harmless intestinal bacterium could make a disease-causing bacterium antibiotic resistant (Kaufman, 2000; Wagner, 2015). Once antimicrobial pressure has been introduced into an environment, resistance can quickly develop and spread by horizontal transfer of plasmids (Salyers *et al*., 2004). Hence, poultry and the poultry rearing environment have been reported to be resistant to erythromycin, lincomycin, and penicillin (Kaufman, 2000; Adeleke and Omafuvbe, 2011).

This increased prevalence of antibiotic resistance is an outcome of evolution. Many microorganisms naturally develop resistance to withstand an antibiotic's attack (Lewis, 2016). Defenseless bacteria species are selectively destroyed with antibiotics leaving behind those that can resist it (Adzitey, 2015). Many factors contribute to the spreading of antibiotic resistance, for example, the movement of carrier animals between herds or flocks and between countries, the assembly of susceptible animals in close confinement and the movement of resistance determinants throughout the ecosystem by means of vectors such as rodents, insects, and birds (Kaufman, 2000; Adeleke and Omafuvbe, 2011;Lewis, 2016). Though bacterial antibiotic resistance is a natural phenomenon, societal factors also contribute to the problem. These factors include increased infection transmission, coupled with inappropriate antibiotic use (Lewis, 2016).

2.3.3 Alternatives to antibiotics

Antibiotics used for growth promotion are typically administered to all animals in a pen, herd, or flock, at a relatively low dosage and over long periods of time. Alternatives such as in fed enzymes, organic acids, prebiotics, probiotics, phytochemicals like essential oils are used commercially for growth promotion and occasionally disease prevention. Antibiotics and their alternatives can also be used to prevent diseases in healthy animals. Disease prevention uses are defined as the administration of a drug to healthy animals in a situation where a specific and increased disease risk is present (FDA, 2016).

2.3.3.1 Enzymes

Feed enzymes are used to improve the utilization of nutrients in raw materials and to reduce feed cost. Improvements in nutrient availability are achieved by one or more of the

following mechanisms; degradation of specific bonds in ingredients not usually degraded by endogenous digestive enzymes; degradation of anti-nutritive factors that lower the availability of nutrients; increased accessibility of nutrients to endogenous digestive enzymes; and supplementation of the enzyme capacity of young animals.(Jacob, 2015). Enzymes widely used in poultry industry are the carbohydrases that cleave the viscous fibre component in cereals an phytases that target the phytic acid-complexes in plant ingredient. Future advances in feed enzyme technology will involve the development of enzymes that can be used to target the anti-nutritive factors in unconventional feedstuffs and improve their feeding value (Jacob, 2015; Ravindran, 2016).

2.3.3.2 Organic acids

Organic acids have been used for decades in feed, to protect feed from microbial and fungal damage. These acids are added to foods as preservative agents and can also be used to control microbial contamination (Kum *et al*., 2010). In fact, the organic acids that have antimicrobial activity are simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing a hydroxyl group on the alpha carbon such as lactic, malic, and tartaric acids. It has been noted that salts of some of these acids have performance benefits (Dibner and Buttin, 2002). Some short chain carboxylic organic acids, such as sorbic and fumaric acids containing double bonds, also have antifungal activity (Dibner and Buttin, 2002). Several of these organic acids can be produced in small quantities as a result of the fermentation activity of the gastro-intestinal tract (GIT) (Dorsa, 1997) where anaerobic microflora are predominant (Dixon and Hamilton, 1981). The effects of organic acids on broiler chickens have been well documented. Hassan *et al*. (2012) proposed that the inclusion of organic acids to broiler chicken diets enhanced growth and

feed utilization and feed conversion ratio. Acidification of broiler chicken diets with different organic acids has been shown to reduce pathogenic bacteria toxicity through controlling pathogenic colonization on the gastrointestinal wall, and by inhibiting damage to epithelial cells (Langhout, 2000). The basic principal mode of action of organic acids is that non-dissociated organic acids can diffuse through lipophilic bacteria and mold membranes, and can disrupt the enzymatic reaction and the transport system of the bacteria (Cherrington *et al*., 1991). As described by Lambert and Stratford (1999), after penetration of organic acids into bacterial cytoplasm the non-ionized organic acids decompose to H (Hþ) ions and (A) ions. By the time of the decline of the pH inside the bacteria, a specific mechanism (Hþ-ATPase pump) will act to return the intracellular levels to normal pH. This process requires energy, which will lead to reduced energy accessibility for the proliferation of cells and eventually stop the bacterial growth or even kill it. Acids are bactericidal for *Salmonella* serotype *Enteritidis* PT4. Chaveerach *et al*. (2004) demonstrated that organic acids in the drinking water of chicks could have a potential effect on *Campylobacter* infection.

Czerwinski *et al*. (2012) found a negative correlation between *Enterobacteriaceae* numbers and the concentration of undissociated propionate, acetate and butyrate in the caeca. Dietary addition of organic acids can also improve the digestibility of protein and amino acids (Afsharmanesh and Pourreza, 2005), calcium, phosphorus, magnesium and zinc (Garcia *et al*., 2007). Also, it has been reported that organic acid supplementation can improve gastrointestinal villi height and cell proliferation (Adil*et al*. 2010). Other activities of organic acids associated with acidification include the increase of pancreatic secretions, microbial phytase activity, and the improvements of digestive enzyme activity (Dibner and Buttin, 2002).

The applications of microencapsulated organic acids on animals are limited. Smulikowska *et al*. (2010) suggested that there is no growth promoting response to coated sodium butyrate or its salt in birds raised under optimized conditions. This finding has been supported by a number of other researchers (Smulikowska *et al*., 2010;Czerwinski *et al*., 2012; Zhang *et al*., 2013). Jerzsele *et al*. (2012) suggested that protected sodium butyrate had no beneficial effects in birds challenged with *C. perfringens*, but a combination of sodium butyrate with essential oils protected with vegetable fat increased body weight gain, villus height and decreased gross lesion scores compared with the control in broiler chickens.

2.3.3.3 Essential oils

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin (Franz and Novak, 2009). The term "essential oils" emerged because "oils" were wishfully believed to be "essential" to life, and have a long history of being used by human for cosmetic and medicinal purposes. The development of essential oils, however, was delayed by the advent of antibiotics in the middle of the 19th century, and was renewed recently. Out of 3,000 known essential oils, 300 were recognized as commercially important and mainly used in the flavours and fragrances market (van de Braak and Leijten, 1994).

Essential oils are a sum of constituent volatiles, and thus the effects of essential oils should be a totality of effects of all components and their interactions. However, 2 or 3 components could account for up to 85% of the total mixture compared with the minors (Miguel, 2010), and thereby contribute to the primary property of the mixture. For example, the phenols

(thymol and carvacrol) constituted about 80% of the essential oils of oregano, the most widespread species of *Lamiaceae* family, and are mainly responsible for its antibacterial and antioxidant activities. Besides thymol and carvacrol, r-cymene was found as another dominant component of oregano (Bouhaddouda *et al*., 2016). Although the r-cymene is not an effective antimicrobial agent by itself, it could facilitate the transport of carvacrol across the cytoplasmic membrane (Oke *et al*., 2009).

Essential oils are perceived as growth promoters in poultry diets (Zhang *et al*., 2014). Many published reviews reported that feed intake in chicks was unchanged or slightly reduced by dietary inclusion of essential oils (Brenes and Roura, 2010; Franz *et al*., 2010; Hashemi and Davoodi, 2010; Hippenstiel *et al*., 2011; Bozkurt *et al*., 2014). Halle *et al*. (2004) and Amad *et al*. (2011) reported that daily feed intake of broiler chickens was numerically decreased by increasing the dietary level of a blend of thyme, star anise, and *Origanum*leaves, and its associated essential oils compared with the control. Similarly, Cabuk *et al*. (2006) noted a significantly reduced feed intake of broiler chickens from young breeders by graded inclusion of a cocktail of essential oils (oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil, and citrus peel oil). Moran (1982) reportedthatpoultry might not be sensitive to flavour as pigs, and Roura *et al*. (2008) reported that birds are more tolerant to exposure of moderate levels of essential oils than pigs,hence, poultry birds may be able to tolerate a level of essential oil feed containing materials.

2.3.4 Benefits of essential oils

Essential oils offer numerous opportunities in livestock production, hence, among other feed additives, their roles in poultry nutrition are numerous.

2.3.4.1 Feed utilization

Unlike feed intake, improvements in weight gain and feed conversion ratio dominate the observations. Two well-accepted mechanisms are the stimulation of digestive enzyme secretion and the stabilization of ecosystem of gut microflora, leading to improved feed utilization and less exposure to growth-depressing disorders associated with digestion and metabolism (Franz *et al*., 2010; Bento *et al*., 2013; Kurekci *et al*., 2014; O'Bryan *et al*., 2015). The positive effects of essential oils on digestive enzyme secretion from the pancreas and intestinal mucosal have been reported in many broiler chicken studies (Jamroz *et al*., 2006; Jang *et al*., 2007; Basmacioglu Malayo glu *et al*., 2010). These effects were confirmed by the increased digestibility of nutrients, but did not translate into improvement in growth performance (Amad *et al*., 2011).

2.3.4.1 Antimicrobial and anticoccidial activity

The antimicrobial activity of essential oils has been explored in many in vitro assays which showed that thymol, eugenol and carvacrol have high antimicrobial activity against pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium*, both of which are potential risk factors of enteric infections (Franz *et al*., 2010; Hippenstiel *et al*., 2011; Bassole and Juliani, 2012). Thymol, eugenol and carvacrol are structurally similar, and have been proved to exert synergistic or additive antimicrobial effects when combined at lower concentrations (Bassole and Juliani, 2012).

In in vivo studies, essential oils used either individually or in combination have shown clear growth inhibition of *Clostridium perfringens* and *E. coli* in the hindgut and ameliorated intestinal lesions and weight loss than the challenged control birds (Jamroz *et al*., 2006;

Jerzsele *et al*., 2012). One well known mechanism of antibacterial activity is linked to their hydrophobicity, which disrupts the permeability of cell membranes and cell homeostasis with the consequence of loss of cellular components, influx of other substances, or even cell death (Brenes and Roura, 2010; Solorzano-Santos and Miranda-Novales, 2012; O'Bryan *et al*., 2015). It is of note that Gram negative bacteria are more tolerant to the actions of essential oil than Gram-positive bacteria due to their hydrophilic constituents in the outer membrane (Brenes and Roura, 2010; Giannenas *et al*., 2013; Seow *et al*., 2014).

Coccidiosis a common parasitosis disease caused by protozoa of the genus *Eimeria*, leads to malnutrition and performance depression in poultry. There is an increasing interest in using essential oils against coccidiosis infection. The supplementation of essential oils led to a significant reduction of coccidian oocyst excretion and an alleviation of intestinal lesions in chicks (Alp *et al*., 2012; Bozkurt *et al*., 2014; Barbour *et al*., 2015).

2.3.4.3 Anti-oxidative activity and carcass hygiene

Chicken body antioxidative stability could be improved by essential oils (Zhai *et al.*, 2018). Habibi *et al*. (2014) and Placha *et al*. (2014) observed that malondialdehyde concentration in liver, duodenal mucosa, and kidney was significantly decreased by supplementing ginger powder and thyme oil to broiler diet. Poultry products are particularly prone to oxidative deterioration due to their high concentrations of polyunsaturated fatty acids. The reviews by Lee *et al*. (2010) and Khan *et al*. (2012) showed that thyme oil is effective in retarding oxidant degradation in poultry derived products, such as meat and eggs. The possible reason might be the antioxidant activity derived from the phenolic OH group which acts as a donor

of hydrogen interacting with peroxy radicals during the initial process in lipid oxidation and thereby inhibiting or retarding the hydroxyl peroxide formation (Lee *et al*., 2010).

Rosemary, oregano, and sage of the Labiate family were also reported for their effective antioxidant activities in broiler meat (Brenes and Roura, 2010; Franz *et al*., 2010). Kohlert *et al*. (2000) reported that the metabolic fates of essential oils are dependent on their chemical structure; essential oil components are quickly eliminated; and accumulation is unlikely due to the high clearance and short half-lives in human, which lends support to no residue of essential oils in animal products.

Carcass hygiene could be improved by essential oils, which should be attributed to their reducing the load of pathogens (Zhai *et al.*, 2018). Alali *et al*. (2013) reported that a blend of carvacrol, thymol, eucalyptol, and lemon reduced *Salmonella* spp. and subsequently reduce the cross contamination in carcass processing. Venkitanarayanan *et al*. (2013) reported that a blend of caprylic acid and essential oils (trans-cinnamaldehyde, eugenol, carvacrol, and thymol) reduced *Salmonella enteritidis* and *Campylobacter Jejuni* in cecal contents of birds, which indicates a less likelihood of microbial contamination of poultry meat and eggs. An interesting study by Witkowska and Sowinska (2013) showed improvement in hygiene conditions in poultry house via air disinfectants using thyme and peppermint oils individually as primary components. The role of gut microbiota, nutrition and environmental factors and how they interplay to affect performance is shown in Figure 2.1 (Juan *et al*., 2019).

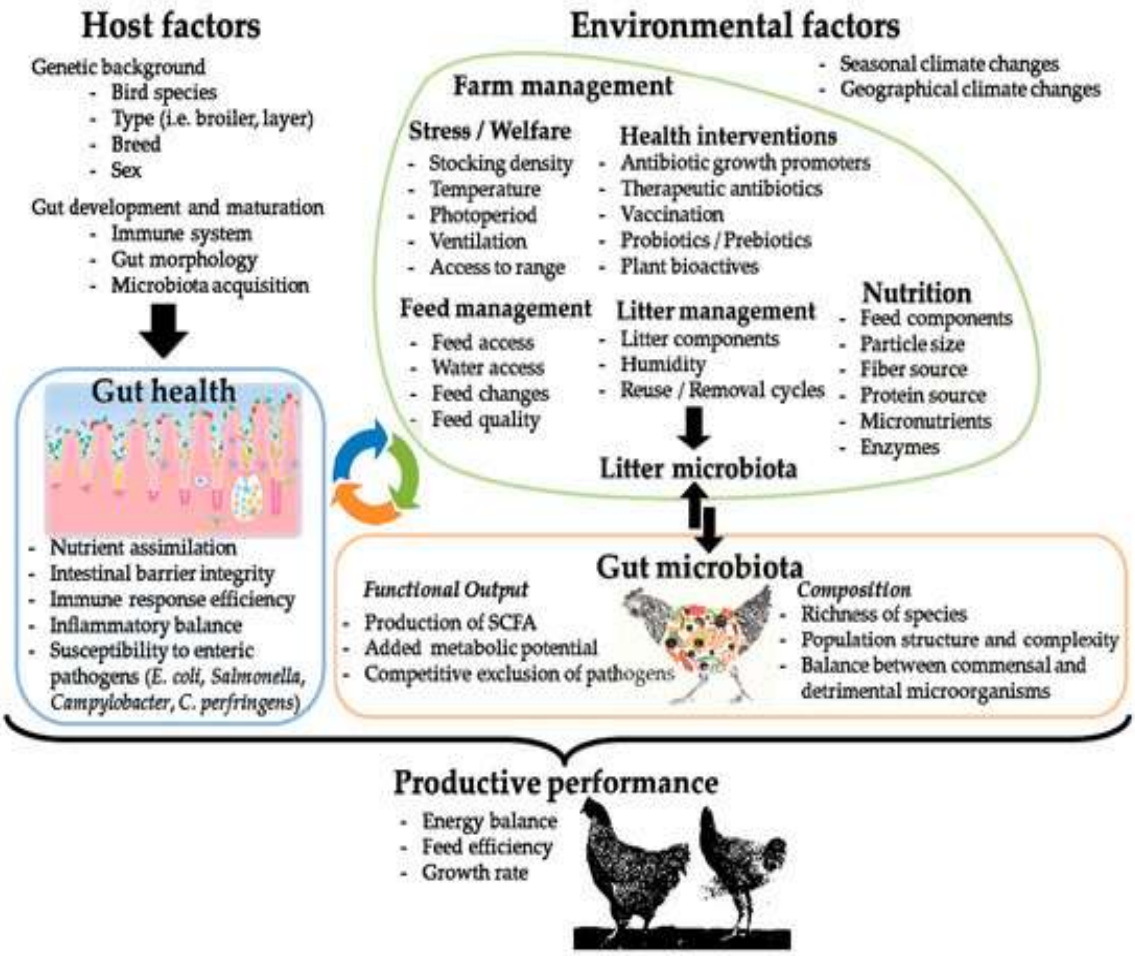


Figure 2.1: Environmental and host-related factors that shape chicken gut microbiota and its interplay with gut health and productive performance (Juan *et al*., 2019).

2.3.3.4 Prebiotics

Prebiotics are generally defined as indigestible feed ingredients that selectively stimulate the growth or activity of beneficial bacteria that are already resident in the GIT (Gibson and Roberfroid, 1995). The potential effects of prebiotics on animals were recognized in the 1980s (Hajati and Rezaei, 2010). For a feed ingredient to be classified as a prebiotic, it must be neither digested nor absorbed in the upper part of the digestive system, and it must be a selective substrate for one or a limited number of useful bacteria in the GIT, stimulate the bacteria to grow, be metabolically activated, be able to alter the intestinal microflora toward a healthier composition, and be palatable as a food ingredient (Collins and Gibson, 1999; Hajati and Rezaei, 2010). Among those, carbohydrates are commonly seen in the literature as prebiotics. For example, lactose, a disaccharide, has been used as a prebiotic in chickens (Hajati and Rezaei, 2010). Other predominantly used prebiotics include, manno oligosaccharides (MOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides, glycol-oligosaccharides, galacto oligosaccharides, gluco oligosaccharides, lactitol, lactulose, malto oligosaccharides, and trans-galacto oligosaccharides (Gibson and Roberfroid, 1995; Patterson *et al*., 1997; Collins and Gibson, 1999).

In general, prebiotics can prevent the colonization of bacterial pathogens in the GIT (Bengmark, 2001), lower the gut pH through short-chain fatty acids (SCFA) production (Gibson and Wang, 1994). MOS is extracted from the yeast cell wall of *Saccharomyces cerevisiae* yeast (Hofacre *et al*., 2003). The yeast cell wall consists of protein, glucans, and mannan (Klis *et al*., 2002). The exact mode of action of MOS is unclear, but b-glucans and mannans are the units with primary functions on bacteria indirectly. The mannans act as a receptor for type-1 fimbriae which are used by some harmful bacteria such as *E. coli* and

Salmonella to attach to the gastrointestinal wall (Oyofo *et al*., 1989). β-glucans act as microbial recognition receptors of the innate immune system (Gantner *et al*., 2003) and both mannans and β-glucans structures stimulate the immune system (Spring *et al*., 2000). The effects of MOS supplementation on the broiler immune system, gut microflora and gut morphology have been well documented. For instance, Shanmugasundaram and Selvaraj (2012) reported that dietary prebiotics (killed whole yeast cells) increased interleukin-10 mRNA by 9 fold in comparison to the control.

Shanmugasundaram *et al*. (2013) also proposed that 0.2% of killed whole yeast cell prebiotics increased body weight gain, macrophage nitric oxide production and caecal tonsil interleukin-1mRNA amounts in broilers challenged with coccidia. Ghosh *et al*. (2011) reported that antibody titer against Newcastle disease was significantly higher in birds fed a yeast cell wall preparation. In the same study, birds were orally infected with *Salmonella pullorum* at 45 days of age. After the oral infection, Salmonella counts were lower in digesta in birds fed the yeast cell wall preparation. This is supported by a number of studies, where dietary MOS were effective in reducing Salmonella infection (Fernandez *et al*., 2000; Spring *et al*., 2000). It has also been reported that the mannan oligosaccharides improved intestinal morphology, such as increasing villi height and altering mucosal architecture (Iji *et al*., 2001), and decreasing the crypt depth of the mucosa of the small intestine (Yang *et al*., 2009).

2.3.3.5 Probiotics

Probiotics are “live microbial feed supplements, which beneficially affect the host animal by improving its microbial balance” (Fuller, 1990). The characteristic of ideal probiotics are

that they must be from host origin, resistant to gastric acids and bile, persist in the intestinal tract, adhere to epithelium or mucus, produce inhibitory compounds, alter immune response and modulate the microflora activity (Patterson and Burkholder, 2003). The modes of action of probiotics include stimulating the immune system, maintaining gut microflora by competitive exclusion, altering metabolism through increased digestive enzyme activity, decreasing bacterial enzyme activity and ammonia production, and neutralizing enterotoxins (Collins and Gibson, 1999; Simmering and Blaut, 2001; Patterson and Burkholder, 2003).

The mechanisms of competitive exclusion of pathogens include competitive use of nutrients and mucosal binding sites, or production of short-chain fatty acids (SCFAs), low pH and bacteriocins, which are bactericidal or bacteriostatic for pathogenic bacteria (Ohland and MacNaughton, 2010). A number of studies have reported the potential of undefined (normal gut flora) or defined (characterized bacterial strain) probiotics on the colonization of pathogenic bacteria. Mead (2000) reported that normal gut flora preparations showed efficacyagainst food borne pathogens such as *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium botulinum*, *C. perfringens*, *Salmonella*, and *Campylobacter jejuni*. Morishita *et al*. (1997) showed that *Lactobacillus acidophilus* and *Streptococcus faecium* reduced the colonization of *C. jejuni* in the jejunum by 27%. Jayaraman *et al*. (2013) found that dietary supplementation of *B. subtilis* in experimentally challenged broiler chickens with *Eimeria* and *C. perfringens* reduced feed conversion ratio (FCR), intestinal lesion scores, and *C. perfringens* counts, and increased villus height to crypt depth ratios compared with an infected control without such supplementation.

2.3.3.6 Phytobiotics

Phytogenic feed additives (PFA) are the plant derived products used to improve performance of livestock and poultry (Windisch *et al*., 2008; Jacela *et al*., 2010). The PFA comprise of a wide variety of herbs, spices and products derived thereof and are mainly essential oils. This class of feed additives is at present used to a great extent as alternatives to the antibiotic growth promoters (AGP) in poultry and swine nutrition. Obviously the ban imposed on the usage of the AGP in diets of food animals is the main driving force that has caused the surge in the use of PFA (Wati *et al*., 2015).

Alternative to the AGP should have the same beneficial effects when included in diets. The dubious mechanism of action of the in-feed antibiotics notwithstanding (Huyghebaert *et al*., 2011), it is generally accepted that the AGP elicit some antibacterial action and thereby reduce the incidence and severity of subclinical infections. In doing so, they reduce the microbial usage of nutrients and improve their absorption from the intestine by thinning the intestinal mucosa (Brennan *et al*., 2003). The indirect impact of all these actions is translated into higher growth rate. The basis of this mechanistic explanation is that the AGP do not exert growth promoting effects in germ-free animals. The general industry practice is to feed the livestock with doses of antibiotics at sub-therapeutic levels which is unlikely to exert any growth inhibitory action on the resident bacteria (Niewold, 2007) although a clear shift in the intestinal microbiota was observed when antibiotics were added to broiler diets at levels below the minimum inhibitory concentration (Pedroso *et al*., 2006; Wise and Siragusa, 2007) and this, at least partly, explains the effects of the AGP.

Furthermore, microbiota shifts affect morphology of the gut wall and induce immune reactions, which by affecting energy expenses of the host animal may promote their growth (Teirlynck *et al*., 2009). Based on the hypothesized mechanism of action of the AGP, the alternative products should have modulatory effects on the gut microbiota and the immune system. Although antimicrobial and growth promoting effects of PFA have been reported by several workers the mode of action of most of the PFA is still not very clear. Possibly, the essential oils present in the PFA promote gut functions by stimulating secretion of digestive enzymes, bile and mucus (Platel and Srinivasan, 2004), the terpenoids and phenolic compounds help the animals to combat with the oxidative stress the animals come across (Aeschbach *et al*., 1994) and compounds like carvacrol, other terpenoids and aldehydes present in most of the PFA exert substantial antimicrobial effects (Burt, 2004; Mathlouthi *et al*., 2012) thus establishing eubiosis in the gut.

2.4 Mechanism of Action of Phytobiotics

Phytogenic feed additives (PFAs) requires some time to bring any shift in gut microbiota (Giannenas *et al*., 2003; Mountzouris *et al*., 2011). The PFA do this by decreasing the production of growth depressing microbial metabolites such as ammonia and biogenic amines (Windisch *et al*., 2008), selection of healthier microbial groups (Castillo *et al*., 2006) and increasing nutrient availability to the host (Anderson *et al*., 1999). This is evidenced by the higher numbers of *Lactobacillus* spp. in the caeca of the PFA supplemented group in the study carried out by Wati *et al.* (2015). A depression in the intra-epithelial leukocyte numbers of the intestinal mucosa of pigs supplemented with essential oils was reported which indicated that PFA might be capable of controlling the inflammatory reactions the intestinal mucosa is always exposed to (Nofrarias *et al*., 2006). Controlling of inflammatory

processes should spare nutrients for absorption (Huang *et al*., 2010; Ahmed *et al*., 2013). Moreover, due to their antibiotic effects essential oils in PFA influence intestinal microbiota and facilitate production of digestive enzymes to increase nutrient digestibility (Stoni *et al*., 2006; Mountzouris *et al*., 2011).

There are reports that essential oils from thymol, eugenol, curcumin, piperin, carvacrol and cinnamaldehyde might reduce proliferation of *Clostridium perfringens* in broiler chicken gut (Jamroz *et al*., 2003; McReynolds *et al*., 2009). Lower counts of *Clostridium, Salmonella and E. coli* and a higher count of *Lactobacillus* had a stabilizing effect on gut microbiology and hence will improve digestibility of nutrients in animals (Brenes and Roura, 2010).

Apart from the effects on intestinal microbiology, the essential oils in PFA reportedly stimulate secretion and activities of digestive enzymes like trypsin, lipase and amylase (William and Losa, 2001; Wenk, 2003; Jang *et al*., 2004; Jamroz *et al*., 2005; Jang *et al*., 2007; Lee *et al*., 2010) and may increase villus height (Cardoso *et al*., 2012). A higher faecal moisture content and shorter digesta time in the PFA supplemented birds group is suggestive of greater secretory activities of the small intestinal mucosa (Wati *et al*., 2015).

2.5 Scent Leave (*Ocimum gratissimum)*

Ocimum gratissimum (*O. gratissimum*) has been used extensively in the traditional system of medicine in many countries. In the North East of Brazil, it is used for medicinal, condiments and culinary purposes. The flowers and the leaves of this plant are rich in essential oil, so it is used in the preparation of teas and infusion (Rabelo *et al*., 2003). In the Coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea (Effraim *et al*.,2003). In the Savannah areas, decoctions of the leaves are used to

treat mental illness (Akinmoladun *et al*.,2007). *O. gratissimum* is used by the *Ibos* of the South Eastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, cold and catarrh (Ijeh *et al*.,2005). Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children (Cristiana *et al*.,2006). People of Kenya and Sub-Sahara Africa use it in the treatment of abdominal pains, sore eyes, infections, coughs, barrenness, fever, convulsions and tooth gargle, regulation of menstruation and as a cure for the prolapsed of the rectum (Matssyoh *et al*., 2007). In India, the whole plant has been used for the treatment of sunstroke, headache and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activities (Oliver, 1980; Tania *et al*.,2006).

In Nigeria, many tribes use the leaves extract in the treatment of diarrhoea, while the cold leave infusions are used for the relief of stomach upset and haemorrhoids (Kabir *et al*.,2005). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, headache, disease of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu and Salau, 2005). The infusion of *O. gratissimum* leaves is used as pulmonary antisepticum, antitussivum and antispasmodic (Ngassoum *et al.*,2003). *Ocimum gratissimum* is not a problem in cultivation, it is mostly a weed of roadsides and wasteland. However, it is also an important pasture. The basic requirements for its growth are moist and fertile soils but it can tolerate drought after flowering (Arhoghro *et al*., 2009). *Ocimum gratissimum* is grown for the essential oils in its leaves and stems (Sulistiarini *et al*., 1999).

2.5.1 Origin, distribution and adaptation of *Ocimum gratissimum*

Ocimum gratissimum also known as tea bush or fever plant is a shrub which is highly exploited in folk medicine (Gege-Adebayo *et al.,* 2013). It also belongs to the family of plant known as *Lamiaceae* with characteristic pleasant aroma due to the presence of volatile oil (Dalziel, 1993). It is a widely used local plant for both nutritional and therapeutic purposes (Arhoghro *et al.,* 2009).

It is a perennial and odouriferous herb which grows to a height of about 1-2 feets with branched stems. It has 1-2 inches long, oval, pointed, sharp and pale greenish yellow leaves which are sub-acuminate to acuminate at apex, cuneate at base with a coarsely crenate, serrated at the margin and dotted on both the sides. The leaves and seeds of *O. gratissimum* is shown in Plate I. It has small black elongated seeds that are round at one end and flattened at the other (Verma and Kothiyal, 2012; Choudhury *et al.,* 2011). The peduncles are densely pubescent with Calyx which is 5-7mm long, campanulate and greenish-white to greenish-yellow in colour (Bhat, 2003). The two surfaces of the leaf epidermal cells are typical of irregular contours with diacytic stomata and secretory glands mostly abundant in the leaf. These are presented in simple *pluricellular* hairs on the leaf veins (Garcia *et al.,* 1998).

The plant is indigenous to the savannah, tropical rain forest and coastal areas of West Africa especially in Kenya and Nigeria. It is also found in the tropical Asia such as India (Akinjogunla *et al*., 2011; Oguanobi *et al*., 2012; Gege-Adebayo *et al.,* 2013). In Nigeria, it is found in the Savannah and coastal areas (Illoh and Awojide, 2000). It has been found to be cultivated in Ceylon, South Sea Islands and also within Nepal, Bengal, Chittagong and Deccan (Nadkarni and Nadkarni, 1999).





PlateI: *Ocimum gratissimum* L. showing leaves and seed

2.5.2 Classification of *Ocimum gratissimum*

Kingdom: *Plantae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Ocimum*

Species: *O. gratissimum*

Binomial Name: *Ocimum gratissimum* (L.)

2.5.3 Antimicrobial and antifungal activities of *Ocimum gratissimum*

An investigation of antifungal activity of the essential oil obtained by steam-distillation (1.1%w/w) of the aerial parts of *O. gratissimum* and of an ethanolic extract from the steam distillation residue has been carried out using the agar diffusion method. The results revealed that the essential oil inhibited the growth of all fungi tested, including the *phytopathogens, Botryoshaeriarhodina, Rhizoctonia sp,*and two strains of *Alternaria sp*., while the extract from the residue was inactive. The antifungal activity of eugenol was evaluated against a species of *Alternaria* isolated from tomato and *Penicillium chrysogenum.* The minimal inhibitory concentrations of eugenol were 0.16 and 0.31mg/disc for *Alternaria sp. and P. chrysogenum,* respectively (Trevisan *et al*., 2006).

The antibacterial activity of different extracts from the leaves of *O. gratissimum* has been tested against *staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Salmonella typhimurium,* pathogenic bacteria that cause diarrhoea. Extracts evaluated included cold

water extract, hot water extract and steam distillation extract. Only the steam distillation extract had inhibitory effects on the selected bacteria and the minimum inhibitory concentration ranged from 0.10 % for *S. aureus* to 0.01% for *E.coli* and *S. typhimurium*, and 0.001% for *S.typhi* (Adebolu and Salau, 2005). Due to these polyvalent performances and the sweet fragrance of this natural product, *O. gratissimum* essential oil containing a high level of ethyl cinnamate seems especially suitable for dermatology and cosmetology (Dubey *et al.*,1997).

An exhaustive study was performed on stem bark parts by Akinyemi*et al*.(2005).The authors attributed antimicrobial activity to the aqueous and ethanolic extract of *O. gratsimum.* Both the extracts were active against *S. aureus* and MRSA. They act as bacteriostatic at lower concentration and bactericidal at higher concentration. Minimum inhibitory concentration and minimum bactericidal concentration were found to be between 18.2 to 24.0g/ml and 30.4 to 37.0µg/ml respectively. Their results offer a scientific basis for the traditional use of water and ethanol extracts of *O. gratissimum* against MRSA-associated diseases.

The essential oil of *O. gratissimum* inhibited *S. aureus* at a concentration of 0.75 mg/ml. The essential oil was also active against members of the family Enterobacteriaceae. The minimal inhibitory concentrations (MICs) for *Shigella flexineri, Salmonella enteritis, Escherichia coli, Klebsiella sp.,* and *Proteus mirabilis* were at concentrations ranging from 3 to 12 µg/ml. The minimum bactericidal concentration of the essential oil was within a twofold dilution of the MIC, for this organism. The compound that showed antibacterial activity in the essential oil of *O. gratissimum* was identified as eugenol (Nakamura *et al.*,2005). In an*in vitro*test of antifungal activity of thirteen essential oil obtained from plants against dermatophytes of the tested oil, *O. gratissimum* was found to be the most active, inhibiting

80% of the dermatophyte strains tested and producing inhibition zones greater then 10mm in diameter(Lima *et al.,* 1993). Hydro-distilled volatile oil from the leaves of *O. gratissimum* from Meru district in Eastern Kenya was evaluated for antimicrobial activity where the essential oil were evaluated against both Gram positive *(S. aureus, Bacillus spp.)* and Gram negative (*E. coli, P. aeruginosae, S. typhi, K. pneumoniae, P. mirabilis)* bacteria and a pathogenic fungus,*Candida albicans*. The minimum inhibitory concentration of the oil for gram negative bacteria ranged from 107 to 750 mg/ml and 93.7 to 150mg/ml for gram positive bacteria. The minimum inhibitory concentration (MIC) for the fungus *C. albicans* was 50mg/ml. The MIC values for chloramphenicol ranged from 22.5 to 31.3mg/ml. The oil had pronounced antibacterial and antifungal activities on all microbes (Matsyoh *et al.,* 2007). The antibacterial effect of *O. gratissimum* extracted from the aromatic plant was investigated against *Listeria monocytogenessero type* 4a. Agar well diffusion and tube dilution method were used and the data recorded demonstrated antibacterial activity of the essential oil against the test bacteria (Mbata and Saikia, 2007) which established a good support for the use of this plant in herbal medicine and a base for the development of new drugs and phytomedicine, which is also applicable to livestock production.

The antibacterial activity of different extracts from the leaves of *O. gratissimum* was tested against *S. aureus, E.coli, S. typhi* and *S. typhimurium*, pathogenic bacteria that cause diarrhoea. The extracts evaluated included cold water extract, hot water extract and steam distillation extract. Only the steam distillation extract had inhibitory effects on the selected bacteria. The MIC ranged from 0.1% for *S. aureus* to 0.01% for *E.coli* and *S. typhimurium*, and 0.001% for *S. typhi* (Adebolu and Salau, 2005). Effects of leaf extracts of *O. gratissimum* on spore germination and mycelia reduction of the most commonly occurring

fungal pathogen causing soft rot of yam tuber were investigated by Okigbo and Ogbonnaya, (2006).Fungi isolated from rotted yams were *Aspergillus niger, A. flavus, Fusarium oxsporium, Rhizopus stolonifer, Botryodiplodia theobromae and penicillium chrysogenum.* They reported that the ethanol leaf extract was most effective followed by cold water extracts. Essential oils extracted by hydro-distillation from local plants in Benin, Western Africa were evaluated *in vitro* and *in vivo* for their efficacy against *Fusarium verticillioides* infection and *fumonisin* contamination. *O. gratissimum* was found to be the most effective *in vitro,* completely inhibiting the growth of *F. verticillioides* in corn and totally inhibited fungal growth at concentrations of 8, 6.4 and 4.8 µg/ml, respectively, over a period of 21 days. At the concentration of 4.8µl/g, these oils did not affect significantly *fumonisin* production. However, a marked reduction of *fumonisin* level was observed in corn stored in closed conditions. The oil adversely affected kernel germination and therefore cannot be recommended for controlling *F. verticillioides* on stored corn used as seeds, when used at this concentration (Fandohan *et al.,* 2004). Cells cultivated in the presence of 100g/ml of essential oil showed a decrease of activity of the succinate cytochrome c reductase enzyme, a typical mitochondrion marker, as compared to untreated cells (Holetz *et al*.,2003). All the bacteria were susceptible on a different scale to the undiluted oils. The inhibition zone of the undiluted oil of *O. gratissimum* is more extensive than that of the other oil.

2.6 Curry Leave (*Ocimum canum*)

Ocimum canum Sims is a semi-woody plant of about 40 cm high that belongs to the family of *Lamiaceae*. It is an aromatic plant and native to tropical Africa (Steel, 2006). It is popularly called African basil, but known as "*Efinrin elewe dudu*" in south-western Nigeria where it is used locally for the treatment of gastrointestinal problems, piles, dysentery, as an

infusion for nursing mothers and as insect repellant and curry by many others in Southern Nigeria.

The leaf is used for the treatment of diabetes in Ghana (Hogarh, 1996; Nyarko *et al*., 2002). The plant is reported to be rich in volatile essential oils of therapeutic importance (Ekundayo *et al*., 1989). The leaves of *O. canum* are used in the preparation of delicious local soups as well as flavouring agent in yam and cocoyam porridges in the Yoruba tribe of Nigeria. The plant is also used as a local condiment because of its aromatic properties (Bassole *et al*., 2005). The plant is collectively called Basil, is a diverse and rich source of aromatic essential oil. These essential oils are being used as pharmaceutical agents because of their antimicrobial, antiemetic, antidiabetic, antifertility, antiasthmatic, antistress and anticancer activities (Makker *et al*., 2007).

2.6.1 Origin, distribution and adaptation of *Ocimum canum*

Ocimum canum is commonly called *Ocimum americanum* Linn. The *Ocimum americanum*, known as American basil or "hoary basil is an annual herb with white or lavender flowers. Despite the misleading name, it is native to Africa, the Indian Subcontinent, China, and Southeast Asia. The species is naturalized in Queensland, Christmas Island, and parts of tropical America (Figueiedo and Smith, 2008; Khanam and Hassan, 2008).The main uses of *O. canum* are antimicrobial, antioxidant and antidiabetic. It is also used in the treatment of skin diseases and Genito-urinary problems. It is known as English camphor basil, American basil or hoary basil. Herbal synergism is definitely a line of exploration in the development of new drugs (Dabo *et al.,* 2018). It is an *a*nnual, herb, 20–60 cm tall, leaf elliptic-lanceolate, leaf surface glabrous except hairy midrib, veinlets and margin, Inflorescence greenish,

Flowers white, Calyx green with sometimes purplish stripe, long hairy, seed black, narrowly ellipsoid, mucilaginous (Chandra, 2016).

Hoary basil is one of the most popular herbs used in Thailand, Lao, Indonesia, Vietnam and also many Arabic countries. Plants grow to 2 to 4 feet high and their young leaves and stems are harvested and used in various cusines. This tropic bush plant is vigorous and grow best in a full sunny area, at least 6 hours direct sunlight, under warm climates. Young leaves are picked before flowering, for promoting more new large leaves and branches. Plants are very hardy and may survive the winter in subtropics and tropic regions. White flowers appear from July to September. Leaves and tips have strong minty aroma and flavour. It is an excellent herb for home garden and fresh market. A cross section showing the leaves and seeds of *Ocimum canum* is presented in Plate II.

2.6.2 Classification of *Ocimum canum*

Kingdom: *Plantae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Ocimum*

Species: *O. canum*

Binomial Name: *Ocimum canum* (Sims.) (Khanam and Hassan, 2008).





Plate II: *Ocimum canum* S. showing leaves and seeds

2.6.3 Antimicrobial and antifungal activities of *Ocimum canum*

Ocimum canum Sims. is a traditional medicinal plant distributed throughout Tamil Nadu and it is commonly known as Nai Tulasi in Tamil, Africa and in Nigeria around the Savanna. The plant branches out from its base, with angle stems and open foliage. The plant has a pungent, aromatic flavour and is commonly cultivated for culinary purposes. *O. canum* is used specially for treating various types of diseases and lowering blood glucose level and also to treat cold, fever, parasitic infestations on the body and inflammation of joints and headaches (Ngassoum *et al*., 2004). Essential oil from the leaves of *O. canum* possess antibacterial and insecticidal properties (Bassole *et al*., 2003).

It is useful in treating various types of diseases and in lowering blood glucose level, especially in type 2 diabetes patients. The herb can be used to treat colds, fevers, parasitic infestations on the body and inflammation of joints and headaches. The traditional medicine recognized its value in the treatment of fevers, dysentery and tooth problems. It was used as an insect repellent to counter the insect damages to post harvest. It is used in various types of religious functions as part of rituals and as incense to protect the home. The leaves are used for flavouring purposes. The oil of the *Ocimum canum* is composed of Linalool. The seeds may provide dietary fibre or reduce constipation. The leaves are used as a seasoning in stews and soups. The leaves are used to treat a number of ailments such as convulsion, fever, urticarial rush and asthma (Agro product, 2013).

2.7 Poultry Birds Gut Health and Performance

Gut health has recently been the subject of intense studies in poultry production (Rinttila and Apajalahti, 2013). The gut is a pivotal organ system which mediates nutrient uptake and use

by the animals. The gut is also a major site of potential exposure to environmental pathogens (Yegani and Korver, 2008). Hence, a well-functioning and healthy gut is the cornerstone of the optimum performances of the birds. When the gut function and health are impaired, digestion and absorption of nutrients are affected and thus the health and performance of birds will be compromised.

Intestinal mucosa of broiler chicken plays an important role in providing an effective barrier between the hostile luminal content and the host internal tissues. In this notion, intestinal mucosa is an important determinant of gut health and performance of chicken (Rinttila and Apajalahti, 2013). To support the intestinal mucosal barrier functions, the dynamic balance between the mucus layer, epithelial cells, microbiota and immune cells in the intestine is of importance (Schenk and Mueller, 2008). A number of factors associated with diet and infectious disease agents have been reported to affect this dynamic balance, and subsequently affect the health status and production performance of the chicken (Yegani and Korver, 2013). A sub-therapeutic use of antibiotics has been widely practiced in the poultry industry for decades to maintain the balance of ecosystem in the gut as well as to improve the growth performance of chicken (Huyghebaert *et al*., 2011).

2.8 Effect of Feed Additives on Performance of Poultry Birds

Plant extracts and essential oils have been reportedly used to improve broiler performance (Jamroz *et al*., 2005; Mountzouris *et al*., 2011). A study carried out by Wati *et al*. (2015), revealed that supplementation of the PFA improved body weight and FCR of broiler chickens inoculated with enteric pathogens.

2.8.1 Effects of feed additives on blood parameters

Values of haematological parameters are widely used to determine systemic relationship and physiological pathological adaptation including the evaluation of general health conditions, diagnosis and prognosis of various types of animal diseases (Shahin *et al*., 2007). Blood constituents change in relation to the physiological conditions of health and they are affected by certain factors such as breed, age, sex, management systems, and nutrition (Etim *et al.,* 2014).

2.8.1.1 Haematological study

Haematological parameters consist of packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin Concentration (MCHC). Each of these parameters signify certain body reaction or interpret certain body reactions.

Red blood cells are produced in the red bone marrow and they contain haemoglobin molecules and functions majorly in transportation of gases; primarily oxygen and some carbon dioxide (OpenStax College, 2013). Thus, a reduced RBC count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Isaac *et al.,* 2013).

The WBC is a major component of the body's defense against diseases and it protects the body against external invasion by microorganisms **(**OpenStax College, 2013**).** Granular leukocytes include heterophils, eosinophils and basophils while agranular leukocytes include monocytes and lymphocytes. Heterophils are rapid responders to the site of infection and are efficient phagocytes with a preference for bacteria. The eosinophils counteract the activities

of inflammatory chemicals produced by basophils and mast cells. Some eosinophils are also capable of phagocytosis and are particularly effective when antibodies bind to the target and form an antigen-antibody complex. High counts of eosinophils are typical of birds experiencing allergies, parasitic worm infestations, and some autoimmune diseases. Low counts may be due to drug toxicity and stress. Lymphocytes provide generalized, nonspecific immunity, against specific pathogens (disease causing microorganisms) and are involved in specific immunity (OpenStax College, 2013). Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al*., 2013) and enhance adaptability to indigenous environmental and disease prevalent conditions (Kabir *etal*., 2011).

Packed cell volume is the volume percentage of erythrocytes present in a sample of centrifuged blood **(**OpenStax College, 2013**).** According to Isaac *et al.* (2013), PCV is involved in the transport of oxygen and absorbed nutrients. Increased PCV shows better transportation and thus results in increased primary and secondary polycythemia.

Mean corpuscular volume give an indication as to the type of anaemia and may also suggest the aetiology of the disease process (Coles, 2007). For most birds the MCV lies between 121–200 fl; an increase in this value indicates regenerative anaemia while in a non-regenerative anaemia, the cells are either normal or reduced in size. An increase in this value does not occur since RBCs do not become supersaturated with haemoglobin. Mean corpuscular haemoglobin (MCH) is not a useful index as the MCHC (Coles, 2007). The normal haematological indices range is presented in Table 2.3.

Table 2.3: Normal range of haematological parameters of chicken

Parameters	Reference value
Pack Cell Volume (%)	24.00-40.00*
Haemoglobin (g/dL)	7.00-15.00*
Red Blood Cell ($\times 10^{12}$/L)	1.59-4.10*
White Blood Cell ($\times 10^{9}$/L)	1.90-9.50**
Heterophils (%)	15.00-40.00**
Lymphocytes (%)	40.00-100.00***
Monocytes (%)	1.00-7.00****
Basophils (%)	0.10-2.00*
Eosinophils (%)	1.50-6.00**
MCH (pg)	33.00-47.00***
MCV (fl)	90.00-140.00**
MCHC (g/dL)	26.00-85.00***

MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin Concentration, MCV: Mean corpuscular volume*Mitruka and Rawnsely, 1997, **Simrak *et al*., 2004, ***Jain, 1986, ****Jain, 1993.

2.8.1.2 Normal range for serum biochemistry parameters

Biochemical blood parameters include enzymes, metabolites and electrolytes. The enzymes include Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatse (ALP); the metabolites include Cholesterol (Chol), Glucose (Glu) and Creatinine (Cre); while the electrolytes include Sodium (Na), Potassium (K), Calcium (Ca) and Chlorine (Cl), (Coles, 2007). Normal plasma values for AST in most birds should normally be below 230 IU/l but can be in the range of 52–270 IU/L. As with the biochemistry of many of the blood constituents, the level varies with the age and seasonal activities (Coles, 2007). AST has a wide distribution in body tissues and although it is not liver specific it is possibly the single most useful enzyme for indicating liver disease. However, soft tissue damage, intramuscular injection, particularly with irritant drugs, hepatopathy and toxic chemicals increases the level of AST (Coles, 2007).

ALT is an enzyme found in the cells of many avian tissues and in other animals. Normal levels of ALT in birds vary considerably from 6.5–263 IU/L and elevations have been shown to be associated with hepatocellular disruption (Harris, 2000 and Coles, 2007). ALP is an enzyme which is found mostly in the duodenum and kidney. According to Coles (2007), ALP is considered to be a good indicator of osteoblastic activity. Its level increases in growing birds, egg laying birds when calcium metabolism is increased in the medullary bone and in some cases in liver disease, such as aflatoxin poisoning. According to Coles (2007), normal values for cholesterol in birds are in the range of 108–330 mg/dl. This substance is the precursor of all steroid hormones and bile acids. The remainder of the cholesterol is produced in the liver and is also gotten from the diet. Plasma levels of cholesterol are increased in cases of fatty liver and kidney syndrome.

Glucose is required for cellular respiration and is the preferred fuel for all body cells (OpenStax College, 2013). Elevations in blood glucose levels are the principal pathological change noted in avian species (Harris, 2000). Increased glucose level results in hyperglycaemia while decreased glucose level results in hypoglycaemia. Creatinine levels in avian serum samples typically fall below a measurable range and certain technical factors contribute to a high incidence of artefactual changes. Elevations have been associated with kidney disease, but creatinine is not considered to be a reliable indicator of renal function (Harris, 2000).

Electrolytes play important roles in various physiological processes, such as osmotic pressure maintenance, synthesis of tissue proteins, intra and extracellular homeostasis maintenance, maintenance of the electrical potential of the cell membrane, acid-base homeostasis, as well as the functioning of enzymes and nerves (Borges *et al.,* 2003). Normal calcium levels in most birds are within the range of 8–12 mg/dl (Coles, 2007). Total blood calcium levels tend to be higher during ovulation in birds (corresponding with increased ALP) and coincident with the transport of protein-bound calcium to the shell gland, but the level of calcium ions in the blood remains constant. Immature birds tend to have lower blood calcium levels. Potassium is considered the most abundant intracellular cation and is involved in many metabolic processes, including nerve conduction, muscle excitation-contraction and cell volume regulation. Consequently, changes in K^+ homeostasis profoundly affect cellular functions. Sodium is also essential in the production of hydrochloric acid in the stomach which is vital for digestion. Changes in sodium values usually reflect serious conditions. According to Harris (2000), elevated levels of sodium occur with salt poisoning, water deprivation and dehydration while decreased levels occur

due to sodium loss through renal disease or diarrhoea. The reported normal range of liver function indices according to Ross *et al.* (1976), Bounous and Stedman (2000) and LAVC (2009) is shown in Table 2.4.

Table 2.4: Normal range of liver function parameters

Parameters	Reference value
Total protein (g/dL)	3.60-5.50*
Albumin (g/dL)	1.10-2.20*
Globulin (g/dL)	1.20-3.20**
Albumin : Globulin	0.35-0.75*
Aspartate-Amino Transferase (IU/L)	100-400**
Alanine-Amino Transferase (IU/L)	-
Alkaline Phosphatase (IU/L)	10-106***

*Ross *et al*. (1976), **LAVC (2009), ***Bounous and Stedman (2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research farm, Ahmadu Bello University, Zaria, Kaduna State. Zaria is in the Northern Guinea Savannah zone, located on longitude 11° 09' 01.78'' N and 7°39'14.79''E 671m above sea level. The climate is characterized by well-defined dry and wet seasons. The area has three distinct seasons; hot dry season from March to May, the warm rainy season from June to September, and a cool dry season from November to February. The climate is relatively dry with a mean annual rainfall of 700-1400mm (IARMS, 2019). The area has an average relative humidity of 36.00 % during the dry season and 78.50 % for the wet season and an average minimum and maximum temperatures of 15.60 and 38.50 °C respectively(Oluwasemire and Alabi, 2004). This study was carried out between May-December, 2019.

3.2 Ethical Approval

Ethical approval was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) before the commencement of the experimental trials.

3.3 Source, Processing and Chemical Analyses of Test Materials

3.3.1 Source and processing of *Ocimum gratissimum* and *Ocimum canum*

Ocimum gratissimum and *Ocimum canum*leaves were purchased from Sabo market in Sabon-Gari Local Government Area of Kaduna State; the samples were removed from the

stem and dried under shade for three (3) days, crushed by grinding using hammer mill and included as part of the experimental diet.

3.3.2 Chemical analysis of *Ocimum gratissimum* and *Ocimum canum*

Samples of *Ocimum gratissimum* and *Ocimum canum* were analyzed for proximate composition using the methods described by AOAC (2005) at the Biochemical Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria.

Phytochemical contents and quantitative determination of essential oil in the experimental materials were analyzed using the standard method described by Ejikeme *et al*. (2014) for plant materials in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria.

3.4Experiment one: Effect of *Ocimum gratissimum* and *Ocimum canum* as Phytobiotics on the Performance of Broiler Chickens

3.4.1 Experimental design and management of birds

Three hundred and ninety six (396) day old Cobb 500 broiler chicks were allotted to six dietary treatments with 3 replicates of 22 birds each in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices. Feed and water were provided *ad libitum* throughout the experimental period (49 days).

3.4.2 Experimental diets

Six treatment diets were formulated as shown in Tables 3.1 and 3.2 for the starter and finisher phases respectively. Scent and curry leaves were added as follows:

Diet 1: Standard diet without experimental materials (Control)

Table 3.1: Composition of broiler starter diets containing varying levels of *Ocimum gratissimum* and *Ocimum canum*

	Inclusion of Experimental Materials (g/100kg Diet)					
		Ocimum gratissimum		*Ocimum canum*		
Ingredients (%)	**0**	**400**	**800**	**400**	**800**	**Oxytet**
Maize	56.75	56.35	55.95	56.35	55.95	56.75
Soyabean cake	28.00	28.00	28.00	28.00	28.00	28.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00	11.00
Scent leave meal	0.00	0.40	0.80	0.00	0.00	0.00
Curry leave meal	0.00	0.00	0.00	0.40	0.80	0.00
Bone Meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.05	0.05	0.05	0.05	0.05	0.05
Vit-min Premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis						
ME (Kcals/Kg)	2,923	2,916	2,910	2,918	2,912	2,923
Crude Protein (%)	23.07	23.04	23.01	23.03	23.01	23.07
Crude Fibre (%)	3.66	3.68	3.70	3.67	3.69	3.66
Ether Extract (%)	3.24	3.37	3.40	3.35	3.39	3.24
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorus (%)	0.87	0.86	0.86	0.86	0.86	0.87
Methionine (%)	0.50	0.50	0.50	0.50	0.50	0.50
Lysine (%)	1.28	1.28	1.28	1.28	1.28	1.28
Feed Cost (₦/kg)	118.10	121.10	124.10	121.30	124.30	121.60

[A] Vitamin- mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2, 000,000 IU; Vit. E, 40,000mg; Vit. K3, 2000mg; Vit. B1, 1,500mg; Vit. B2, 5,000mg, Vit. B6, 4,000mg; Vit. B12, 20mg; Niacin, 40,000mg; Calpan, 10,000mg; Folic acid, 1,000mg; Biotin, 100mg; Choline Chloride, 30,000mg; Manganese, 80,000mg; Iron, 40,000mg; Zinc, 60,000mg; Copper, 80,000mg; Iodine, 800mg; Cobalt, 300mg; Selenium, 200mg. Oxytet = Oxytetracycline.

Table 3.2: Composition of broiler finisher diets containing varying levels of *Ocimum gratissimum* and *Ocimum canum*

	Inclusion of Experimental Materials (g/100kg Diet)					
		Ocimum gratissimum		*Ocimum canum*		
Ingredient (%)	**0**	**400**	**800**	**400**	**800**	**Oxytet**
Maize	58.00	57.60	57.20	57.60	57.20	58.00
Soyabean cake	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00	11.00
Scent leaf	0.00	0.40	0.80	0.00	0.00	0.00
Curry leaf	0.00	0.00	0.00	0.40	0.80	0.00
Maize offal	6.80	6.80	6.80	6.80	6.80	6.80
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.35	0.35	0.35	0.35	0.35	0.35
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vit-min Premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis						
ME (Kcals/Kg)	2,923	2,920	2,917	2,920	2,917	2,923
Crude Protein (%)	20.09	20.06	20.08	20.05	20.07	20.09
Crude Fibre (%)	4.07	4.12	4.17	4.10	4.14	4.07
Ether Extract (%)	4.55	4.63	4.71	4.65	4.73	4.55
Calcium (%)	1.27	1.27	1.27	1.27	1.27	1.27
Phosphorus (%)	0.57	0.57	0.57	0.57	0.57	0.57
Methionine (%)	0.55	0.55	0.55	0.55	0.55	0.55
Lysine (%)	1.04	1.04	1.04	1.04	1.04	1.04
Feed Cost (₦/kg)	105.10	108.10	111.10	108.30	111.30	109.10

[A] Vitamin- mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2, 000,000 IU; Vit. E, 40,000mg; Vit. K3, 2000mg; Vit. B1, 1,500mg; Vit. B2, 5,000mg, Vit. B6, 4,000mg; Vit. B12, 20mg; Niacin, 40,000mg; Calpan, 10,000mg; Folic acid, 1,000mg; Biotin, 100mg; Choline Chloride, 30,000mg; Manganese, 80,000mg; Iron, 40,000mg; Zinc, 60,000mg; Copper, 80,000mg; Iodine, 800mg; Cobalt, 300mg; Selenium, 200mg. Oxytet = Oxytetracycline.

Diet 2: Standard diet with scent leaf meal additive 400 g/100kg

Diet 3: Standard diet with scent leaf meal additive 800 g/100kg

Diet 4: Standard diet with curry leaf meal additive 400 g/100kg

Diet 5: Standard diet with curry leaf meal additive 800 g/100kg

Diet 6: Standard diet with Oxytetracycline as feed additive (dosage was as recommended by manufacturer).

3.4.3 Growth study

Initial and final weights of birds were taken at the beginning and at the end of both starter and finisher phases. Weight gain and feed intake were measured weekly while feed/gain ratio and cost per Kg gain were computed for both phases. Mortality was recorded as they occurred.

3.4.4 Haematological study

At the end of the feeding trial (7^{th} week), 2 ml blood samples were taken from three birds per treatment into sterilized sample bottles containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determination of haematological parameters such as Packed cell volume (PCV) which was determined by the microhaematocrit method, haemoglobin concentration (Hb) which was determined photometrically at the wavelength of 540 nm, the erythrocyte (RBC) and leucocyte (WBC) counts were determined using the improved Neubauer haemocytometer and Differential counts were determined by the thin slide method (Lamb, 1991).

3.4.5 Liver function test

Another set of 2 ml blood samples were taken from three birds per treatment into sterilized sample bottles containing no anticoagulant. The samples were allowed to clot and then centrifuged, serum was separated and stored at -20°C at the Clinical Pathology laboratory of the Ahmadu Bello University veterinary Teaching Hospital Zaria for determination of parameters related to liver function; blood glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), globulin and albumin, according to the methods described by Lamb (1991).

3.4.6 Determination of lipid profile parameters

Another 2 ml blood samples were taken from three birds per treatment into sterilized sample bottles containing no anticoagulant and taken to the Clinical Pathology Laboratory of the Ahmadu Bello University Teaching Hospital, Zaria for determination of parameters related to lipid profile (cholesterol, triglyceride, high density lipoprotein and low density lipoprotein) using standard laboratory procedures (Lamb, 1991).

3.4.7 Determination of antioxidant capacity

At the end of the trial (7^{th} week), the antioxidant status of the birds were measured in the blood using three birds per treatment. Malondialdehyde haemolysate, superoxide dismutase (SOD); catalase (CAT) and glutathione peroxidase (GPx) activity were determined using spectrophotometry methods based on the Northwest Life Specialist (NWLSS^{TM}) (Palgia and Valentine, 1967) at the Clinical Pathology laboratory of the Ahmadu Bello University veterinary Teaching Hospital, Zaria, Nigeria.

3.4.8 Intestinal microbial study

At the end of the 7 weeks experimental period, bacterial cell species and numbers in the ileum and caecum were determined using one chicken per replicate hence three chickens per treatment. This was carried out at the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria. The total bacteria load, and isolation of *Salmonella, Staphylococcus* and *Escherichia coli* from the samples were done using different selective media for isolation of bacteria groups and characterization based on sugars fermentation using Microbact 12E kit and conventional biochemical methods. The media used were Eosin Methylene Blue Agar, Mannitol Salt Agar and Salmonella Shigella Agar (OMP, 2015[abc]).

3.3.9 Carcass analysis

At the end of the finisher phase, 3 chickens with representative weights from each treatment were selected for carcass evaluation. The selected birds were starved of feed but given water *ad libitum* overnight. They were bled by severing the jugular vein and then scalded in hot water to remove the feathers. Live weight for each chicken was taken before slaughter. Dressed weight, standard cut parts and weight of organs were measured while dressing percentage was calculated. The organ weights: liver, lungs, kidney, gizzard and heart and abdominal fat content were expressed as percentage of live weight while the cut parts: thigh, drumstick, breast and back were expressed as percentage of dressed weight.

3.4.10 Villi morphometric study

Intestinal segment samples (approximately 2cm in length) were taken from the birds used for carcass evaluation to ascertain villi morphometric. Intestinal tissues were harvested and fixed in 10% formolsaline. They were histologically processed according to the method of

Bancroft and Stevens (2010). They were dehydrated through ascending grades of alcohol (70 %, 90 % and 100 %) for 2 hours each. The tissues were cleared in xylene for 2 hours then were impregnated and embedded in paraffin wax. They were sectioned at 5 micron thickness using Rotary microtome machine (Leica RT 25 made in England). Sectioned tissues were mounted on slides, dried and stained using Hematoxylin and Eosin (H and E) stain. Stained slides of the tissues were photomicrographed using Amscope Digital Camera for microscope version 2.0, made in Japan. Histomorphometric analysis for the villus was carried out using Digimizer image analysis software version 4.5 made in USA (Bancroft and Stevens, 2010). The morphometric indices that were evaluated include villi height, villi width, crypt depth, surface area, perimeter and the ratio of villi height to villi depth at the Histology Laboratory, Department of Human Anatomy, Ahmadu Bello University, Zaria.

3.4.11 Sensory evaluation

The carcass quality and acceptability were assessed for flavour, tenderness, juiceness, texture, colour and overall acceptability with a structured questionnaire on a 5-point Hedonic scale (5-like extremely, 4-like moderately, 3-neither like nor dislike, 2-dislike moderately, 1-dislike extremely) (Appendix 2) which involved a total of 30 respondents (semi-trained panelists) in the Faculty of Agriculture, Ahmadu Bello University, Zaria. All needed accessories were provided; clean water, tooth-picks, detergent, biscuit and serviette were provided for the panelists before the evaluation. Each sample was blind coded to reduce bias (Appendix 3). The samples were processed by grilling at the animal product and processing laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria.

3.4.12 Economic analysis

The economic benefit of feeding broiler chickens with the test materials was determined using gross margin analysis, using the following formulae:

$$\text{Return per naira invested} = \frac{Revenue}{Total\ variable\ cost}$$

$$\text{Gross margin} = \text{Revenue} - \text{Total variable cost}$$

$$Revenue\ per\ chicken = Cost\ of\ live\ chicken\ per\ kg\ \times average\ weight\ (kg)$$

$$Total\ variable\ cost = \sum All\ variable\ production\ costs$$

$$Feeding\ cost = Cost\ of\ feed\ per\ kg\ \times Feed\ intake\ (kg)$$

3.5Experiment Two: Effect of Combinations of *Ocimum gratissimum* and *Ocimum canum* as Phytobiotics on the Performance of Broiler Chickens

3.5.1 Experimental design and management of birds

Four hundred and five (405) day old Cobb 500 broiler chicks were allotted to five dietary treatments with 3 replicates of 27 birds each in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices. Feed and water were provided *ad libitum* throughout the experimental period (49 days).

3.5.2 Experimental diets

Five treatment diets were formulated as shown in Tables 3.3 and 3.4 for the starter and finisher phases respectively. Scent and curry leaves were added in combinations as follows:

Diet 1: Standard diet without experimental materials (Control)

Diet 2: Standard diet containing*Ocimum gratissimum*: *Ocimum canum*(75:25)

Diet 3: Standard diet containing*Ocimum gratissimum*: *Ocimum canum* (50:50)

Diet 4: Standard diet containing*Ocimum gratissimum*: *Ocimum canum*(25:75)

Diet 5: Standard diet with Oxytetracycline as feed additive (dosage was as recommended by manufacturer).

3.5.3 Growth study

Same as in experiment 1.

3.5.4 Haematological study

Same as in experiment 1.

3.5.5 Liver function test

Same as in experiment 1.

3.5.6 Determination of lipid profile parameters

Same as in experiment 1.

3.5.7 Determination of antioxidant capacity

Same as in experiment 1.

Table 3.3: Composition of broiler starter diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

	Inclusion of Experimental Materials (g/100kg Diet)				
		Ocimum gratissimum : *Ocimum canum*			
Ingredients (%)	**0**	**600:200**	**400:400**	**200:600**	**Oxytet**
Maize	56.80	56.00	56.00	56.00	56.80
Soyabean cake	28.00	28.00	28.00	28.00	28.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00
Scent leaf	0.00	0.60	0.40	0.20	0.00
Curry leaf	0.00	0.20	0.40	0.60	0.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.45	0.45	0.45	0.45	0.45
Common Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.05	0.05	0.05	0.05	0.05
Vit-min Premix[A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcals/Kg)	2,923	2,918	2,916	2,910	2,923
Crude Protein (%)	23.07	23.04	23.03	23.01	23.07
Crude Fibre (%)	3.66	3.68	3.70	3.67	3.66
Ether Extract (%)	3.24	3.37	3.40	3.35	3.24
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorus (%)	0.87	0.86	0.86	0.86	0.87
Methionine (%)	0.50	0.50	0.50	0.50	0.50
Lysine (%)	1.28	1.28	1.28	1.28	1.28
Feed Cost (₦/kg)	118.10	124.10	124.30	124.45	121.60

[A] Vitamin- mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2, 000,000 IU; Vit. E, 40,000mg; Vit. K3, 2000mg; Vit. B1, 1,500mg; Vit. B2, 5,000mg, Vit. B6, 4,000mg; Vit. B12, 20mg; Niacin, 40,000mg; Calpan, 10,000mg; Folic acid, 1,000mg; Biotin, 100mg; Choline Chloride, 30,000mg; Manganese, 80,000mg; Iron, 40,000mg; Zinc, 60,000mg; Copper, 80,000mg; Iodine, 800mg; Cobalt, 300mg; Selenium, 200mg. Oxytet = Oxytetracycline.

Table 3.4: Composition of broiler finisher diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

	Inclusion of Experimental Materials (g/100kg Diet)				
		Ocimum gratissimum : *Ocimum canum*			
Ingredient (%)	**0**	**600:200**	**400:400**	**200:600**	**Oxytet**
Maize	58.00	58.00	58.00	58.00	58.00
Soyabean cake	19.00	19.00	19.00	19.00	19.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00
Scent leaf	0.00	0.60	0.40	0.20	0.00
Curry leaf	0.00	0.20	0.40	0.60	0.00
Maize offal	7.80	7.00	7.00	7.00	7.80
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.40	0.40	0.40	0.40	0.40
Common Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vit-min Premix[A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcals/Kg)	2,923	2,921	2,921	2,921	2,923
Crude Protein (%)	20.09	20.07	20.07	20.06	20.09
Crude Fibre (%)	4.07	4.14	4.12	4.11	4.07
Ether Extract (%)	4.55	4.62	4.64	4.67	4.55
Calcium (%)	1.27	1.27	1.27	1.27	1.27
Phosphorus (%)	0.57	0.57	0.57	0.57	0.57
Methionine (%)	0.55	0.55	0.55	0.55	0.55
Lysine (%)	1.04	1.04	1.04	1.04	1.04
Feed Cost (₦/kg)	105.10	109.60	109.80	109.95	109.10

[A] Vitamin- mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2, 000,000 IU; Vit. E, 40,000mg; Vit. K3, 2000mg; Vit. B1, 1,500mg; Vit. B2, 5,000mg, Vit. B6, 4,000mg; Vit. B12, 20mg; Niacin, 40,000mg; Calpan, 10,000mg; Folic acid, 1,000mg; Biotin, 100mg; Choline Chloride, 30,000mg; Manganese, 80,000mg; Iron, 40,000mg; Zinc, 60,000mg; Copper, 80,000mg; Iodine, 800mg; Cobalt, 300mg; Selenium, 200mg. Oxytet = Oxytetracycline.

3.5.8 Intestinal microbial study

Same as in experiment 1.

3.5.9 Villi morphometric study

Same as in experiment 1.

3.5.10 Carcass analysis

Same as in experiment 1.

3.5.11 Sensory evaluation

Same as in experiment 1.

3.5.12 Economic analysis

Same as in experiment 1.

3.6 Data Analysis

All data obtained from the study were statistically analysed using the General Linear Model Procedure of Statistical Analysis Systems software package (SAS, 2002) while significant differences between treatment means were separated using Tukey Procedure.

3.6.1 Experimental model

The model for the study is as shown below:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = Performance of the chickens (j^{th} observation)

μ = Population mean

T_i = Effect of *Ocimum* spp. (i^{th} treatment)

e_{ij} = Error term

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of *Ocimum gratissimum* and *Ocimum canum* leaves

The proximate composition of *Ocimum gratissimum* and *Ocimum canum* is presented in Table 4.1.The results showed that the crude protein (CP), crude fibre (CF), ether extract (EE), nitrogen free extract (NFE) and ash content of *Ocimum gratissimum* were 5.25%, 10.90%, 5.89%, 69.44% and 4.82% respectively. This indicates that the plant contains some considerable levels of nutrients other than their phytogenic values. The values obtained for *Ocimum gratissimum* in this study is above 3.33% and 9.52% for crude protein and crude fibre reported by Ekwe *et al*. (2017) but lower in EE and ash contents of 8.50% and 13.67% respectively that they reported. However, the results obtained showed lower values in CP and CF than values of 6.38% and 18.52 % respectively reported for *Ocimum gratissimum leaves* (Nworgu, 2016). Adewole (2014) also reported protein content of 16.51±0.40 %, CF content of 9.07±0.27 %, EE content of 2.78±0.01 % and NFE content of 58.89±4.49 %.

The proximate composition of *Ocimum canum* as shownindicates that the CP, CF, EE, NFE and ash content were 5.19%, 10.81%, 6.13%, 72.07% and 4.28% respectively. This indicates that the plant contains some considerable levels of nutrients other than their nutritive and phytogenic values. The CP obtained in this study is higher than 4.00% and CF lower than 17.00% reported by Aluko *et al*. (2012). EE was also lower compared to the value of 7.00% that was reported for *Ocimum gratissimum* leaves (Aluko *et al*., 2012). Lower CP values reported may be attributed to differences in environment.

Table 4.1: Proximate composition of *Ocimum gratissimum* and *Ocimum canum*leaves

Parameters	Composition (%)	
	Ocimum gratissimum	***Ocimum canum***
Crude protein	5.25	5.19
Crude fibre	10.90	10.81
Ether extract	5.89	6.13
Nitrogen free extract	69.44	72.09
Ash	4.82	4.28

4.2 Phytochemical and essential oil Composition of *Ocimum gratissimum* and *Ocimum canum* leaves

The phytochemical content and quantitative composition of essential oil in *Ocimum gratissimum* and *Ocimum canum*is presented in Table 4.2. The phytochemical analysis of *Ocimum gratissimum* showed that tannins, saponins, glycosides, flavonoids, alkaloids and phenol contents were 0.47, 1.60, 0.17, 10.00, 2.01 and 2.66 mg/kg, respectively. These values were higher than values reported by Ekwe *et al*. (2017) which may be due to varietal difference, location and stage of harvest.

The screening of *Ocimum canum* in this study showed that tannins, saponins, glycosides, flavonoids, alkaloids and phenol contents were 0.86, 2.50, 0.11, 7.43, 1.90 and 4.25 mg/kg, respectively. The result of phytochemical analysis showed that the plant material is very rich in secondary metabolites and were present in different concentrations. The values obtained for the phytochemicals present in *Ocimum canum* were lower than the values of alkaloid (11.43±0.09), phenol (7.50±0.06), tannin (10.90±0.06), flavonoid (8.20±0.06) and saponin (12.87 ±0.19) mg/kg reported by Adewole (2014). Differences in result obtained may be due to difference in location and cutting stage. High flavonoid content in *Ocimum gratissimum* and *Ocimum canum* may be responsible for their high aromatic properties.

The quantitative determination of the essential oil (EO) content of *O. gratissimum* and *O. canum* showed that they contain 2.66% and 2.74 % EO, respectively. This considerable higher content of EO in these botanicals may be responsible for their high medicinal value and aromatic properties. The essential oil content obtained in this study is higher than the value of 0.8-1.2% reported by Orwa *et al*. (2009) for *O. gratissimum*.

Table 4.2: Phytochemical composition of *Ocimum gratissimum* and *Ocimum canum*leaves

Components	Concentrations (mg/kg)	
	Ocimum gratissimum	***Ocimum canum***
Tannins	0.47	0.86
Saponins	1.60	2.50
Glycosides	0.17	0.11
Flavonoids	10.0	7.43
Alkaloids	2.01	1.90
Phenols	2.04	4.25
Essential Oil*	2.66	2.74

*expressed in percentage (%) per experimental material.

This implies that *Ocimum gratissimum* used in this study had higher essential oil content which may also offer higher benefits. Higher EO reported may be due to environmental differences, stage and time of cutting.

4.3 Experiment 1: Effect of Diets containing*Ocimum gratissimum* and *Ocimum canum* as Phytobiotics on the Performance of Broiler Chickens

4.3.1 Growth performance of broiler chicks fed diets containing *Ocimum gratissimum* and *Ocimum canum* leaves (0-4 weeks)

The growth performance of broiler chicks fed diets containing *Ocimum gratissimum* and *Ocimum canum* leaves as phytobiotics is presented in Table 4.3. The results showed that there were significant differences in final weight, weight gain, feed intake, feed conversion ratio (FCR) and feed cost per kg gain. Final weight, weight gain and feed intake were higher ($p<0.05$) in broiler chicks fed diets supplemented with oxytetracycline, *Ocimum gratissimum* at 800g/100kg diet and *Ocimum canum* at 400g and 800g respectively.

FCR and feed cost per kg gain were better ($p<0.05$) in birds fed diets containing oxytetracycline and the experimental materials compared to those in the control group. Feed intake was highest ($p<0.05$) in broiler chicks fed diet containing 800g of *Ocimum gratissimum*. Feed cost per kg weight gain were reduced by ₦15.89 (Oxytet), ₦12.02 (800 g of *O. canum*), ₦10.75 (400 g of *O. canum*), ₦7.81 (400 g of *O. gratissimum*) and ₦2.53 (800 g of *O. canum*) treatment groups indicating higher economic gain to farmers by inclusion of the experimental materials in broiler chickens diets.

The findings of this study showed that inclusion of *Ocimum gratissimum* and *Ocimum canum* in the diets of broiler chicks had positive effect in improving the growth and economic efficiency of the birds. In several studies, researchers have also stated that feed

Table 4.3: Performance of broiler chicks fed diets supplemented with *Ocimum gratissimum* and *Ocimum canum* (0-4weeks)

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
	0	400	800	400	800	Oxytet	
Initial weight (g/b)	46.46	46.46	46.46	46.47	46.42	46.53	0.34
Final weight (g/b)	943.15^{c}	952.78^{bc}	1000.38^{ab}	1000.56^{ab}	1042.94^{a}	1030.98^{ab}	43.30
Weight gain (g/b)	896.69^{b}	906.32^{b}	953.91^{ab}	954.10^{ab}	996.52^{a}	984.45^{a}	43.21
Feed intake (g/b)	1497.63^{a}	1421.85^{b}	1501.41^{a}	1474.42^{ab}	1492.33^{ab}	1462.54^{ab}	38.64
Feed conversion ratio	1.68^{b}	1.57^{a}	1.58^{a}	1.54^{a}	1.50^{a}	1.50^{a}	0.05
Feed cost (₦/kg)	118.10	121.10	124.10	121.30	124.30	121.60	NA
Feed cost/kg gain (₦/kg)	197.93^{b}	190.12^{ab}	195.40^{b}	186.80^{a}	186.45^{a}	182.04^{a}	6.11
Mortality (%)	2.98	2.98	2.98	1.49	0.00	1.49	1.66

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, g/b: gram/bird, NA: not analyzed.

additives may stimulate early gut development and improve overall efficiency of the chicken's gastro intestinal tract (Donoghue, 2003; Apajalahti *et al.,* 2004). The high performance of the broiler chicks fed diets containing AGP showed that antibiotics improved feed efficiency and growth rate. This could be attributed to its antimicrobial effect especially in handling the pathogenic strains of bacteria. In a similar study where the diets of broiler chickens were supplemented with chlortetracycline, oxytetracycline and penicillin, it was reported to improve growth rate (Anonymous, 2015). There was no significant difference ($p>0.05$) in mortality rate across the treatments.

4.3.2 Growth performance of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum* leaves (5-7 weeks)

The growth performance of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum* leaves as phytobiotics is presented in Table 4.4. The results showed that there were significant ($p<0.05$) differences in final weight, weight gain, feed intake, feed conversion ratio (FCR) and feed cost per kg gain. Final weight, weight gain and feed intake were significantly ($p<0.05$) higher in broiler chickens fed diets supplemented with oxytetracycline, *Ocimum gratissimum* and *Ocimum canum* at 800g/100kg diets respectively. FCR were better in broiler chickens fed diets containing *Ocimum canum* at 800g per 100kg diet (1.71) and oxytetracycline (1.77) while feed cost per kg gain was significantly($p<0.05$) better in birds fed diets containing oxytetracycline and the experimental materials. Mortality rate was significantly ($p<0.05$) least in broiler chickens fed diets containing 800 g of *Ocimum gratissimum*, 400 g and 800 g of *Ocimum canum* and oxytetracycline compared to birds fed diet containing 400 g of *Ocimum gratissimum* and the control diet.

Table 4.4: Growth performance of broiler chickens fed diets supplemented with *Ocimum gratissimum* and *Ocimum canum* (5-7weeks)

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
	0	400	800	400	800	Oxytet	
Initial weight (g/b)	1001.11	1001.36	1009.96	1015.91	1018.33	1016.19	10.32
Final weight (g/b)	2426.72^{b}	2478.03^{b}	2650.74^{a}	2513.88^{b}	2689.36^{a}	2645.54^{a}	65.68
Weight gain (g/b)	1425.61^{c}	1476.67^{c}	1640.78^{a}	1497.96^{bc}	1671.03^{a}	1629.35^{ab}	69.89
Feed intake (g/b)	2853.20^{ab}	2771.50^{b}	3011.70^{a}	2772.90^{b}	2865.20^{ab}	2877.70^{ab}	103.59
Feed conversion ratio	2.00^{c}	1.88^{bc}	1.85^{b}	1.85^{b}	1.71^{a}	1.77^{ab}	0.07
Feed cost (₦/kg)	105.10	108.10	111.10	108.30	111.30	109.10	NA
Feed cost/kg gain (₦/kg)	210.51^{b}	203.14^{ab}	205.22^{ab}	200.56^{ab}	190.32^{a}	193.28^{a}	8.47
Mortality (%)	1.51^{b}	1.51^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.45

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, NA: not analyzed.

The higher final weight and total weight gain observed for broiler chickens fed diets containing the experimental material and oxytetracycline could be as a result of exclusive elimination of harmful bacterial load in the gut of the birds leading to higher concentration of beneficial bacterial that helped in effective feed digestion and nutrient utilization. Better FCR and feed cost per kg gain observed in broiler chickens fed diets containing 800 g of *Ocimum canum* and oxytetracycline and other levels of *Ocimum gratissimum* and *Ocimum canum* compared to the control groups may indicates increased proliferation of absorptive surfaces leading to higher feed utilization and better growth performance. Feed cost per kg gain were reduced in the treatment groups by ₦20.19 (800 g of *O. canum*), ₦17.23 (Oxytet), ₦9.95 (400 g *O. canum*), ₦7.37 (400 g of *O. gratissimum*) and ₦5.29 (800 g of *O. gratissimum*). This indicates higher return to farmers by use of *Ocimum spp.* in broiler chickens diet through reduction and cost of feeding per weight gain. Hence, addition of these materials accounts for higher economic gain.

Phytogenic feed additives had been shown to have antimicrobial effect in the gut of livestock (Khan *et al*., 2009; Pandey *et al*., 2015). In another study by Weerasingha and Atapattu (2013), NGPs was reported to improve growth rate and efficiency of feed utilization. Feed intake was highest ($p<0.05$) for birds fed diet containing 800 g of *Ocimum gratissimum* (3011.70g/bird). The higher feed intake by birds on higher levels of *Ocimum gratissimum* could be as a result of the appetite stimulating effect of the essential oil of the materials. The result of this study agrees with the findings of Grashorn (2010) and Pandey *et al*. (2015) who stated that phytogenic feed additives stimulates higher feed intake in livestock. Lower mortality rate recorded in broiler chickens fed diets containing 800 g of *Ocimum gratissimum*, 400 g and 800 g of *Ocimum canum* and oxytetracycline compared to

birds fed diet containing 400 g of *Ocimum gratissimum* and the control diet may be attributed to higher antimicrobial activities of the test materials and oxytetracycline in reducing the populations of pathogenic strains of bacteria thereby increasing liveability.

The findings of this study showed that inclusion of *Ocimum gratissimum* and *Ocimum canum* in the diets of broiler chickens exerts a positive effect in the growth performance of broiler chickens. The high performance of the broiler chickens fed diet containing AGP showed that antibiotics improved feed efficiency and growth rate. This could be attributed to its antimicrobial effect especially in increasing the population of beneficial strains of bacteria. In several studies, researchers have also stated that feed additives may stimulate early gut development and improve overall efficiency of the chicken gastro intestinal tract and thereby improve the overall performance and economic efficiency of the enterprise (Donoghue, 2003; Apajalahti *et al.*, 2004).

4.3.3 Haematological indices of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

Table 4.5 shows the haematological parameters of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*. Significant differences ($p<0.05$) were observed among haematological parameters measured except for red blood cell and white blood cell counts which were not significantly ($p>0.05$) different across the treatments. Pack cell volume (PCV), Haemoglobin (Hb), Heterophils, lymphocytes, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were significantly ($p<0.05$) higher for broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*.

Table 4.5: Haematological parameters of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM	Reference Value
		Ocimum gratissimum		*Ocimum canum*				
	0	400	800	400	800	Oxytet		
Pack Cell Volume (%)	31.31^{b}	34.13^{b}	40.75^{a}	39.32^{a}	40.79^{a}	36.20^{a}	1.73	24.00-40.00*
Haemoglobin (g/dL)	14.43^{ab}	12.77^{b}	16.10^{a}	13.63^{ab}	14.53^{a}	12.90^{b}	1.31	7.00-15.00*
Red Blood Cell ($\times 10^{12}$/L)	3.23	2.47	3.56	3.37	3.85	2.50	0.68	1.59-4.10*
White Blood Cell ($\times 10^{9}$/L)	5.04	5.40	6.13	4.39	4.43	4.65	1.26	1.90-9.50**
Heterophils (%)	6.83^{b}	7.03^{b}	7.50^{b}	11.50^{a}	11.13^{a}	6.33^{b}	1.31	15.00-40.00**
Lymphocytes (%)	86.70^{b}	86.00^{b}	87.87^{b}	92.27^{a}	87.90^{b}	86.43^{b}	1.99	40.00-100.00***
Monocytes (%)	4.80^{ab}	5.23^{a}	5.27^{a}	4.20^{b}	5.63^{a}	5.47^{a}	0.43	1.00-7.00****
Basophils (%)	0.20^{ab}	0.23^{ab}	0.20^{ab}	0.23^{ab}	0.37^{a}	0.13^{b}	0.09	0.10-2.00*
Eosinophils (%)	1.47^{ab}	1.50^{ab}	1.20^{b}	1.33^{b}	1.33^{b}	1.97^{a}	0.30	1.50-6.00**
MCH (pg)	41.30^{c}	49.57^{b}	55.43^{a}	55.10^{b}	50.47^{b}	55.73^{a}	1.82	33.00-47.00***
MCV (fl)	139.53^{b}	148.60^{ab}	161.27^{a}	150.83^{a}	156.83^{a}	155.23^{a}	8.23	90.00-140.00**
MCHC (g/dL)	31.10^{d}	34.67^{bc}	36.67^{ab}	37.47^{a}	37.43^{a}	32.93^{cd}	1.41	26.00-85.00***

[abc]Means with the same superscripts along the rows are not significantly different ($p>0.05$), SEM: standard error of means, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin Concentration, MCV: Mean corpuscular volume*Mitruka and Rawnsely, 1997, **Simrak *et al*., 2004, ***Jain, 1986, ****Jain, 1993.

The observed values in this trial across the treatment groups however fell within the normal range reported for healthy chickens as reported by Mitruka and Rawnsley (1997). This indicates that the diets were suitable and adequate for broiler chickens and that the diets did not have adverse effect on the health status of the birds.Packed cell volume (PCV) and haemoglobin count were significantly ($p<0.05$) higher in birds fed diets containing *Ocimum gratissimum* at 800 g/100kg and *Ocimum canum* at 400 g and 800 g per 100kg diet respectively. PCV reported in this study ranges from 31.31-40.79%. Packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell and total protein (TP) are important blood parameters used to assess the health status of animals. It has been established that reduction in the levels of nutrients in feeds result in decrease in PCV and Hb of animals (Oladele and Ayo, 1999). PCV is an index of toxicity; for the value to fall within the normal range suggests that the broiler birds had the ability to tolerate the phytochemicals in the diet.

The haemoglobin content indicates the oxygen carrying capacity of the birds' circulatory system (Iheukwumere and Herbert, 2003). Birds with low Hb can be said to have low oxygen carrying capacity and can easily succumb to any form of respiratory disease while birds with high Hb concentration can be regarded as having high oxygen carrying capacity and such birds are likely to withstand some levels of respiratory stress. The blood parameters did not deviate from established ranges and hence the health of the birds were not compromised by inclusion of the experimental materials.

4.3.4 Liver function indices of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The liver function indices of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum* is presented in Table 4.6. Significant ($p<0.05$) differences were observed across the dietary treatments for all parameters measured except for albumin, AST and ALT levels which were not different ($p>0.05$). Total protein and globulin levels were significantly ($p<0.05$) higher in birds fed dietary supplement of *Ocimum canum* and increased numerically as the inclusion levels increases indicating higher blood clotting ability which according to Moses *et al*. (2017) will aid in preventing haemorrhage. Albumin to globulin ratios ranges from 0.50-0.79 which falls within the normal range reported for healthy birds by Bounous and Stedman (2000).

ALP was significantly ($p<0.05$) higher in birds in the control group, those fed diets containing 400 g and 800 g of *Ocimum gratissimum* and 400 g of *Ocimum canum* respectively compared to the other treatments. However, values obtained were all within normal range of 10-106 IU/L as stated by Ross *et al*. (1976). High amount of liver enzymes in the blood indicates liver damage (WebMed, 2016).

Harper *et al*. (1997) and Ewuola and Egbunike (2008) reported that increase in aspartate amino transferase are clinical indication of diagnosing state of damage done to the visceral organs by toxic substances or infections. Values obtained in this study were within normal range reported for healthy birds by Clinical Diagnostic Division (1990), Bounous and Stedman (2000), LAVC (2009) and Ajuonuma *et al.* (2013) except for AST which were below reference value of 100-400µL (LAVC, 2009). The result of this study therefore indicates that the birds were in healthy condition.

Table 4.6: Liver function parameters of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)							Reference value
		Ocimum gratissimum		*Ocimum canum*				
	0	400	800	400	800	Oxytet	SEM	
Total protein (g/dL)	3.77[ab]	3.63[b]	3.67[b]	3.93[ab]	4.20[a]	3.70[b]	0.22	3.60-5.50*
Albumin (g/dL)	1.60	1.50	1.47	1.33	1.40	1.57	0.16	1.10-2.20*
Globulin (g/dL)	2.17[b]	2.13[b]	2.20[b]	2.60[a]	2.80[a]	2.13[b]	0.20	1.20-3.20**
Albumin : Globulin	0.79[a]	0.70[ab]	0.66[ab]	0.52[b]	0.50[b]	0.74[a]	0.11	0.35-0.75*
Aspartate-Amino Transferase (IU/L)	54.70	57.17	65.87	54.63	58.6	59.93	5.85	100-400**
Alanine-Amino Transferase (IU/L)	13.90	13.07	14.47	14.87	14.50	13.87	0.93	-
Alkaline Phosphatase (IU/L)	78.30[a]	79.60[a]	81.47[a]	78.70[a]	63.37[b]	67.57[b]	3.69	10-106***

[abc]Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline, Reference values: *Ross *et al*. (1976), **LAVC (2009), ***Bounous and Stedman (2000).

4.3.5 Serum lipid profile of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The results in Table 4.7 shows the lipid profile of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*. There were significant ($p<0.05$) differences in total cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL) across the dietary treatments while triglyceride levels were not different. Cholesterol and LDL levels were reduced ($p<0.05$) by inclusion of *Ocimum gratissimum* at 400 g and 800 g, *Ocimum canum* at 800 g and AGPs in the diets as phytobioticsources. HDL levels were also significantly ($p<0.05$) increased across the treatments except for broiler chickens fed diet containing 800 g of *Ocimum gratissimum* which had lower HDL of 150.20 mg/dl and the birds in the control group with HDL of 1555.90mg/dl.

Collins (2018) stated that the body synthesizes about 1500-2000 mg of new cholesterol each day with an additional intake ranging from 28 – 116mg/dl which is far against the desirable cholesterol level of 200mg (<130 LDL, >90 HDL and <135mg/dl triglyceride). Excess of cholesterol in the bile can crystalize to form gallstones that may block the bile duct leading to the development of atherosclerosis (fatty deposits that form inside the blood vessels) leading to heart attack.This reduction in cholesterol levels may also result in reduced occurrence of cardiovascular diseases which is in agreement with the report of Sturkie (2000) who opined that the concentration of cholesterol is influenced by physical and nutritional status of the bird and hence concluded that low cholesterol reduces the occurrence of cardiovascular disease (Jessie, 2016).

Table 4.7: Lipid profile of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM	Reference value
		Ocimum gratissimum		*Ocimum canum*				
	0	400	800	400	800	Oxytet		
Cholesterol (mg/dl)	128.10[a]	116.60[b]	113.40[b]	135.10[a]	107.20[b]	106.50[b]	5.38	129-237
Triglyceride (mg/dl)	38.17	41.40	41.08	37.52	38.85	42.79	2.88	<135.00
High Density Lipoprotein (mg/dl)	155.90[c]	180.10[b]	150.20[c]	230.80[a]	192.20[b]	196.30[b]	11.48	>90.00
Low Density Lipoprotein (mg/dl)	58.71[a]	39.23[b]	38.36[b]	52.42[a]	29.93[bc]	24.49[c]	5.87	<130.00

[abc]Means with the same superscripts along the rows are not significantly different (p>0.05),

Oxytet: Oxytetracycline**,** SEM: standard error of means, Reference value: Clinical Diagnostic laboratory (1990); Collins (2018).

Kirkpinar *et al.* (2011) reported that broilers fed with diets supplemented with oregano had significantly lower cholesterol and triglycerides compared to the control treatments. Elson and Qureshi (1995) also reported that plant extracts may lower the blood cholesterol level in broilers via inhibition of the controlling enzyme for cholesterol synthesis 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.

4.3.6 Antioxidant capacity of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The antioxidant capacity of broiler chickens fed diets containing varying levels of *Ocimum gratissimum* and *Ocimum canum*is presented in Table 4.8. Significant ($p<0.05$) differences were observed across the dietary treatments for superoxide dismutase (SOD), glutathione peroxidase (GPx) catalase (CAT) and malondialdehyde (MDA). There was a significant ($p<0.05$) increase in SOD activity as the levels of experimental material increases in the diet as well as a significant ($p<0.05$) reduction of MDA content across the treatment as dietary supplementation of the treatment materials increases indicating that these phytogenic feed additives possesses potential for improving antioxidant capacity.

Studies have reported that the essential oils from *Ocimum gratissimum* and *Ocimum canum* could perform anti-microbial and antioxidant roles (Rao *et al*., 2011; Satish and Gopu, 2015). The result of this study is in agreement with the study of Ko *et al*. (2004) who reported that excess ROS in body was removed by the antioxidant enzymes.Increased catalase activities recorded in broiler chickens fed diets containing 400 g and 800 g of *Ocimum canum* indicates high ability of the material against numerous oxidative stress related diseases as a therapeutic agent.

Table 4.8: Antioxidant capacity of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
	0	400	800	400	800	Oxytet	
Malondialdehyde (µmol/mg)	22.96[a]	14.55[b]	14.11[b]	22.20[a]	22.06[a]	21.06[a]	1.63
Superoxide dismutase (IU/ml)	15.45[cd]	20.09[b]	26.91[a]	17.94[bc]	19.68[b]	14.43[d]	1.70
Catalase (IU/mg)	26.84[c]	29.54[bc]	29.50[bc]	38.64[a]	32.92[ab]	29.26[bc]	2.83
Glutathione peroxidase (IU/L)	16.35[c]	19.46[b]	21.19[b]	20.91[b]	25.06[a]	17.27[bc]	1.53

[abcd]Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet: Oxytetracycline

Singhal *et al*. (2013) and Ankita *et al*. (2019) also reported that nanoparticle-loaded catalase may be used as a therapeutic agent in oxidative stress-related neurological diseases as an antioxidant enzyme.Glutathione peroxidase activities were higher in broiler chickens fed diets containing 800 g of *Ocimum canum* and other levels of *Ocimum gratissimum* and *Ocimum canum* above the control groups. This indicates the efficacy of these materials against oxidative damage and accumulation of free-radical products (Tappel*et al*., 1982).

Some feed additives have been reported to be beneficial in oxidation resistance, scavenging ROS and promoting antioxidant capability (Capcarova *et al*., 2010; Wen *et al*., 2011). With regards to antioxidant capacity, the endogenous antioxidant defence system in animals do not only rely on internal but also on the other external sources, such as probiotics, prebiotics, and plant extracts which are the natural sources to prevent the body from oxidative stress induced by the ROS (Rajput *et al*., 2013).

Oxidative stress is a common process in life, and it could produce varieties of reactive oxygen species (ROS) in the body, such as hydroxyl free radicals and superoxide anions. Numerous studies (Tappel *et al.*, 1982; Jones, 2006; Deng *et al*., 2012) have shown that excess of ROS can damage the proteins, nucleic acids, and other biological macromolecules, and produce large amounts of MDA leading to tissue damage, thus contributing to the development of diseases. The MDA content in the body indirectly reflects the lipid peroxidation, which is the consequence of the attenuated antioxidant protection when ROS increases (Yang *et al*., 2008).

4.3.7 Effect of *Ocimum gratissimum* and *Ocimum canum* inclusion in broiler chickens diets on ileal microbial count

The microbial count in the ileal of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*is presented in Table 4.9. The population of *Bacillus* and *Lactobacillus* spp. were significantly ($p<0.05$) increased by the inclusion of *Ocimum gratissimum* and *Ocimum canum* in the diets of broiler chickens. *Bacillius spp.* are one of the beneficial harmless microbes in the microbiota. Essential oil of oregano oil could improve the intestinal ecosystem of animals via promoting the growth of beneficial bacteria species and suppressing the growth of potential pathogenic bacterial species (Lin *et al*., 2011). *Lactobacillus spp.* are commensal bacteria that have long been known for their ability to activate the intestinal immune system and increase resistance to diseases. These bacteria have been reported to produce a wide variety of short chain fatty acids which are bacteriostatic for a subset of bacteria spp. Many essential oils stimulate growth of beneficial microbes and limit the number of pathogenic bacteria in poultry (Wenk, 2000;Lan *et al*., 2003).

The results showed that addition of *Ocimum gratissimum* and *Ocimum canum* significantly ($p<0.05$) reduced the concentration of *Escherichia coli, Clostridium spp., Salmonella spp., Enterobacteriaeae* and *Pseudomonas spp.* in broiler chickens compared to those in the control group.Some studies have revealed that Essential oils such as oregano and garlic oil inhibit microorganisms like *E. coli* and other Enterobacterial counts, *Clostridium* count, *Staphylococcus aureus, Salmonella typhimurium* and *Listeria monocytogenes* (Aligiannis *et al*., 2001; Friedman *et al*., 2002; Kirkpinar *et al*., 2011). In another study, levels of pathogenic bacteria and by implication incidence of digestive diseases were reduced by an essential oil, CRINA® Poultry Plus (DSM, 2013).

Table 4.9: Ileal bacteria count of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Bacteria	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
(×10^3 CFU/g)	0	400	800	400	800	Oxytet	
Bacilli spp.	4.17^{c}	5.53^{ab}	6.10^{a}	4.90^{b}	6.10^{a}	4.80^{bc}	0.33
Lactobacilli spp.	13.80^{b}	16.20^{a}	16.30^{a}	16.23^{a}	15.57^{a}	16.03^{a}	0.76
Clostridium spp.	1.27^{c}	0.97^{bc}	0.90^{abc}	0.47^{ab}	0.23^{a}	0.77^{ab}	0.37
Salmonella spp.	0.03^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.01
Escherichia coli	0.40^{b}	0.03^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.03^{b}	0.15
Pseudomonas spp.	0.13^{b}	0.07^{a}	0.00^{a}	0.03^{a}	0.00^{a}	0.07^{a}	0.06
Enterobacteriaceae	3.03^{b}	2.13^{ab}	1.55^{a}	2.20^{ab}	1.87^{ab}	1.83^{a}	0.60

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

4.3.8 Effect of *Ocimum gratissimum* and *Ocimum canum* inclusion in broiler chickens diets on caecal microbial count

Table 4.10 shows the microbial count in the caecal of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum.* Significant ($p<0.05$) differences were observed in all microbial counts for all treatment means except for *Clostridium spp.* that was not detected. The population of *Bacillus* and *Lactobacillus* spp. were increased by the inclusion of *Ocimum gratissimum* at 800 g/100kg diet and *Ocimum canum* at 400 g/100kg and 800 g/100kg diets respectively in the diets of broiler chickens. This implies that the plant materials were able to reduce the proliferation of harmful bacteria thereby increasing the population of beneficial bacteria.In a similar study by Lin *et al*. (2011) essential oil of oregano oil was reported to promote the growth of beneficial bacteria species and suppress the growth of potential pathogenic bacterial species. *Lactobacillus spp.* have been reported for their ability to activate the intestinal immune system and increase resistance to disease. Many essential oils either synthetic or of plant origin have also been shown to stimulate the growth of beneficial microbes and limit the number of pathogenic bacteria in poultry (Wenk, 2000;Lan *et al*., 2003).

Similarly, *Salmonella spp.* were completely eliminated by the addition of these PFA which may be due to their secondary metabolites that have been reported to have both bacteriostatic and bacteriocidal effect on a wide range of pathogenic microorganisms. This is in agreement with studies of Kirkpinar *et al.* (2011) and Moses *et al.* (2017) who reported that essential oil blends inhibits the proliferation of harmful microbes in broiler chickens. The addition of *Ocimum gratissimum* at 400 g/100kg diet reduced the population of *Escherichia coli* than other levels of inclusion though no specific trend was observed.

Table 4.10: Caecal bacteria count of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Bacteria ($\times 10^3$ CFU/g)	Inclusion of Experimental Materials (g/100kg Diet)						
		Ocimum gratissimum		*Ocimum canum*			
	Control	400	800	400	800	Oxytet	SEM
Bacilli spp.	2.50^{d}	4.77^{c}	5.47^{ab}	5.57^{a}	5.47^{ab}	4.89^{bc}	0.29
Lactobacilli spp.	4.43^{d}	14.67^{c}	16.57^{a}	16.20^{ab}	16.20^{ab}	15.03^{bc}	0.74
Clostridium spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella spp.	0.33^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.02
Escherichia coli	5.67^{d}	3.33^{a}	5.67^{d}	4.67^{c}	5.67^{d}	4.13^{b}	0.13

[abcd]Means with the same superscripts along the rows are not significantly different ($p>0.05$), SEM: standard error of means, Oxytet-Oxytetracycline

4.3.9 Villi morphometric of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

Table 4.11 showed the results of the effect of *Ocimum gratissimum* and *Ocimum canum* villi morphometry of cross section of the ileum. Significant ($p<0.05$) differences were observed in all parameters measured. Villi area was higher ($p<0.05$) in birds fed dietary supplement of 400 g of *Ocimum gratissimum,*400 g and 800 g of *Ocimum canum* and AGP (oxytetracycline) than those in the control group. Villi perimeter, height, width, crypt depth and villi height/crypt depth ratio were higher ($p<0.05$) for birds fed diets containing 800 g of *Ocimum canum* and AGP (oxytetracycline) comparedto the control group. The morphology of intestinal villi and crypts has been associated with intestinal function and growth in chickens. Higher intestinal villi are associated with increased absorptive surface area of the intestine and thus, an increased absorptive capacity with resultant higher body weight gain (Kanduri *et al.,* 2013). A lower villus/crypt ratio has been associated with the presence of toxins, poor nutrient absorption and increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue (Xu *et al*., 2003).

The results obtained in this study showed that increase in the surface area of the gut morphology may be responsible for the higher growth performance and better nutrient utilization of the broiler chickens fed dietary supplements of *Ocimum gratissimum* and *Ocimum canum.* Beneficial bacteria such as *Bacillus Subtilis* aid in maintaining optimum villi height and crypt depth supporting the gut's ability to absorb nutrients for animal maintenance and growth (Awad *et al.,* 2009; Lee *et al*., 2010). Presence of longer villi has been reported to increase crypt depth of the mucosa of the small intestine resulting in better feed conversion (Sherief *et al*., 2011).

Table 4.11: Villi morphometry of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
	0	400	800	400	800	Oxytet	
Area (µm)^2	12,767.57^{c}	17,138.91^{ab}	14,306.34^{bc}	18,785.00^{a}	17,445.63^{ab}	17,877.63^{ab}	2,023.23
Perimeter (µm)	667.35^{b}	792.81^{a}	633.83^{b}	819.88^{a}	777.38^{a}	688.58^{b}	63.75
Villi height (µm)	295.64^{bc}	356.06^{ab}	266.09^{c}	365.00^{a}	360.99^{a}	298.69^{bc}	30.95
Villi Width (µm)	81.75^{bc}	91.71^{ab}	98.46^{a}	98.28^{a}	105.82^{a}	75.73^{c}	7.29
Crypt depth (µm)	93.00^{c}	99.70^{bc}	116.88^{a}	101.38^{bc}	109.19^{ab}	117.27^{a}	7.63
Villi Height/Crypt Depth	3.27^{a}	3.69^{a}	2.25^{b}	3.53^{a}	3.45^{a}	2.63^{b}	0.30

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

4.3.10 Carcass characteristics of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The carcass characteristics of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum* is presented in Table 4.12. There were significant ($p<0.05$) differences in all parameters measured except for thigh, back, gizzard and lungs percentages. The results obtained showed that birds fed dietary supplements of *Ocimum canum* at 800 g/100kg diet and AGPs had higher ($p<0.05$) dressed weight and dressing percentage than those fed diets containing *Ocimum gratissimum* and the control group. Result of the cut parts showed that birds on 400 g of *Ocimum gratissimum*, 800 g of *Ocimum canum* and AGPs had significantly ($p<0.05$) higher drumstick and breast cut percentages. Organ weights were significantly ($p<0.05$) higher in birds fed diets containing *Ocimum gratissimum* and *Ocimum canum* at 800 g/100kg diets. The enlargement of these organs may be due to their involvement in detoxification of the materials.

Studies have reported that the use of antibiotics as a growth promoter has the ability to improve weight gain as well as carcass yield of birds (Yang *et al.,* 2009; Al-Kassie, 2010). The result is in agreement with the report of Khaksar *et al*. (2012) who observed significant improvements in some carcass traits due to inclusion of thyme essential oils. However, Schulze *et al*. (2008) did not observe a significant effect in the broiler carcass yield due to the dietary inclusion of essential oil blend based on thyme and cinnamon. Abdominal fat were lower inbirds fed diets containing *Ocimum gratissimum* and *Ocimum canum* at 800 g/100kg diets. Percent abdominal fats significantly ($p<0.05$) reduced as the inclusion levels of the experimental materials increased. The findings of this study agrees with the observation of Myandoab and HosseiniMansoub (2012) who reported that licorice extract lowered abdominal fat levels in Japanese quails.

Table 4.12: Carcass and organs characteristics of broiler chickens fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						SEM
		Ocimum gratissimum		*Ocimum canum*			
	0	400	800	400	800	Oxytet	
Live wt. (g/bird)	2350.00^{c}	2400.00^{bc}	2533.33^{a}	2416.67^{bc}	2466.67^{ab}	2516.67^{a}	50.46
Dressed weight (g/bird)	1702.33^{c}	1722.00^{c}	1828.00^{ab}	1775.67^{bc}	1860.00^{a}	1868.00^{a}	45.33
Dressing percent (%)	72.43^{b}	71.77^{b}	72.14^{b}	73.49^{ab}	75.44^{a}	74.20^{a}	1.14
Cut parts expressed as percentage of dressed weight (%)							
Thigh	12.33	11.67	12.23	12.36	12.17	12.69	0.29
Drum stick	9.59^{b}	10.20^{a}	9.48^{b}	9.65^{b}	10.33^{a}	9.78^{ab}	0.30
Breast	24.57^{a}	23.98^{ab}	24.92^{a}	22.50^{b}	24.77^{a}	25.53^{a}	0.75
Back	11.58	12.02	12.10	12.16	11.70	11.35	0.34
Organ weights expressed as percentage of live weight (%)							
Liver	2.27^{b}	2.28^{b}	2.87^{a}	2.34^{b}	2.50^{ab}	2.17^{b}	0.25
Lungs	0.61	0.69	0.76	0.70	0.70	0.70	0.14
Kidney	0.67^{b}	0.71^{ab}	0.78^{a}	0.73^{ab}	0.74^{a}	0.64^{b}	0.05
Gizzard	2.24	2.32	2.41	2.29	2.43	2.23	0.10
Heart	0.45^{c}	0.55^{ab}	0.51^{bc}	0.51^{bc}	0.64^{a}	0.50^{bc}	0.05
Abdominal fat	1.29^{c}	0.95^{b}	0.63^{a}	1.01^{b}	0.76^{a}	1.01^{b}	0.13

^{abc}Means with the same superscripts along the rows are not significantly different ($p<0.05$), SEM: standard error of means, Oxytet-Oxytetracycline.

4.3.11 Sensory attributes of meat from broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The results of the sensory analysis for the meat of broiler chickens fed dietary supplement of *Ocimum gratissimum* and *Ocimum canum* is presented in Table 4.13. The organoleptic evaluation of food products to any food processing technology is very important in determining the consumer acceptability. The results obtained shows that there were significant ($p< 0.05$) differences in all the sensory parameters evaluated. Meat of the broiler chickens in the treatment groups supplemented with *Ocimum gratissimum* and *Ocimum canum* had higher eating and sensory attributes. Sensory parameters had higher organoleptic attributes for the birds in the treatment groups fed diets supplemented with *Ocimum gratissimum* and *Ocimum canum* compared to the control and AGP groups. This implies that addition of these phytogenic feed materials improved the eating quality of broiler chicken meat which may be due to higher aromatic fluids and essential oil in these materials which made them to maintain desirable culinary properties. Okubanjo *et al*. (2010) in their study, also reported higher culinary properties for spent layers chicken meat tenderized with calcium chloride as additive.Similarly, Olusola (2018), it was reported that *Ocimum gratissimum* extract increased the organoleptic properties of meat floss (*Dambu nama*).

4.3.12 Economic analysis of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The economic benefit analysis of dietary inclusion of *O. gratissimum* and *O. canum* in the diet of broiler chickens is presented in Table 4.14. The results showed that broiler chickens fed the control diet had the least economic gain with a gross margin of ₦820.04 while birds fed diet containing *Ocimum canum* at 800 g/100kg diet had the best economic gain with a gross margin of ₦974.78 which is ₦154.74 above what was obtained for the control.

Table 4.13: Sensory evaluation of broiler chicken meat fed diet containing *Ocimum gratissimum* and *Ocimum canum*

Parameters	Inclusion levels of experimental materials (g/100kg diet)						
		Ocimum gratissimum		*Ocimum canum*			
	0	**400**	**800**	**400**	**800**	**Oxytet**	**SEM**
Colour	4.13^{bc}	4.68^{ab}	4.87^{a}	4.61^{ab}	4.84^{a}	3.87^{c}	0.34
Texture	3.97^{bc}	4.65^{ab}	4.87^{a}	4.55^{ab}	4.77^{a}	3.84^{c}	0.35
Flavour	3.45^{c}	4.85^{a}	4.87^{a}	4.58^{ab}	4.81^{a}	3.77^{bc}	0.42
Tenderness	3.74^{c}	4.42^{b}	4.87^{a}	4.74^{ab}	4.65^{ab}	3.97^{c}	0.34
Juiciness	3.32^{c}	4.55^{a}	4.88^{a}	4.61^{a}	4.84^{a}	4.03^{b}	0.37
Taste	3.29^{b}	4.74^{a}	4.94^{a}	4.65^{a}	4.81^{a}	3.65^{b}	0.39
Acceptability	3.45^{b}	4.77^{a}	4.94^{a}	4.84^{a}	4.94^{a}	3.48^{b}	0.37

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline**.**

Table 4.14: Economic analysis of production of broiler chickens fed diets supplemented with *Ocimum gratissimum* and *Ocimum canum*(0-7weeks)

		Inclusion of Experimental Materials (g/100kg Diet)				
		Ocimum gratissimum		*Ocimum canum*		
Parameters	**Control**	**400**	**800**	**400**	**800**	**Oxytet**
Variable costs						
Day-old chicks	220.00	220.00	220.00	220.00	220.00	220.00
Starter feed cost/kg (₦/kg)	118.10	121.10	124.10	121.10	124.10	121.60
Starter feed intake (kg)	1.49	1.42	1.50	1.47	1.49	1.46
Feeding cost starter (₦)	176.91	172.20	186.27	178.50	185.16	177.90
Finisher feed cost/kg (₦/kg)	105.10	108.10	111.10	108.30	111.30	109.10
Feed intake (kg/b)	2.85	2.77	3.01	2.77	2.87	2.88
Finisher feeding cost (₦)	299.85	299.65	334.63	300.32	318.87	313.99
Total feeding cost (₦/kg)	476.76	471.86	520.91	478.82	504.03	491.89
Water (₦)	12.37	12.37	12.37	12.37	12.37	12.37
Wood shaving (₦)	5.68	5.68	5.68	5.68	5.68	5.68
Labour (₦)	60.60	60.60	60.60	60.60	60.60	60.60
Brooding (₦)	50.51	50.51	50.51	50.51	50.51	50.51
Vaccination (₦)	9.59	9.59	9.59	9.59	9.59	9.59
Repair (₦)	25.25	25.25	25.25	25.25	25.25	25.25
Transportation (₦)	20.19	20.19	20.19	20.19	20.19	20.19
Total variable cost (TVC) (₦)	880.96	876.05	925.10	883.01	908.22	896.08
Revenue (R)						
Average weight (kg/b)	2.43	2.48	2.65	2.51	2.69	2.65
Cost of live chicken (₦/kg)	700.00	700.00	700.00	700.00	700.00	700.00
Revenue per chicken (₦)	1701.00	1736.00	1855.00	1757.00	1883.00	1855.00
Gross margin (R-TVC)	820.04	859.95	929.90	873.99	974.78	958.92
Return/naira invested	1.93	1.98	2.01	1.99	2.08	2.07

Oxytet; oxytetracycline, kg: kilogram, kg/b: kilogram/ bird, TVC: total variable cost, Gross margin: Revenue-TVC, Return/naira invested: Revenue/TVC

This accounts for 18.87% additional gain by using *Ocimum canum* at 800 g/100kg diet.The economic gain on the basis of gross margin showed that birds fed diets containing *O. gratissimum* at 400 g and 800 g/100kg diet had 4.86% and 13.40% economic gain above the control diet containing no additive while birds fed dietary supplement of *O. canum* at 400 g and 800 g/100kg diets had 6.58% and 18.87% economic gain above the birds in the control group. This implies that inclusion of *O. gratissimum* and *O. canum* in broiler diets offer higher economic gain. Similarly, birds fed diet containing *O. canum* at 800 g/100kg diet had an economic gain of 1.65% above those fed diets supplemented with oxytetracycline as AGPs.

The result also showed that broiler chickens fed diets containing oxytetracycline and *Ocimum canum* at 800 g/100kg diet had better economic returns with higher return per naira invested of 2.07 and 2.08, respectively meaning that for every ₦1 invested in broiler chickens production with the inclusion of these feed additives, there was a corresponding return of ₦2.07 and ₦2.08 with a gain of ₦1.07 and ₦1.08 respectively while those fed diets containing *Ocimum gratissimum* at 800 g/100kg diets was 2.01 implying an additional gain of ₦1.01 for every ₦1 invested. Hence, feeding broiler chickens diets supplemented with PFA increases over all economic gain. This corresponds to the report of Akbarian *et al.* (2012) who stated that the use of feed additives accounted for higher economic gain in addition to other numerous purposes in poultry production. In another study on the economic benefit of broiler chickens fed diets containing PFA, higher economic gain was reported (Maidala *et al.*, 2017).

4.4 Experiment 2: Effect Diets containing of Combinations of *Ocimum gratissimum* and *Ocimum canum* as Phytobiotics on the Performance of Broiler Chickens

4.4.1 Growth performance of broiler chicks fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum* leaves (0-4 weeks)

The growth performance of broiler chicks fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum* leaves as phytobiotics is presented in Table 4.15. The results showed that there were significant ($p<0.05$) differences in final weight, weight gain, feed intake, feed conversion ratio (FCR) and feed cost per kg gain across the treatments. Final weight and weight gain were higher ($p<0.05$) in broiler chicks fed diets supplemented with combinations of *O. gratissimum* and *O. canum* at 600:200, 400:400 and 200:600 g/100kg diets respectively.

Feed intake was significantly ($p<0.05$) higher in birds fed diets containing the combinations of *O. gratissimum* and *O. canum*compared to those on the control and AGP diets. Higher feed intake recorded for birds fed diets containing the experimental materials in combination showed that the aromatic nature of the PFAs stimulates appetite, hence, resulting in higher feed intake. FCR and feed cost per kg gain were better ($p<0.05$) in broiler chicks fed diets containing the PFA in the ratio of 400:400 and 200:600 g/100kg and the diet containing AGP than those fed diet containing combinations of *o. gratissimum* and *O. canum* at 600:200 g/100kg diet and the control diet. Feed cost per kg weight were reduced by ₦21.20 (in Oxytet), ₦10.90 (in 400:400 and 200:600 combinations of *Ocimum gratissimum* and *Ocimum canum*) and ₦5.80 (in 600:200 combinations of *Ocimum gratissimum* and *Ocimum canum*) treatment groups respectively. Mortality of the broiler chicks were significantly ($p<0.05$) reduced by the inclusion of *O. gratissimum* and *O. canum*.

Table 4.15: Performance of broiler chicks fed diets supplemented with combinations of *Ocimum gratissimum* and *Ocimum canum* (0-4weeks)

		Mixtures of *O. gratissimum* and *O. canum* (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Initial weight (g/b)	53.64	53.70	53.64	53.33	53.70	0.95
Final weight (g/b)	1269.00^{b}	1445.00^{a}	1432.00^{a}	1415.00^{a}	1467.00^{a}	37.48
Weight gain (g/b)	1215.36^{b}	1391.30^{a}	1378.36^{a}	1361.67^{a}	1413.30^{a}	37.43
Feed intake (g/b)	2068.00^{b}	2187.00^{a}	2110.00^{ab}	2084.00^{ab}	2093.00^{b}	28.13
Feed conversion ratio	1.71^{c}	1.58^{b}	1.53^{a}	1.53^{a}	1.48^{a}	0.04
Feed cost (₦/kg)	118.10	124.10	124.30	124.50	121.60	NA
Feed cost per kg gain (₦/kg)	201.40^{c}	195.60^{bc}	190.50^{b}	190.50^{b}	180.20^{a}	5.12
Mortality (%)	2.47^{b}	0.00^{a}	1.24^{ab}	0.00^{a}	1.24^{ab}	0.96

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), Oxytet: oxytetracycline, SEM: standard error of means, NA: not analyzed.

Feed intake has been reported to increase with inclusion of PFAs which corresponds with the findings of this study (Pandey *et al*., 2015). However, there are several studies that reported decline in total feed intake as a result of feeding diets containing essential oils from PFAs (Jimoh *et al*., 2017, Odetola *et al*., 2017).Better growth performance of broiler chicks fed diets containing combinations of *O. gratissimum* and *O. canum* compared to those fed diet with AGP can be attributed to better gut development and enhanced nutrient assimilation.

Superior performance of birds fed diets containing combinations of *O. gratissimum* and *O. canum* indicates positive synergetic effect between the combined PFAs since they contain different essential oils. Thymol, eugenol and carvacrol are structurally similar, and have been shown to exert synergistic or additive antimicrobial effects when combined at lower concentrations (Bassole and Juliani, 2012). Essential oils used in combination have shown clear growth inhibition of pathogenic bacteria in the hindgut and ameliorated intestinal lesions in birds (Jamroz *et al*., 2006; Jerzsele *et al*., 2012). The findings of this study is in line with the studies of Donoghue (2003) and Apajalahti *et al.* (2004) who reported that feed additives stimulate early gut development and hence, improve the overall efficiency of poultry production.

4.4.2 Growth performance of broiler chickens fed diets containing combinations *Ocimum gratissimum* and *Ocimum canum* leaves (5-7 weeks)

Table 4.16 shows the growth performance of broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum* leaves as phytobiotics at the finisher phase.

Table 4.16: Growth performance of broiler chickens fed diets supplemented with combinations of *Ocimum gratissimum* and *Ocimum canum* (5-7weeks)

		Mixtures of *O. gratissimum* and *O. canum* (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Initial weight (g/b)	1398.00	1455.00	1431.00	1404.00	1462.00	39.15
Final weight (g/b)	2983.00^{b}	3424.00^{a}	3406.00^{a}	3447.00^{a}	3512.00^{a}	88.75
Weight gain (g/b)	1571.00^{b}	1969.00^{a}	1976.00^{a}	2043.00^{a}	2049.00^{a}	79.46
Feed intake (g/b)	3279.00^{b}	3821.00^{a}	3670.00^{a}	3645.00^{a}	3620.00^{a}	125.76
Feed conversion ratio	2.09^{c}	1.94^{b}	1.87^{ab}	1.79^{a}	1.77^{a}	0.06
Feed cost (₦/kg)	105.10	109.60	109.80	110.00	109.10	NA
Feed cost per kg gain (₦/kg)	219.37^{c}	212.80^{bc}	205.10^{ab}	196.70^{a}	192.80^{a}	7.23
Mortality	2.56^{b}	0.00^{a}	0.00^{a}	1.28^{ab}	0.00^{a}	0.81

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), Oxytet: oxytetracycline, SEM: standard error of means, NA: not analyzed.

The results showed that there were significant ($p<0.05$) differences in final weight, weight gain, feed intake, feed conversion ratio (FCR) and feed cost per kg gain (₦/kg). Final weight, weight gain and feed intake were higher ($p<0.05$) for broiler chickens fed diets supplemented with oxytetracycline and combinations of *O. gratissimum* and *O. canum* at 600:200, 400:400 and 200:600 g/100kg diets respectively. FCR and feed cost per kg gain were significantly ($p<0.05$) better in broiler chickens fed diets containing oxytetracycline and combinations of *O. gratissimum* and *O. canum*.Liveability was also improved by inclusion of AGP and combinations of the experimental materials. Feed intake was highest ($p<0.05$) in birds fed diet containing 600:200 g of *O. gratissimum* and *O. canum* (3821.00 g/bird) and least in the birds on control diets (3279.00 g/bird). The higher feed intake by birds on higher levels of *O. gratissimum* to *O. canum* (600:200 g/100kg diet) could be as a result of the appetite stimulating effect of the essential oil of *O. gratissimum*. The result of this study agrees with the findings of Grashorn (2010) and Pandey *et al*. (2015) who stated that phytogenic feed additives stimulates higher feed intake in livestock. Feed cost per kg weight were reduced by ₦26.95 (in Oxytet), ₦22.67 (in 200:600 combinations of *Ocimum gratissimum* and *Ocimum canum*), ₦14.27 (in 400:400 combinations of *Ocimum gratissimum* and *Ocimum canum*) and ₦6.57 (in 600:200 combinations of *Ocimum gratissimum* and *Ocimum canum*) treatment groups respectively; indicating a higher economic return to farmers.

The higher final weight and total weight gain observed in broiler chickens fed diets containing the experimental material and oxytetracycline could be as a result of exclusive elimination of harmful bacterial load in the gut of the birds leading to higher concentration of beneficial bacterial that helped in effective feed digestion, higher nutrient availability and

better nutrient utilization. The findings of this study showed that inclusion of combinations of *O. gratissimum* and *O. canum* in the diets of broiler chickens exerts a positive effect in the growth performance of broiler chickens.

The higher performance of the broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum*at 600:200, 400:400 and 600:200 and those fed diet containing oxytetracycline showed that antibiotics improved feed efficiency and growth rate. This could be attributed to its antimicrobial effect especially in increasing the population of beneficial strains of bacteria. Thymol (chief EO in *O. canum*), eugenol (chief EO in *O. gratissimum*) and carvacrol (found in appreciable amount in both *O. gratissimum* and *O. canum*) have been reported to exert synergistic or additive antimicrobial effects when combined at lower concentrations due to their similarity structurally (Bassole and Juliani, 2012), hence, has higher growth promoting effect in broiler chickens due to the bacteriocidal and bacteriostatic effects of their EO (Jamroz *et al*., 2006; Jerzsele *et al*., 2012). Phytogenic feed additives have been shown to have antimicrobial effect in the gut of livestock (Khan *et al*., 2009; Pandey *et al*., 2015) and also improves growth rate and efficiency of feed utilization (Weerasingha and Atapattu, 2013).

4.4.3 Haematological indices of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Table 4.17 shows the haematological indices of broiler chickens fed diets containing combinations *O. gratissimum* and *O. canum*. Significant ($p<0.05$) differences were observed in all haematological parameters measured except for eosinophils, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume which were not significantly ($p>0.05$) different across the treatments.

Table 4.17: Haematological parameters of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Parameters	Control	Mixtures of *O. gratissimum* and *O. canum* (g/100kg Diet)			Oxytet	SEM	Reference Value
		600:200	400:400	200:600			
Pack Cell Volume (%)	39.33^{ab}	36.47^{ab}	34.13^{b}	41.77^{a}	38.53^{ab}	2.78	24.00-40.00*
Haemoglobin (g/dL)	12.00^{ab}	11.13^{ab}	10.60^{b}	12.73^{a}	12.03^{ab}	0.84	7.00-15.00*
Red Blood Cell ($\times 10^{12}$/L)	2.51^{ab}	2.38^{ab}	2.23^{b}	2.67^{a}	2.54^{ab}	0.20	1.59-4.10*
White Blood Cell ($\times 10^{9}$/L)	8.50^{a}	7.96^{ab}	7.56^{b}	8.70^{a}	8.11^{ab}	0.48	1.90-9.50**
Heterophils (%)	2.70^{a}	2.30^{a}	1.87^{ab}	1.73^{b}	1.53^{b}	0.44	15.00-40.00**
Lymphocytes (%)	91.70^{b}	92.37^{ab}	92.53^{ab}	93.57^{a}	93.67^{a}	0.81	40.00-100.00***
Monocytes (%)	0.13^{b}	0.07^{b}	0.47^{a}	0.13^{b}	0.17^{b}	0.12	1.00-7.00****
Basophils (%)	1.80^{ab}	1.53^{bc}	2.17^{a}	1.03^{d}	1.20^{cd}	0.24	0.10-2.00*
Eosinophils (%)	47.93	46.77	47.73	47.60	47.40	0.99	1.50-6.00**
MCH (pg)	36.17	37.30	32.90	35.33	34.63	4.26	33.00-47.00***
MCV (fl)	157.00	153.40	153.60	156.20	152.70	2.59	90.00-140.00**
MCHC (g/dL)	30.53	30.50	31.07	30.50	31.07	0.29	26.00-85.00***

^{abc}Means with the same superscripts along the rows are not significantly different ($p>0.05$), SEM: standard error of means, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin Concentration, MCV: Mean corpuscular volume*Mitruka and Rawnsely, 1997, **Simrak *et al*., 2004, ***Jain, 1986, ****Jain, 1993.

Most of the parameters measured were significantly ($p>0.05$) higher in broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum* though no specific trends were observed.The observed values of all the parameters measured in this trial across the treatment groups however fell within the normal range reported for healthy chickens (Mitruka and Rawnsley, 1997). This indicates that the diets were suitable and adequate for broiler chickens, and the birds were all healthy and not anaemic. Packed cell volume (PCV), haemoglobin count (Hb), white blood cell (WBC) and red blood cell (RBC) were significantly ($p<0.05$) lower in birds fed diets containing combination of *O. gratissimum* and *O. canum* at 400:400 g/100kg though within reference range. PCV value reported in this study ranges from 34.13-41.77%, Hb count within 10.60-12.73 g/dL, WBC values obtained ranges from 7.56-8.70$\times10^{9}$/L and

RBC ranges from 2.23-2.67$\times10^{12}$/L. PCV, Hb, WBC, RBC and total protein (TP) are important blood parameters used to assess the health status of animals. It has been established that reduction in the levels of nutrients in feeds result in decrease in PCV and Hb of animals (Oladele and Ayo, 1999). PCV is an index of toxicity; for the value to fall within the normal range suggests that the broiler chickens had the ability to tolerate the phytochemicals in the diet.

The aim of estimating the haemoglobin content was to determine the oxygen carrying capacity of the birds' circulatory system (Iheukwumere and Herbert, 2003) since birds with low Hb can be said to have low oxygen carrying capacity and can easily succumb to any form of respiratory disease while birds with high Hb concentration can be regarded as having high oxygen carrying capacity and such birds are likely to withstand some levels of respiratory stress. The blood parameters did not deviate from established ranges and hence the health of the birds were not compromised by inclusion of the experimental materials in combinations.

4.4.4 Liver function indices of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The liver function indices of broiler chickens fed diets containing *O. gratissimum* and *O. canum* is presented in Table 4.18. Significant ($p<0.05$) differences were observed across the dietary treatments for all parameters measured except for total protein which was not different ($p>0.05$). Albumin and globulin levels were highest ($p<0.05$) in birds fed dietary supplement of combination of *O. gratissimum* and *O. canum* at 200:600 g/100kg diet. Albumin to globulin ratios ranges from 0.60-0.94 and most were within the normal range reported for healthy birds by Bounous and Stedman (2000) except for those in the AGP groups.

The serum protein levels reported in this work implies the adequacy of the diets in meeting the nutritional needs of the birds. This is an indication of higher blood clotting ability thereby preventing occurrence of haemorrhage (Moses *et al.*, 2017). The liver enzymes, aspartate-amino transferase (AST), alanine-amino transferase (ALT) and alkaline phosphatase (ALP) were significantly ($p<0.05$) higher in broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum* at a ratio of 600:200, 400:400 and 200:600 g/100kg diet. However, values obtained were all within normal range of 10-106 IU/L for ALP as stated by Ross *et al*. (1976) but AST were below reference range of 100-400 IU/L. High amount of liver enzymes in the blood indicates liver damage (WebMed, 2016) as also Harper *et al*. (1997) and Ewuola and Egbunike (2008) observed in their separate studies that increase in aspartate amino transferase are clinical indication of diagnosing state of damage done to the visceral organs by toxic substances or infections.

Table 4.18: Liver function parameters of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Parameters	Control	Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)			Oxytet	SEM	Reference value
		600:200	400:400	200:600			
Total protein (g/dL)	2.80	3.08	2.81	2.72	3.13	0.22	3.60-5.50*
Albumin (g/dL)	1.16^{b}	1.16^{b}	1.07^{b}	1.57^{a}	1.52^{a}	0.09	1.20-3.20**
Globulin (g/dL)	1.64^{b}	1.92^{ab}	1.71^{ab}	2.03^{a}	1.61^{b}	0.18	1.10-2.20*
Albumin : Globulin	0.71^{ab}	0.60^{b}	0.63^{ab}	0.77^{ab}	0.94^{a}	0.17	0.35-0.75*
Aspartate-Amino Transferase (IU/L)	54.70^{b}	61.03^{ab}	60.57^{ab}	67.93^{a}	53.03^{b}	5.89	100-400**
Alanine-Amino Transferase (IU/L)	11.23^{ab}	11.50^{ab}	9.87^{b}	12.37^{a}	13.67^{a}	1.43	-
Alkaline Phosphatase (IU/L)	86.80^{b}	85.10^{b}	96.03^{a}	89.87^{ab}	85.63^{b}	4.32	10-106***

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline, Reference values: *Ross *et al*. (1976), **LAVC (2009), ***Bounous and Stedman (2000).

Values obtained in this study were within normal range reported for healthy birds by Clinical Diagnostic Division (1990), Bounous and Stedman (2000), LAVC (2009) and Ajuonuma *et al.* (2013) except for AST which were below reference value of 100-400 IU/L (LAVC, 2009). The result of this study therefore indicates that the birds were in healthy condition and were not negatively affected by the experimental diets.

4.4.5 Serum lipid profile of broiler chickens fed diets containing *Ocimum gratissimum* and *Ocimum canum*

The results in Table 4.19 shows the lipid profile of broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum*. There were significant differences ($p<0.05$) in total cholesterol, triglycerides and high density lipoproteins (HDL) across the dietary treatments while low density lipoproteins (LDL) levels were not affected by dietary treatments.

Total cholesterol and triglycerides levels were significantly ($p<0.05$) reduced by the inclusion of combinations of *O. gratissimum* and *O. canum* in the diet of broiler chickens as phytobiotics. HDL and LDL levels were not affected by supplementation of combinations *O. gratissimum* and *O. canum* in the diet of broiler chickens. Collins (2018) stated that the body synthesizes about 1500-2000 mg of new cholesterol each day with an additional intake ranging from 28 – 116mg/dl which is far against the desirable cholesterol level of 200mg (<130 LDL, >90 HDL and <135mg/dl triglyceride). Excess of cholesterol in the bile can crystalize to form gallstones that may block the bile duct leading to the development of atherosclerosis leading to heart attack.

Table 4.19: Lipid profile of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Parameters	Control	Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)			Oxytet	SEM	Reference value
		600:200	400:400	200:600			
Cholesterol (mg/dl)	111.80^{b}	102.40^{bc}	93.54^{c}	100.50^{bc}	130.50^{a}	7.49	129-237
Triglyceride (mg/dl)	81.92^{a}	76.78^{ab}	58.92^{bc}	52.53^{c}	80.90^{a}	9.74	<135.00
High Density Lipoprotein (mg/dl)	174.33^{b}	160.99^{c}	157.27^{c}	165.16^{bc}	187.72^{a}	4.92	>90.00
Low Density Lipoprotein (mg/dl)	21.13	26.08	24.49	24.86	26.58	6.25	<130.00

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), Oxytet-Oxytetracycline**,** SEM: standard error of means, Reference value: Clinical Diagnostic laboratory (1990); Collins (2018).

This reduction in cholesterol levels may also results in reduced occurrence of cardiovascular diseases which is in agreement with the report of Sturkie (2000) who opined that the concentration of cholesterol is influenced by physical and nutritional status of birds and hence concluded that low cholesterol reduces the occurrence of cardiovascular disease (Jessie, 2016). PFA has also been reported to lower cholesterol and triglycerides compared to the control diets (Kirkpinar *et al.,* 2011).

4.4.6 Antioxidant capacity of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The antioxidant capacity of broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum* at varying ratio is presented in Table 4.20. The results showed that significant ($p<0.05$) differences were observed across the dietary treatments in all the parameters measured; superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA). There was significant ($p<0.05$) increase in SOD activity with inclusion of the combinations of *Ocimum gratissimum* and *Ocimum canum* as well as a significant ($p<0.05$) reduction of MDA content at higher levels of each of the combinations of *O. gratissimum* to *O. canum* (600:200 and 200:600 g/100kg diets) respectively indicating that these PFAs when used in combinations possesses potential for improving antioxidant capacity. This confirms earlier reports that the essential oil from *O. gratissimum* and *O. canum* performs antimicrobial and antioxidant role (Rao *et al*., 2011; Satish and Gopu, 2015).

Oxidative stress is a common process in life, and it could produce varieties of reactive oxygen species (ROS) in the body, such as hydroxyl free radicals and superoxide anions. Numerous studies has shown that excess of ROS can damage the proteins, nucleic acids, and

Table 4.20: Antioxidant capacity of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

		Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Malondialdehyde (µmol/mg)	21.40[b]	17.74[c]	23.19[b]	18.85[c]	26.12[a]	1.21
Superoxide dismutase (IU/ml)	15.77[b]	16.43[ab]	16.43[ab]	17.37[a]	15.20[b]	0.80
Catalase (IU/mg)	25.56[b]	38.36[a]	23.58[b]	25.79[b]	23.82[b]	3.39
Glutathione peroxidase (IU/L)	18.13[a]	17.53[a]	18.13[a]	15.57[b]	16.93[a]	1.15

[abc]Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

other biological macromolecules, and produce large amounts of MDA leading to tissue damage, thus contributing to the development of diseases. The MDA content in the body indirectly reflects the lipid peroxidation, which is the consequence of the attenuated antioxidant protection when ROS increase (Yang *et al*., 2008).

Some feed additives has been reported to be beneficial in oxidation resistance, scavenging ROS and promoting antioxidant capability (Capcarova *et al*., 2010 and Wen *et al*., 2011). With regards to antioxidant capacity, the endogenous antioxidant defence system in animals do not only rely on internal but also on the other external sources, such as probiotics, prebiotics, and plant extracts which are the natural sources to prevent the body from oxidative stress induced by the ROS (Rajput *et al*., 2013).

4.4.7 Ileal microbial count of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Table 4.21 shows the microbial count in the ilea of broiler chickens fed diets containing combinations of*Ocimum gratissimum* and *Ocimum canum*at varying ratio. The population of*Lactobacillus spp*. were significantly ($p<0.05$) increased by the inclusion of the combinations of *O. gratissimum* and *O. canum* in the diets of broiler chickens. *Bacillius spp.* were not affected. *Lactobacillus spp.* are commensal bacteria that have long been known for their ability to activate the intestinal immune system and increase resistance to diseases. These bacteria have been reported to produce a wide variety of short chain fatty acids which are bacteriostatic for a subset of bacteria spp. (Gibson and Wang, 1994; Dibner and Buttin, 2002; Ohland and MacNaughton, 2010).

Table 4.21: Ileal bacteria count of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Bacteria ($\times 10^3$ CFU/g)	Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)				
	Control	600:200	400:400	200:600	Oxytet	SEM
Bacilli spp.	6.47	6.33	6.90	5.77	6.43	0.66
Lactobacilli spp.	12.27^{b}	15.17^{ab}	16.17^{a}	14.83^{ab}	14.90^{ab}	0.75
Clostridium spp.	0.20	0.00	0.00	0.00	0.00	0.16
Salmonella spp.	0.06^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.03
Escherichia coli	0.03^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.01
Pseudomonas spp.	0.00	0.07	0.07	0.07	0.00	0.06
Enterobacteriaceae	3.47	3.13	3.77	3.53	3.67	0.72

^{ab}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

Essential oil of oregano oil could improve the intestinal ecosystem of animals via promoting the growth of beneficial bacteria species and suppressing the growth of potential pathogenic bacterial species (Lin *et al*., 2011). Many essential oils stimulate growth of beneficial microbes and limit number of pathogenic bacteria in poultry (Wenk, 2000;Lan *et al*., 2003).

The results showed that addition of combinations of *O. gratissimum* and *O. canum* significantly ($p<0.05$) reduced the concentration of *Salmonella spp.* and *E. coli* compared to the control group. Essential oil of oregano and garlic have also been reported to reduce and inhibit the growth of pathogenic strains of bacteria (Aligiannis *et al.*, 2001, Friedman *et al.*, 2002; Kirkpinar *et al.*, 2011). Reduction in levels of pathogenic bacteria by implication will reduce the risk of digestive diseases by the use of combinations of essential oils of *O. gratissimum* and *O. canum* which is similar to the report by CRINA® Poultry Plus (DSM, 2013).

4.4.8 Caecal microbial count of broiler chickens fed diets containing combinations *Ocimum gratissimum* and *Ocimum canum*

The microbial count in the caeca of broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum*in different proportion is presented in Table 4.22.Significant ($p<0.05$) differences were observed in all the parameters measured except for *Enterobacteriaceae* that was notsignificantly ($p>0.05$) different across the dietary treatments.

Table 4.22: Caecal bacteria count of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

Bacteria (×10^3 CFU/g)	Control	Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet) 600:200	400:400	200:600	Oxytet	SEM
Bacilli spp.	5.33^{b}	6.10^{a}	5.93^{ab}	4.60^{c}	5.30^{b}	0.33
Lactobacilli spp.	8.67^{c}	10.23^{ab}	11.63^{a}	9.33^{bc}	9.57^{bc}	0.76
Clostridium spp.	0.12^{b}	0.07^{ab}	0.00^{a}	0.00^{a}	0.03^{ab}	0.05
Salmonella spp.	0.10^{b}	0.00^{a}	0.10^{b}	0.10^{b}	0.10^{b}	0.01
Escherichia coli	0.40^{b}	0.10^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.07
Pseudomonas spp.	0.13^{b}	0.03^{ab}	0.00^{a}	0.03^{ab}	0.13^{b}	0.06
Enterobacteriaceae	1.77	2.20	3.47	3.90	3.03	1.60

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

The population of *Bacillus* and *Lactobacillus* spp. were significantly ($p<0.05$) increased by the inclusion of combinations of *O. gratissimum* and *O. canum* at 600:200 and 400:400 g/100kg diets respectively. Similarly, pathogenic strains of bacterial species such as *Salmonella spp., Clostridium spp., Pseudomonas spp.* and *E. coli* were significantly ($p<0.05$) reduced or completely eliminated by the addition of the PFA in combinations which may be due to their secondary metabolites that have been reported to have both bacteriostatic and bacteriocidal effect on a wide range of pathogenic microorganisms. This is supported by the studies of Kirkpinar *et al.* (2011) and Moses *et al.* (2017) who reported that essential oil blends inhibits the proliferation of harmful microbes.

The results showed that addition of combinations of *O. gratissimum* and *O. canum* reduced the concentration of *Salmonella spp.* and *E. coli* compared to the control group. Essential oil of oregano and garlic have also been reported to reduce and inhibit the growth of pathogenic strains of bacteria (Aligiannis *et al.*, 2001, Friedman *et al.*, 2002; Kirkpinar *et al.*, 2011). Reduction in levels of pathogenic bacteria by implication will reduce the risk of digestive diseases by the use of combinations of essential oils of *O. gratissimum* and *O. canum* which is in agreement with the report by CRINA® Poultry Plus (DSM, 2013).

This implies that the plant materials were able to reduce the proliferation of harmful bacteria thereby increasing the population of beneficial bacteria.In a similar study by Lin *et al.* (2011), essential oil of oregano oil was reported to promote the growth of beneficial bacteria species and suppressed the growth of potential pathogenic bacterial species. *Lactobacillus spp.* have been reported for their ability to activate the intestinal immune system and increase resistance to diseases. Many essential oils either synthetic or of plant origin

havealso been shown to stimulate the growth of beneficial microbes and limit number of pathogenic bacteria in poultry (Wenk, 2000;Lan *et al*., 2003).

4.4.9 Villi morphometry of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The results of the effect of combinations of *O. gratissimum* and *O. canum* on villi morphometry of cross section of the ileum is presented in Table 4.23. Significant ($p<0.05$) differences were observed in all parameters measured across the dietary treatment. Villi area, villi perimeter, height, width, crypt depth and villi height/crypt depth ratio were higher ($p<0.05$) in birds fed dietary supplement of combinations of *O. gratissimum* and *O. canum* at a ratio of 600:200 g/100kg diet compared to the control and other treatment means. Villi perimeter, height and width were also higher in birds fed diets containing AGP as an additive. The morphology of intestinal villi and crypts is associated with intestinal function and growth in chickens. Higher intestinal villi are associated with increased absorptive surface area of the intestine and thus, an increased absorptive capacity with resultant higher body weight gain (Kanduri *et al.*, 2013). A lower villus/crypt ratio has been associated with the presence of toxins, poor nutrient absorption and increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue (Xu *et al*., 2003). The result obtained in this works showed that the improvement in the gut morphology may be responsible for the higher growth performance in birds fed diets containing combinations of *O. gratissimum* and *O. canum.*

Table 4.23: Villi morphometry of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

		Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Villi Area (µm)^2	14,020.00^{c}	38,999.00^{a}	21,572.00^{b}	22,424.00^{b}	21,484.00^{b}	3619.93
Villi perimeter (µm)	716.60^{c}	1076.00^{a}	892.50^{bc}	882.60^{bc}	916.20^{ab}	91.35
Villi height (µm)	309.20^{c}	465.80^{a}	394.30^{ab}	377.30^{b}	400.70^{ab}	37.35
Villi width (µm)	91.90^{c}	149.40^{a}	84.79^{c}	114.60^{bc}	135.00^{ab}	16.51
Crypt depth (µm)	117.40^{cd}	101.00^{d}	166.50^{a}	139.80^{b}	136.70^{bc}	10.18
Villi height/ crypt depth	2.65^{b}	4.83^{a}	2.38^{b}	2.71^{b}	2.94^{b}	0.36

^{abcd}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

Beneficial bacteria such as *Bacillus subtilis* aid in maintaining optimum villi height and crypt depth supporting the gut's ability to absorb nutrients for animal maintenance and growth (Awad *et al.,* 2009; Lee *et al*., 2010). Presence of longer villi has been reported to increase crypt depth of the mucosa of the small intestine resulting in better feed conversion (Sherief *et al*., 2011). Similarly, Harish (2008) also reported that feed additives and their combinations gives a synergistic effect in promoting gut health, improving nutrient uptake and preventing enteric pathogens.

4.4.10 Carcass characteristics of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The carcass characteristics of broiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum* is presented in Table 4.24. There were significant ($p<0.05$) differences in live weight, dressed weight and organ weights while the cut parts showed no significant differences across the dietary treatments. The results obtained showed that birds fed dietary supplements of combinations of *O. gratissimum* and *O. canum* and those diets containing the AGP had higher ($p<0.05$) live weight and dressed weight compared to those of other dietary treatments. Organ weights were significantly ($p<0.05$) higher in birds fed diets containing combinations of *O. gratissimum* and *O. canum* in all levels of combinations. The enlargement of these organs may be due to their involvement in detoxification of the materials and hence may also confer superior benefits.

Studies have reported that the use of antibiotics as a growth promoter has ability to improve weight gain as well as carcass yield of birds (Yang *et al.,* 2009; Al-Kassie, 2010; Vitor*et al.,* 2010). The result is in agreement with the observation of Khaksar *et al*. (2012) who reported significant improvements in some carcass traits due to inclusion of thyme essential

Table 4.24: Carcass and organs characteristics of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

		Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Live weight (g/b)	2650.00^{c}	2900.00^{ab}	2783.00^{b}	2800.00^{ab}	2917.00^{a}	60.09
Dressed weight (g/b)	1933.00^{b}	2186.00^{a}	2085.00^{a}	2078.00^{a}	2179.00^{a}	56.28
Dressing (%)	72.94	75.38	74.92	74.21	74.70	1.32
Cut parts expressed as percentage of dressed weight (%)						
Breast	25.48	25.75	25.93	25.12	26.56	1.40
Back	14.31	14.82	15.13	14.73	14.46	0.55
Thigh	10.83	11.23	11.43	10.69	11.13	0.38
Drumstick	9.20	9.61	9.43	9.18	9.08	0.45
Organ weights expressed as percentage of live weight (%)						
Gizzard	2.33^{a}	1.99^{b}	2.10^{ab}	2.11^{ab}	2.26^{ab}	0.17
Heart	0.57^{ab}	0.72^{a}	0.53^{ab}	0.54^{ab}	0.42^{b}	0.08
Lungs	0.42^{c}	0.49^{bc}	0.67^{a}	0.56^{ab}	0.63^{a}	0.07
Liver	2.05^{ab}	2.26^{a}	2.40^{a}	2.39^{a}	1.89^{b}	0.18
Kidney	0.63^{b}	0.60^{b}	0.74^{a}	0.65^{b}	0.64^{a}	0.04
Abdominal Fat	1.32^{b}	0.45^{a}	0.70^{ab}	0.97^{ab}	0.97^{ab}	0.38

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline, g/b: gram per bird.

oil. However, Schulze *et al*. (2008) did not observe a significant effect in the broiler carcass yield due to the dietary inclusion of essential oil blend based on thyme and cinnamon. Also Muhl and Liebert (2007) did not find differences in the carcass yield of broilers fed plant extracts and combinations of plant extracts. Abdominal fat were significantly ($p<0.05$) loweredinbroiler chickens fed diets containing combinations of *O. gratissimum* and *O. canum*at different proportions and oxytetracycline, while the control had the highest level of abdominal fat. Numerically, the higher the level of *Ocimum gratissimum* in the combination, the lower the level of abdominal fat. Reduction in the levels of abdominal fat with inclusion of these leaves meals implies that they have the ability to reduce the consumption of excess fats in broiler chicken meat. The findings of this study agrees with that of Myandoab and HosseiniMansoub (2012) who reported that licorice extract lowered abdominal fat levels in Japanese quails.

4.4.11 Sensory attributes of meat from broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The results of sensory analysis for the meat of broiler chickens fed dietary supplement of *Ocimum gratissimum* and *Ocimum canum* in combinations is presented in Table 4.25. The result obtained showed that there were significant ($p< 0.05$) differences for all the sensory parameters evaluated. Meat of the broiler chickens in the treatment groups supplemented with combinations of *O. gratissimum* and *O. canum* had higher eating and sensory attributes compared to that of both positive and negative control groups. The organoleptic evaluation of food products to any food processing technology is very important in determining the consumer acceptability since the consumers are the final authority in any product though the last point in the value chain. Sensory parameters had higher organoleptic attributes for the birds in the treatment groups fed diets supplemented with higher levels of *O. gratissimum* to *O. canum* than the control and AGP

Table 4.25: Sensory evaluation of meat of broiler chickens fed diet containing combinations of *Ocimum gratissimum* and *Ocimum canum*

		Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)				
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**	**SEM**
Colour	3.22^{b}	4.67^{a}	3.94^{ab}	4.06^{ab}	3.94^{ab}	0.51
Texture	3.22^{b}	4.78^{a}	4.17^{ab}	4.11^{ab}	3.61^{b}	0.50
Flavour	3.56^{c}	4.44^{ab}	4.61^{a}	4.06^{ab}	3.67^{bc}	0.42
Tenderness	3.94^{ab}	4.56^{a}	4.17^{ab}	4.28^{ab}	3.50^{b}	0.44
Juiceness	3.22^{b}	4.17^{ab}	4.44^{a}	3.83^{ab}	3.11^{b}	0.58
Acceptability	3.72^{b}	4.56^{a}	4.06^{ab}	4.17^{ab}	3.61^{b}	0.34

^{abc}Means with the same superscripts along the rows are not significantly different (p>0.05), SEM: standard error of means, Oxytet-Oxytetracycline

treatment groups implying that the higher the level of *O. gratissimum* in the diet of broiler chickens, the better the eating quality.

This implies that addition of these phytogenic feed materials improved the eating quality of broiler chicken meat which may be due to higher aromatic compound content and essential oil in these botanical which makes them to maintain desirable culinary properties. Okubanjo *et al*. (2010) in their study, also reported higher culinary properties for spent layers chicken meat tenderized with Calcium chloride as additive. This observation in the present study is in agreement with literature that essential oils of basils have been used extensively for many years in flavouring of confectionaries, baked foods, condiments, sausages and meat (Panuwat *et al.*, 2003). It also agrees with the findings ofOyadeyi *et al.* (2014) and Olusola (2018) who reported that *O. gratissimum* extracts increased organoleptic properties of meat floss (*Dambu nama*).

4.4.12 Economic analysis of the production of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*

The economic analysis of dietary inclusion of combinations of *O. gratissimum* and *O. canum* in the diets of broiler chickens is presented in Table 4.26. The result showed that broiler chickens fed diets containing combinations of these PFAs and AGPs had higher economic gain with gross margin of ₦1270.78 (600:200 g/100kg diets of *O. gratissimum* and *O. canum*), ₦1283.13 (400:400), ₦1316.95 (200:600) and 1373.12 (AGPs) while birds fed control diet had a gross margin of ₦1063.41. The gross margin indicates that as levels of *O. canum* increases in the combinations, marginal gains also increased. Economic gain from birds fed diets containing AGP above the standard control group was ₦309.71 (29.12% above what was obtained for the control group).

Table 4.26: Economic analysis of production of broiler chickens fed diets containing combinations of *Ocimum gratissimum* and *Ocimum canum*(0-7weeks)

		Mixtures of *O. gratissimum* and *O. canum* Inclusion (g/100kg Diet)			
Parameters	**Control**	**600:200**	**400:400**	**200:600**	**Oxytet**
Variable costs					
Day-old chicks (₦)	250.00	250.00	250.00	250.00	250.00
Starter feed (₦/kg)	118.10	124.10	124.30	124.45	121.60
Starter feed intake (kg)	2.07	2.19	2.11	2.08	2.09
Feeding cost starter (₦)	244.23	271.41	262.27	259.35	254.51
Finisher feed (₦/kg)	105.10	109.60	109.80	109.95	109.10
Feed intake (kg/b)	3.28	3.82	3.67	3.65	3.62
Finisher feeding cost (₦)	344.62	418.78	402.97	400.77	394.94
Total feeding cost (₦/kg)	588.85	690.19	665.24	660.12	649.45
Water (₦)	12.37	12.37	12.37	12.37	12.37
Wood shaving (₦)	5.68	5.68	5.68	5.68	5.68
Labour (₦)	60.60	60.60	60.60	60.60	60.60
Brooding (₦)	52.15	52.15	52.15	52.15	52.15
Vaccination (₦)	9.59	9.59	9.59	9.59	9.59
Repair (₦)	25.25	25.25	25.25	25.25	25.25
Transportation (₦)	20.19	20.19	20.19	20.19	20.19
Total variable cost (TVC) (₦)	1024.69	1126.02	1101.07	1095.95	1085.28
Revenue					
Average weight (kg/b)	2.98	3.42	3.41	3.45	3.51
Cost of live chicken (₦/kg)	700.00	700.00	700.00	700.00	700.00
Revenue per chicken (₦)	2088.10	2396.80	2384.20	2412.90	2458.40
Gross margin (₦)	1063.41	1270.78	1283.13	1316.95	1373.12
Return/naira invested	2.04	2.13	2.17	2.20	2.27

Feeding cost= cost of feed (₦/kg)×Feed intake (kg), oxytet: oxytetracycline, Gross margin: Revenue-TVC, Return/naira invested: Revenue/TVC

Benefits from birds fed combinations of *O. gratissimum* and *O. canum* were ₦207.37 (19.50%), ₦219.72 (20.66%) and ₦253.54 (23.84%) for 600:200, 400:400 and 200:600 combinations respectively above what was obtained for the control group.

The results also showed that broiler chickens fed diets containing oxytetracycline and combinations of *O. gratissimum* and *O. canum* had higher returns per naira invested compared to the control group. Higher return per naira invested of 2.27, 2.20, 2.17 and 2.13 were obtained for AGP groups, 200:600, 400:400 and 600:200 groups respectively meaning that for every ₦1 invested in broiler chickens production with the inclusion of combinations of these PFAs, there is a corresponding return of ₦2.27 with a gain of ₦1.27 (AGP), ₦2.20 with a gain of ₦1.20 (200:600), ₦2.17 with a gain of ₦1.17 (400:400) and ₦2.13 with a gain of ₦1.13 (600:200) which were better than the control group with a return per naira invested of ₦2.04 meaning a gain of ₦1.04 for every ₦1 invested. The findings of the study is in agreement with the observationsof earlier studies that feed additives increases economic gain of broiler production (Akbarian *et al.*, 2012; Maidala *et al.*, 2017).The use of PFAs do not only impart positive influence on growth performance and gut morphology but also have economic benefit in poultry production.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Two feeding trials were conducted to evaluate the comparative advantage of *Ocimum gratissimum* and *Ocimum canum* singly and in combinations as alternatives to antibiotic growth promoters (AGPs) in broiler chickens. In the first feeding trial, a total of 396 day-old Cobb 500 broiler chicks were allotted randomly to six dietary treatments each replicated thrice, with 22 chicks per replicate in a completely randomized design. Experimental materials were included at 0g, *Ocimum gratissimum* and *Ocimum canum* at 400 g and 800 g respectively (T1-T5) while oxytetracycline was used as positive control (T6). In the second feeding trial, a total of 405 day-old Cobb 500 broiler chicks were allotted randomly to five dietary treatments each replicated thrice, with 27 chicks per replicate. Mixture of *Ocimum gratissimum* and *Ocimum canum* were included at 0g, 75:25, 50:50 and 25:75 (T1-T4) while oxytetracycline was used as positive control (T5). Data were collected on growth parameters, carcass characteristics, haematological parameters, liver function indices, lipid profile, antioxidant capacity, villi morphometric, gut microbial contents and organoleptic properties. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using Tukey procedures.

In the first feeding trial, the growth performance result showed that final weight, weight gain, feed conversion ratio and feed cost per kg gain were significantly higher ($p<0.05$) for birds fed diets containing *O. gratissimum* and *O. canum* at 800 g per 100kg diets at both the starter and finisher phases. Haematological parameters, liver function parameters and lipid profile were significantly improved and were within normal range reported for healthy

chickens. Antioxidant capacity was significantly improved by supplementation of *O. gratissimum* and *O. canum*; with an increase ($p<0.05$) in SOD activity (17.94-26.91µ/ml as against control with 15.45 µ/ml and 14.43 µ/ml for AGP and reduction in MDA content. The population of *Bacillus* and *Lactobacillus* spp. were increased while the concentration of *Escherichia coli*, *Clostridium spp., Salmonella spp., Enterobacteriaeae* and *Pseudomonas spp* were significantly ($p<0.05$) reduced by the inclusion of *Ocimum spp.* Villi area, villi perimeter, height, width, crypt depth and villi height/crypt depth ratio were higher ($p<0.05$) for birds fed dietary supplement of 400 g of *O. gratissimum*,400 g and 800 g of *O. canum* and AGP (Oxytetracycline) than the control. Carcass characteristics of cut parts showed that birds on 400 g of *O. gratissimum*, 800 g of *O. canum* and AGPs had higher ($p<0.05$) drumstick and breast cut percentages. Organ weights were significantly higher ($p<0.05$) for birds fed diets containing *Ocimum spp.* at 800 g/100kg diets. Abdominal fat were lower for birds fed diets containing *O. gratissimum* and *O. canum* at 800 g/100kg diets. The result of sensory analysis showed that meat of the broiler chickens in the treatment groups supplemented with *O. gratissimum* and *O. canum* had higher eating and sensory attributes.

In the second feeding trial, the growth performance result showed that final weight, weight gain, feed conversion ratio and feed cost per kg gain were significantly higher ($p<0.05$) for birds fed diets containing combinations of *O. gratissimum* and *O. canum* at 600:200, 400:400 and 200:600 g per 100kg diets at both the starter and finisher phases. Haematological parameters, liver function parameters, lipid profile and antioxidant activities were significantly improved. Ilea and caecal microbial population of *Bacillus* and *Lactobacillus* spp. were increased while the concentration of pathogenic bacteria were reduced by the inclusion of *Ocimum spp.* Villi area, villi perimeter, height, width, crypt

depth and villi height/crypt depth ratio were higher ($p<0.05$) for birds fed dietary combinations of *O. gratissimum* and *O. canum* and AGP (Oxyteracycline) than the control. Cut parts were not different but organs weights were increased while abdominal fat concentrations were reduces for birds fed combinations of the PFAs ($p<0.05$). Organoleptic and eating quality of the meat of broiler chickens were ranked higher for birds fed higher levels of *O. gratissimum* followed by *O. canum* above the control groups (positive and negative).

5.2 Conclusions

From the results obtained, the following conclusions are made:

Ocimum gratissimum and *Ocimum canum* inclusion in broiler chickens diets at 800 g/100kg diets, respectively significantly improved growth performance, lipid profile, antioxidant activities, gut microbial count, villi morphometric, carcass characteristics, organoleptic properties, economic benefits with no adverse effect on haematological parameters and liver function indices.

Combinations of *Ocimum gratissimum* and *Ocimum canum* at 600:200, 400:400 and 200:600 g/100kg, respectively in broiler chickens diets significantly improved growth performance, antioxidant activities, gut microbial count, villi morphometric, carcass characteristics, organoleptic properties, economic benefits with no detrimental effect on haematological parameters, liver function indices and lipid profile.

5.3 Recommendations

Poultry farmers and feed millers can use:

1. *Ocimum gratissimum* and *Ocimum canum* at 800 g/100kg diets as an alternative to synthetic antibiotic growth promoters for improved growth performance, better eating quality of broiler meat and higher economic returns.
2. Combinations of *Ocimum gratissimum* and *Ocimum canum* at 600:200 g/100kg diets as an alternative to synthetic antibiotic growth promoters at the starter phase for better growth performance and at the finisher phase for better eating quality of broiler meat.
3. Combinations of *Ocimum gratissimum* and *Ocimum canum* at 200:600 g/100kg diets as an alternative to synthetic antibiotic growth promoters at the finisher phase for better growth performance, better eating quality of broiler meat and higher economic returns.
4. Further research should be conducted using *Ocimum gratissimum* and *Ocimum canum* on other poultry species; and also their combinations with other phytogenic feed additives.

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APPENDICES

Appendix 1: Identification of phytochemicals in experimental materials

Constituents	Experimental materials		Tests
	Ocimum gratissimum	*Ocimum canum*	
Carbohydrates	+	+	Molisch
Anthraquinones	-	-	Bontragers
Glycosides	+	+	Fehling
Cardiac glycosides	+	+	Kene Killiami
Saponins	+	+	Fehling
Tannins	+	+	Feric chloride
Flavonoids	+	+	Shiroda
Alkaloids	+	+	Dragendorff
Steroids	+	+	Lieberman Burchard
Triterpenes	+	+	Lieberman Burchard

+: present, -: absent

Appendix 2: Questionnaire for experiment 1

DEPARTMENT OF ANIMAL SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
SENSORY EVALUATION SCORE SHEET

SECTION A: Demography of the Assessor Date: …….....
Instruction: Please tick the most appropriate

1. Sex: male ( ) Female ( )

2. Average Age; 15-25( ) 26-40( ) 41 and above ( )

3. Marital Status; Single ( ) Married ( )

4. Occupation; Civil Servant ( ) Students ( ) Others ( ) Specify: ……

5. Level of Education: Non formal ( ) Primary ( ) Secondary ( ) Tertiary ( )

6. Which meat type do you prefer? Beef ( ) Mutton ( ) Chevon ( ) Rabbit ( ) Chicken ( ) Snail ( ) Fish ( ) Others: specify please: ……

7. Are you allergic to chicken meat products? Yes ( )No ( )

SECTION B: Consumers' Perception of the Products

Instruction: Please choose your level of preference for each trait of the given samples by choosing the terms that best reflect your rating of the products displayed before you, assess the sample by writing the appropriate number under the product code. Ensure you rinse your mouth after each tasting.

5-point Hedonic scale

5-like very much
4-like
3-neither like nor dislike
2-dislike
1-dislike very much

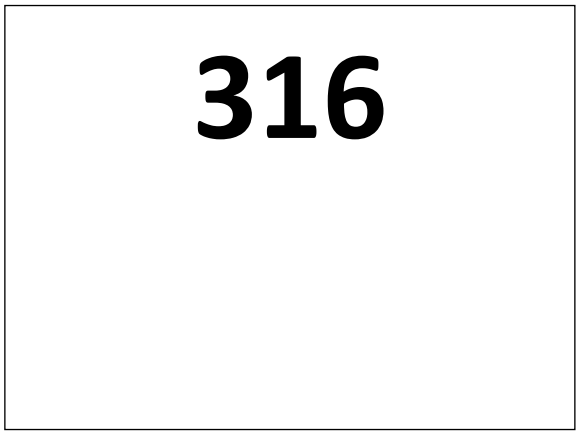
	Samples					
Parameters	**563**	**251**	**146**	**431**	**316**	**612**
Colour						
Texture						
Flavour						
Tenderness						
Juiciness						
Acceptability						

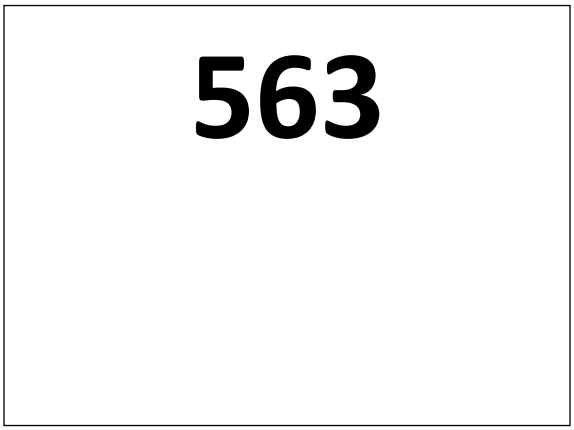
Additional comment (If any)
……...
……...

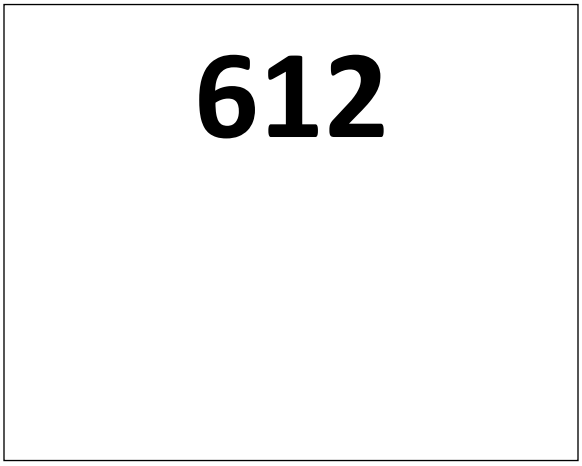
THANK YOU FOR YOUR TIME!

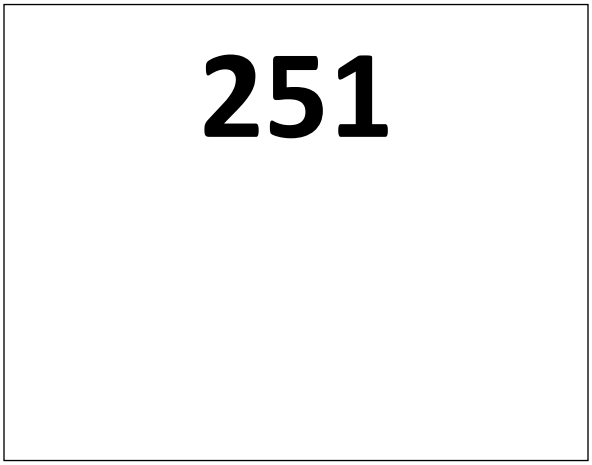
Appendix 3: Product code for experiment 1

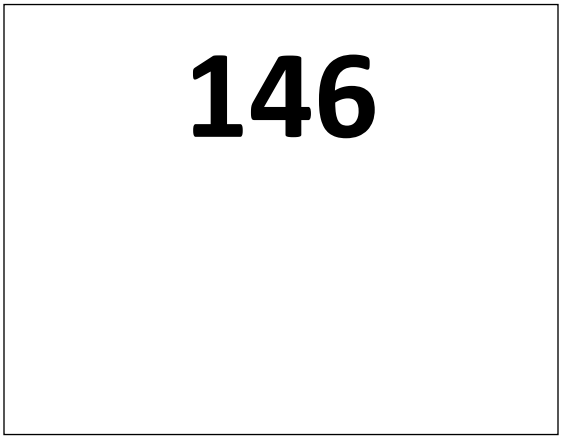
PRODUCTS FOR ASSESSMENT

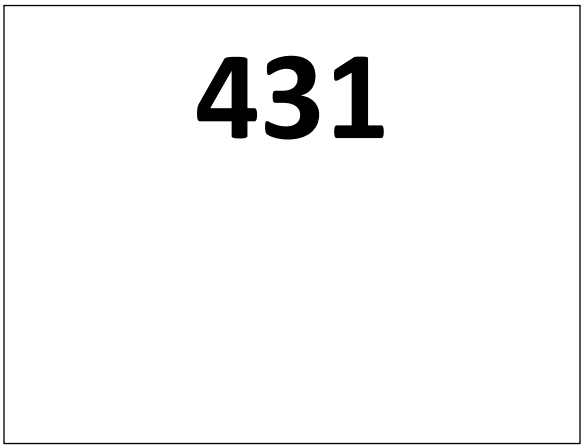












Appendix 4: Questionnaire for experiment 2

DEPARTMENT OF ANIMAL SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
SENSORY EVALUATION SCORE SHEET

SECTION A: Demography of the Assessor Date: …….....
Instruction: Please tick the most appropriate

1. Sex: male ( ) Female ( )

2. Average Age; 15-25( ) 26-40( ) 41 and above ( )

3. Marital Status; Single ( ) Married ( )

4. Occupation; Civil Servant ( ) Students ( ) Others ( ) Specify: ……

5. Level of Education: Non formal ( ) Primary ( ) Secondary ( ) Tertiary ( )

6. Which meat type do you prefer? Beef ( ) Mutton ( ) Chevon ( ) Rabbit ( ) Chicken ( ) Snail ( ) Fish ( ) Others: specify please: ……

7. Are you allergic to chicken meat products? Yes ( )No ( )

SECTION B: Consumers' Perception of the Products

Instruction: Please choose your level of preference for each trait of the given samples by choosing the terms that best reflect your rating of the products displayed before you, assess the sample by writing the appropriate number under the product code. Ensure you rinse your mouth after each tasting.

5-point Hedonic scale

5-like very much
4-like
3-neither like nor dislike
2-dislike
1-dislike very much

	Samples				
Parameters	**563**	**251**	**146**	**431**	**316**
Colour					
Texture					
Flavour					
Tenderness					
Juiciness					
Acceptability					

Additional comment (If any)
……...
……...

THANK YOU FOR YOUR TIME!

Appendix 5: Product code for experiment 2

PRODUCTS FOR ASSESSMENT

