USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

ASSESSMENT OF GROWTH AND MEAT QUALITY OF SPENT LAYERS FED DIET SUPPLEMENTED WITH BASIL, MINT, AND PAWPAW LEAVES POWDER IN SOKOTO, NIGERIA

A Dissertation
Submitted to the
Postgraduate School,

USMANU DANFODIYO UNIVERSITY, SOKOTO NIGERIA

In Partial Fulfillments of the Requirements
For the Award of the Degree of
MASTERS OF SCIENCE (ANIMAL SCIENCE)

BY

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NOVEMBER, 2018

DEDICATION

This research is dedicated to my parents; Buba Bislava and Aisha Jutha for all their support, prayers and encouragement.

CERTIFICATION

This dissertation by **BUBA, Mohammed Bislava(Adm. No. 15210603001**) has met the requirements for the award of the Degree of Master of Science (Animal Science) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

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ACKNOWLEDGEMENTS

I am most grateful to Almighty Allah for His guidance and protection throughout my life. May peace and blessing of Allah (SWT) be upon His Prophet Muhammad (SAW). Alhamdullilah!

It is with deep sorrow I acknowledge the efforts of my former supervisorslate Dr. A. L. Yusuf and late Prof. Y. A. Bashar who not only initiated and designed the plan of this research, but also for their parental manner and encouragement, through correcting the script and offering useful suggestions at every stage in the course of this research. May Almighty Allah grant their souls eternal rest. Ameen.

It is with a great pleasure I acknowledge my Major supervisor Prof. M. Jibir, who also double as Head of Department, Animal Science, for his fatherly role in realising success of this research. My appreciation also goes to my Co-supervisor I, Dr. Y. Na-Allah, who also double as Departmental PG coordinator and Co-supervisor II, Prof. S. B. Manga, for their advices and words of encouragement which contributed immensely to achieving success in this research. My appreciation also goes to the Dean Faculty of Agriculture, Prof. B. Z. Abubakar, all the staff in the Department of Animal Scienceand the Faculty of Agriculture in general for their support in one or the other.

My special appreciation goes to the Manager, Labana FarmsLimited, Dr. Aliyu Sa'adu who helpedsourcing my experimental stock. My gratitude will be incomplete without mentioning the entire laboratory staff of the Departments of Animal Science, Biochemistry,Biological Science, Microbiology and Soil Science for their assistance, guide and support during my laboratory analyses. I am sincerely grateful for the gesture.

My special gratitude and appreciation also go to my mentors to be precise Dr. M.A. Ghuluze, Prof. M.B. Sastawa, Prof. M.B. Ardo, Prof. J.U. Igwebuike, Prof. I.D.

Mohammed, Dr. S.B. Shamaki, Dr. N. Muhammad and Engr. M.A. Husain for their advices, encouragement and motivation towards the completion of this research.

I will not forget the encouragements, prayers and supports I received from my brothers, friends and well-wishers particularly Abba Ibrahim Bukar (Grandfather), Ba-Tujja, Mohammed Kala, Suleiman Buba, Auwwal M. Yusuf, Suleiman Abubakar, Comrade Adam 3040, Mallam Usman Kolo and Mallam Yusuf Tilde to mentioned but few.

My immeasurable appreciation goes to my parent, siblings and relatives, who have always been luminary icons to my life, for supporting me financially, physically and whose prayers and encouragements culminated in to my academic success.

Finally, since no human venture can succeed without the active support of others, the accomplishment of this research has become possible with the advices, assistance and helpof number of people whom I cannot thank individually as I should like. My sincerely gratitude to goes you all.

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ABSTRACT

The objectives of this study were to assess the growth and meat quality of spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations. This study was conducted in three phases: The first phase was evaluation of phytochemical composition of the test ingredients and proximate composition of the experiment diets. The second phase was a feeding trial with 210 spent layer birds at the age of 115 weeks which lasted for 21 days. The third phase was evaluation of performance characteristics, meat quality and lipid profile of spent layer birds on the test ingredients. The birds were randomly assigned to 7-dietary treatments in completely randomised design (CRD) experiment. The result of bioactive compoundswere found in varying quantity. Saponins, phenols and phenols, tannins, terpenoids were not detected in BLP, MLP and PLP respectively. Results revealed average feed intake and average daily gain per bird per day differed significantly among treatments. Yield of carcass and primal parts showed difference ($p<0.05$) among treatments. Lipid components showed difference ($p<0.05$) among treatments except HDL. Physico-chemical properties, bacteria load, revealed difference ($p<0.05$) among treatments. Sensory evaluation showed significant difference in aroma and tenderness among treatments. The finding of this studyshowed that basil, mint and pawpaw leavesand their combinations are economically cheaper, viable and health wise safer than synthetic chemicals and antibiotics. However inclusion of Basil leaves at 2 % level is the best supplementation level to improve meat quality. Therefore the study recommended use of Basil leaves at 2 % inclusionin the diet of finishing spent layers should be taken to cognisance.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Poultry products (egg and meat) are very popular food commodities around the world and their consumption has increased over the last few decades in many parts of the world (Sharma *et al*., 2015).Poultry eggs have been identified as rich dietary source of cholesterol,which many people have been watchful of in their diets(Adeniyi *et al*., 2016). Excessive expansion of egg production has resulted in the abundance of spent layers (Chueachuaychoo *et al*., 2011b). Globally, there are about 2.6 billion spent layers that are used mainly in the pet food industry, and not much for direct human consumption, because the reduced quality of the meat; usually very tough and chewy, non-juicy and low in fat(Chueachuaychoo *et al*., 2011a; Navid*et al*., 2011). Due to theunacceptable toughness, which is one of the most important determinant of eating quality and acceptability of meat by consumers (Miller *et al.*, 2001), the use of spent layer for meat has long been a problem for the poultry industry (Abdalla *et al*., 2013).

Generally, fatty acids in meat contribute to the quality attributes of the meat by improving its palatability through enhancing tenderness, colour stability, juiciness, aroma, flavour and shelf life(Rhee, 2007; Luciano *et al*., 2009). Poultry meat is very high in polyunsaturated fatty acids (PUFA), which have been one of the most unstable fatty acids that are highly susceptible to lipid oxidation (Cai *et al.*, 2012). During lipid oxidation, fats are broken down causing flavour deterioration, and resulting in the development of off-flavour that are undesirable to consumers (Gray *et al.*, 1996). The high protein and moisture content of meat provides suitable media for the proliferation of microbes during processing and storage.

Lipid oxidation and microbial proliferation are the major causes of quality deterioration in meat and meat products in fresh, cold or frozen conditions(Mario and Cava, 2004; Akarpat*et al.*, 2008). These are some of the factors that lead to concerns with the consumption of spent layers' meat (Kumar *et al*., 2015).Biswas *et al*. (2012) reported that synthetic antioxidants like butylated hydroxytuluene (BHT) and butylated hydroxyanisole (BHA) have successfully been used to delay lipid oxidation in meat but recent reportshave indicated that, consumption of these synthetic chemicals have negative health consequences and these have compelled researches on their alternatives, particularly from natural sources. With the recent increase in preference for complete or partially naturally certified foods including egg, meat and their products, the livestock industries are demanding antioxidants, antimicrobials and tenderisers from natural sources to replace synthetic ones because of the negative health consequences or other beliefs regarding these synthetic agents.It is therefore necessary to assess the potentials of some natural sources of antioxidants, antimicrobials and tenderisers on spent layer meat to improved their quality and acceptability of their meat.

1.2 Problem Statement

Spent layers have disadvantage of having tough and chewy meat. The toughness of spent layers meat is primarily due to the increased actin-myosin cross bridge linking in the muscles of older animals (Archile-Contreras *et al*., 2011).Navid*et al*. (2011)reported that spent layers used for meat purpose are been discarded by quality conscious consumers due to inherent quality differences especially tenderness of the meat. Furthermore, during slaughter, processing, storage and market display, the quality attributes of spent layer meat

also deteriorate faster due to lipid oxidation and microbial proliferation as a result of high PUFA,proteinand moisture contents of the meat.

Lipid oxidation is responsible for reduction in the nutritional quality as well as changes in the flavour, colour and other sensory attributes of meats (Aguirrezabal *et al*., 2000).Microbial contamination cause a major consumers health hazards and economic loss to the meat retailers in terms of food poisoning and meat spoilage.As a result, a significant portion of meat and meat products are spoiled every year. Kantor *et al*. (1997) reported that approximately 3.5 million tons of poultry and other meats were wasted annually at the consumer, retailer and foodservice levels, which have substantial economic and environmental impacts. According to Cerveny *et al*. (2009) if only half (50%) of the meat lossesis preserved,it could satisfy the daily needs of approximately 3.2 million people for poultrymeat.

The synthetic antioxidants and antibiotics used in animal productionto improve meat quality have residual effects in tissues and organs of animals long after withdrawal, causing toxicity and microbial resistance allergy in the consumers (Rolfe, 2000). The hazardous effects of these synthetic chemicals justified the ban on their use in animal feed in 2006by the European Union (EU) (Chiquette, 2009). Attempts to improve meat quality by improving tenderness through post-slaughter processes are not only costlyand labour intensive, but also require large storage area and longer storage time. They are, therefore, impractical and not economically viable (Abdulla *et al*., 2013). These, therefore, have created the need to look for better alternatives that enhance the quality of spent layer meat without having negative impacts on the consumers.

1.3 Justification of the Study

Numerous studies have been conducted to enhance the quality and shelf life of meat and meat products by creating unfavourable environment for the proliferation of spoilage organisms using antibiotics (Moon *et al*., 2011; Muhammad *et al.,* 2011), by delaying onset of lipid oxidation using synthetic antioxidants; such as BHT and BHA (Biswas *et al*., 2012) and enhancing tenderness using chlorides and phosphates (Sachdev and Verma, 1990). Despite all the benefits derived from using these synthetic chemicals, their use is associated with a lot of risks with regards to human health (Jan, 2007).The use of these synthetic agents, apart from reducing cholesterol levels in blood and egg of chickens, delaying lipid oxidation, microbial inhibition and enhancing tenderness, they may also affect other quality attributes of egg, meat and their products negatively, which ultimately affect consumer acceptability of the products (McCarthy *et al*., 2001).

According to Bibitha *et al*.(2002)and Viuda-Martos *et al*.(2010), the use of natural preservatives to improve the quality and shelf-life of egg, meat and their products is a promising technology. Many herbs, plants, fruits and vegetable extracts or powders have been found to have antimicrobial, antioxidant and tenderising properties.Among the tested natural feed substances in poultry nutrition were leaf meal ofPawpaw (*Carica papaya*),Basil (*Ocimum basilicum*), Spearmint (*Mentha spicata*)etc. (Esonu *et al*., 2003; Odunsi, 2003; Ekenyem and Madubuike, 2006; Akande *et al*., 2007).

Dietary supplementation of natural plant substances that have antimicrobial, antioxidant and tenderising effects in animal feeds provide means of direct deposition of theirbioactive compounds into the egg, meatand their products, which could give a permanent quality to theseproducts without any further exogenous addition. Efficient utilisation of these plants in poultry production may transform egg and meat industries by standardising appropriate and

economic technology for processing the huge underutilised spent layer into highly valued meat products that are palatable and economically viable (Jin *et al*., 2007). The present study, set to assess the growth and meat quality of spent layers fed diets supplemented with basil, mint and pawpaw leaves powder in Sokoto, Nigeria, sound justifiable. Findings from this study will add to the existing information for future research on the use of basil, mint and pawpaw leaves and their mixture as natural antimicrobial, antioxidant and tenderising agents in diets of spent layer for improving the egg, meat and their products qualities elsewhere.This will enable farmers sell them at attractive price and earn more income.

1.4 Objectives of the Study

The broad objective of this study is to assess the growth and meat quality of spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.

The specific objectives include to;

i. Assess the growth performanceof spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.
ii. Assess the carcass and meat yield characteristicsof spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.
iii. Evaluate the lipid profile ofspent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.
iv. Evaluate the physico-chemical properties of musclesand meat from spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.
v. Evaluate the sensory propertiesof musclesand meat from spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.
vi. Assess microbial counts onmusclesand meat from spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.

vii. Identify the bacterial species on musclesand meat from spent layers fed diets supplemented with basil, mint and pawpaw leaves and their combinations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Production and Utilisation

Poultry productionis a cheap source of animal protein,and has taken a great change in the last three decades; from a near backyard practice to a venture of industrial promotion. Consumption of egg and poultry meat is increasingworldwide, due to high demand, as a result of increase in population. Eggs and poultry meat are source of complete protein, which man needs in his diet to enable the body make new cells and repair damaged ones, hence it is regarded as part of a healthy diet(Sanda, 2015).According to Sefcik (2010), dietary protein is vital during certain stages of growth, hence protein in egg is a unique type of protein.The level of animal protein in the food is a primary indicator of the quality of life and standard of living (Ogundipe, 1996).

Poultry meat is a highly valued protein food with a relatively low cost, and this has led to its increased consumption worldwide (del Puerto *et al*., 2016). Meat encloses small amount of carbohydrates and a high amount of purine, creatine, creatinine and minerals like phosphorus and iron. Meat is rich in soluble vitamins-B complex and contains approximately 20% proteins(Das, 2013).Thus, poultry meat is a valuable alternative to red meat, especially in regions where under-nutrition or low-income earners are increasing (FAO, 2004). These have led to an increase in poultry production worldwide which led to the development of new “ready to eat” poultry products with effects for meat quality, an important economic factor in the poultry industry (Barbut *et al*., 2008). This quality attributes affect marketing and conservation while nutritional quality influences the consumer decisions (Andersen *et al*., 2005; Cabrera *et al*., 2007).

Nevertheless, according to Ugwu and Onyimonyi (2008), spent layers have remained a good source of meat for most Nigerians and sale of the spent layers at the end of the laying cycle is one important source of income to the poultry farmer. However, meat value is very essential in maintaining consumer health. The factors that affect meat quality are enormous and complex, and they occur from farm to processing. In reality, these factors may present themselves as oxidative stress or as oxidative rancidity and microbial spoilage (Bamidele, 2015) and proper meat handling.

2.2 Impactof Plant Products in Meat Production

The importance of plant products in meat production can never be overemphasis due to its vast contribution to the meat industry. The utilisation of local, cheap and readily available plant feed materials,especially those that are not readily utilised by man, has received particular attention as one of the viable alternative to the use of synthetic products (Nwakpu *et al*., 2000; Odunsi, 2003; Ekenyem, 2006). This is especially nowadays that the price of the conventional protein sources has turned so high in recent times, due to stiff competition between the Nigerian feed industry and man for the utilisation of conventional ingredients such as groundnut, soybean, fish meal etc. Moreover, people are becoming more concerned in healthily produced poultry products because of the negative health consequences of using the hazardous inorganic substances in the production of egg, meat and their products.The World Agro-forestry centre (WAC, 2006) reported that leaf meal processed from fodder shrubs is helping small – scale poultry farmers in Tanzania to boast their income. It does not only serve as protein source but also provide some necessary vitamins and minerals (Opara, 1996). The protein from leaves may be preserved and fed to farm animals in form of leaf meal protein concentrates (Farinu *et al*., 1992).

2.2.1 Utilisation of leaf meal in animal production

2.2.1.1 Basil

Basil plant (*Ocimum basilicum L.*) also called (*dun duya*) in Hausa, is a member of the *Lamiaceae* family; an annual herb that grows in several regions around the world. Basil is an essential oil crop which is cultivated commercially in many countries (Sajjadi, 2006) and has been extensively utilised in food as a flavouring agent, in the perfumery and medical industries (Telci *et al*., 2006). The green leaves contain high concentration of vitamins, minerals and oils (Khare, 2007). Sakr (2003) reported that the volatile oil of basil leaf is predominantly rich in ocimene, methyl chavecol and linalool. Basil leaf powder (BLP) is a good source of feed for fish growth with high feed/protein conversion and nutrient retention efficiencies. In addition, basil has shown a very highantimicrobial and antioxidant properties due to its content of aromatic compounds (Gutierrez *et al*., 2008).

Phytochemical screening of *Ocimum basilicum* revealed the presence of glycoside, gums, mucilage, proteins, amino acids, tannins, triterpenoids, steroids, sterols, saponins, flavones and flavonoids in it (Bihari *et al*., 2011). The essential oils content of basil have been well reviewed by (Hussain *et al*., 2008; Makri and Kintzios, 2008). List and Ho¨ rhammer(1977) reported that basil leaves contain 0.17% oleic acid and a small amount of ursolic acid. Tomar *et al*. (2010) reported that basil has exceptionally high content of β-carotene and lutein-zeaxanthin.Odoemelam *et al*. (2013) established that inclusion of basil (*Ocimium gratissimum*) leaf at 1.00% level in broiler diets generally improved body weight gain, dressing percentage and promoted higher dressed weight and carcass quality. Further organoleptic assessment of the meat also showed that meat from birds fed the *Ocimium gratissimum* containing diets were generally preferred in terms of flavour, juiciness and general acceptability.

2.2.1.2 Mint

Mint plant (*Mentha spicata*) known as spearmint,also called (*na'a na'a*) in Hausais species of mint native to North Africa, Egypt and Morocco (MRH, 2010). It has long tradition medicinal use. It is taken as a tea to treat general digestive problems. It is also widely used in many commercially manufactured products, for cooking and medicine due to its aromatic and flavouring qualities (Harley *et al*., 2004). Mint leaves are extensively used in Indian cuisine and for curing several common ailments (Choudhury *et al*., 2006). Mint extracts were found to have very good antioxidant activity, which were comparable to that of the synthetic antioxidant BHT (Kanatt *et al*., 2008).Abdel Moneim *et al*. (2011) also indicated that spearmint and spearmint oil can be used as antibacterial, antifungal and antiseptic, so that they can be used in food perseveration.

2.2.1.3 Pawpaw

Pawpaw plant (*Carica papaya L.*),also called (*gwanda*) in Hausais native to tropical America; andbelongs to the family *Caricaceae*. It is popular in the tropics and subtropics for its easy cultivation, rapid growth, quick economic returns and adaptation to diverse soils and climates (Harkness, 1967; Campbell, 1984; Islam, 2005). The parts that are usually used include the leaves, fruit, seed, latex and root. The plant has been reported to act as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge (Afolayan, 2003). Pawpaw leaves are known to contain proteolytic enzymes (papain, chymopapain), alkaloids (carpain, carpasemine), sulfurous compounds (benzyl isothiocyanate), flavonoids, triterpenes, organic acids and oils (Osuna-Torres *et al*., 2005).

Pawpaw leaf have shown antimicrobial activity against both gram-negative and gram-positive bacteria which shows indication that the plant is a potential source for production

of drugs with a broad spectrum of activity (Anibijuwon and Udeze, 2009; Okunola *et al*., 2012). In the food industry, papain is used as an active ingredient in many commercial meat tenderisers, because it is more active than other proteases.Studies conducted by Nath *et al*. (2017) using concentrate feed with Pawpaw(*Carica papaya),* Bahera or beleric*(Artocarpus heterophyllus)* and Jackfruit (*Terminalia bellirica)* showed better carcass characteristics and meat composition of goats. Ugwu and Onyimonyi(2008) reported that pawpaw leave meal incorporated at 2% in the diet of finishing broilers improved performance of the birds, carcass and organoleptic indices of the meat. In the same manner Navid *et al*. (2011) reported that supplementation of 2% pawpaw leave meal (PLM) in combination with vitamin D_3 in spent layer diet two weeks before slaughter, improved the meat quality. In consonance, inclusion of 10% pawpaw leaves powder to the feed of the spent layer birds had a positive effect on their meat tenderness. Also wrapping of the spent layer meat with fresh papaya leaves for one hour before cooking increased its level of tenderness. *Carica papaya* leaves juice or extract and vinegar had lower effect on tenderness compared with pawpaw leaves. Navid *et al*. (2011) also reported that moist cooking had greater effect on tenderness compared with oven cooking using pawpaw leaves marinades. Abdulla *et al*. (2013) noted that application of pawpaw leaves marinades one hour before cooking was enough for meat tenderisation. Similarly, Saulawa *et al*. (2015) established that feeding PLM at 10% inclusion level improved the growth performance of rabbits. They further suggested that PLM could be a suitable ingredient in feeding rabbits and further research is required to determine the highest quantity that the rabbits can tolerate.However, Ganzon-Naret (2015), reported that a mixture of water spinach*(Ipomoeaaquatica)*and pawpaw (*Carica papaya)* leaf meals as replacement of fish meal (FM) at 40-50% in sea bass diets resulted in adverse effects on fish growth and reduced its feed efficiency.

2.3 Concept of Meat Production

Meat of any animal has a composition related to the age and nutritional status of the animal (Nath *et al*., 2017). In addition to its nutritive value, meat has other important characteristics, including its attractive sensory properties. Nowadays, some kind of meat could be considered healthier than the other,based on composition and proper handling of the meat (Cascone, 2005). The strategies for producing healthier meat and meat products involve modifications at the farm and the meat processing levels. Different reformulation strategies are being applied to make meat a functional food, modifying its lipid and fatty acid content, and/or by incorporating a series of functional ingredients like fibre vegetable proteins, phytochemicals, prebiotics and other natural antioxidants (Fernandez *et al*., 2004) to achieve the desired purpose. These strategies are fundamental especially now that consumers are aware of the hazardous nature of less healthier meats and are in dear need of healthier meat products.

2.3.1 Metabolic conversion of muscle to meat

The makeover from muscle to meat is a complex process that requires some hours to accomplish, depending on the species, age and the different body parts of the animal. Immediately after slaughter, the transport of nutrients and oxygen within the body of the animal is blocked due to the massive loss of blood. This lead to changes in the metabolic processes within the animal; when aerobic metabolism stops and anaerobic metabolism proceeds over. This has consequences on the biochemistry and structure of the muscle as it enters the rigor mortis state to become meat (Lomiwes, 2008). During these processes of muscle conversion to meat, a series of biochemical events add to the enhancement of many meat value traits (Koohmaraie, 1996; Renand *et al*., 2001; Ouali *et al*., 2006),and in these processes, the action and impact of the calpain enzyme system has been

highlighted(Purintrapiban *et al*., 2001; Koohmaraie *et al*., 2002; Koohmaraie and Geesink, 2006), and in the programmed cell death (Herrera-Mendez *et al*., 2006).However, the activities of these metabolic enzymes are numerously lost within a few hours or days of post-mortem due to the termination of the circulatory system which transports oxygen and glucose to the muscle.

In the absence of oxygen, pyruvate can no longer be metabolised via the aerobic tricarboxylic cycle (TCA cycle), but is anaerobically metabolised to lactic acid (Lomiwes, 2008). Although much less ATP is produced from the anaerobic metabolism of glucose than by the complete aerobic process, yet is enough to maintain muscle extensibility for some hours. This generation of ATP is an attempt to maintain the ATP concentrations to preserve muscle homeostasis (Hedrick*et al*., 1994). One significant post-mortem changes in muscle due to anaerobic metabolism is lowering of the pH in the muscle. During anaerobic metabolism, muscles preferentially utilise glycogen over the remaining free glucose in the muscle. This is possible when glucose has to come into the muscle via the blood stream which is no longer functioning (Lomiwes, 2008), while the glycogen is muscle in situ. Another proposed reason for the preferential utilisation of glycogen over glucose for anaerobic metabolism is that the phosphorylation of glycogen-derived glucose does not require ATP as does hexokinase-catalysed phosphorylation of glucose. The generation of ATP through the anaerobic metabolism of glycogen results in lactic acid as the terminal metabolite. In live animals, any excess production of lactic acid due to temporary oxygen deprivation is transported away from the muscle via the circulatory system (Hedrick*et al*., 1994),but in carcasses where the circulatory system has been terminated, lactic acid necessarily accumulates in the muscle. The glycolysis usually ceases before all glycogen has been used up due to the low pH that develops within the muscle.In a well-fed,

unstressed animal, the pH fall is typically from 7.2 to an ultimate pH (pHu) of 5.5 (Warriss, 2000). Under this condition,the muscle shows extensible properties while ATP is still abundant in the carcass. However, as the pH increases and the metabolism of glycogen is halted, ATP concentrations finally fall below a threshold required to maintain relaxation in muscles (Greaser, 2001). When this occurs, the actin and myosin combine to form permanent cross bridges resulting in rigor mortisand the muscle tends to shorten, as the name rigor suggests, and extensibility is lost (Marsh and Carse, 1974). This anaerobic depletion of glycogen and ATP are observed only within a few hours' post-mortem.

2.3.2 Meat tenderisationprocess

During the onset of rigor in muscle and then after in meat, muscle proteins are selectively and progressively hydrolysed by endogenous enzymes. This process would manifest as the softening of the rigor rigidity, known as tenderisation of the meat as perceived after cooking and during consumption. The tenderisation process is also known as ageing, whichdepending on temperature and other factors, can take several weeks for a muscle to reach maximum tenderness. At the core of tenderisation there is the weakening of the myofibrillar structure due to hydrolysis of certain structural proteins (Lomiwes, 2008). However, the breakdown of intramuscular connective tissuealso plays a minimal role in the tenderisation of meat during ageing (Warriss, 2000). Tenderisation during ageing of meat is due to the activity of proteolytic enzymes within the meat. While the actomyosin structure remains intact during ageing, other myofibrillar proteins are degraded. Ageing is mainly observed in the degradation of the muscle structure as the proteins associated with the Z disks and other myofibrillar structures become extensively degraded with increasing storage time. These proteins include desmin (Young *et al*., 1981), titin (Locker, 1987) and connectin (Maruyama *et al*., 1977). The Z disks keep the ultrastructure of meat intact by

keeping thin filaments (actin)and indirectly the thick filaments (myosin) in their organised longitudinal arrangement.

During ageing, the degradation of the Z disk and its associated proteins lead to fragmentation of the myofibrils resulting in meat tenderness. The proteolytic activities of the calpain and cathepsin enzymes are known to be primarily involved in this process. Factors such as temperature, pre-slaughter conditions and electrical stimulation are known to affect the rate of ageing (Hedrick*et al*., 1994; Devine, 2004).

2.4 Meat Quality Concept

Meat quality is very challenging to define simply due to numerous factors that collectively upset meat qualityproperties with regards to the meat production. Meat quality attributes are important in animal production to meet consumer demands which include such characteristics as meat colour, flavour, tenderness and nutritional value (Liu *et al*., 1995).Functional quality refers to the attributes in meat that affect its appearance and palatability (Cascone, 2005). The three dominant attributes by which consumers judge meat quality are appearance and flavour (Faustman and Cassens, 1990).

Meats from maleanimals usually contain higher percentage of moisture, protein and ash than from females while meats from female animals contain greater amounts of fatsthan from males (El-Dashlouty*et al*., 1978). Also there were differences in the protein, fat and moisture contents between the muscle of the breast and thigh. Breast muscles contain more protein and less fat and moisture than those of the thigh (E-Pelczynska, 1974a). On the other hand, percentage of protein and fat increases while the moisture content decreases with advancing age in poultry (El-Dashlouty*et al*., 1978; E-pelczynska,1974b). El-Dashlouty *et al*. (1978) and E-pelczynska (1974b) also indicated that there were no

differences in the level of protein in thigh muscles at different ages, but the level of protein in the breast muscles was lower in broilers than other chickens. Also it has been shown that fat content increased with age in all tissues, whereas moisture content of breast, thigh and skin decreases with age (E-Pelczynska, 1974a; Singh and Essary, 1974; El- Dashlouty, 1978; Grey *et al*., 1982; Warriss, 2000). E-pelczynska (1974b) also indicated that the content of connective tissue is related to the age of chicken as the difference in the muscles of the thigh and breast, and showed that the influence of age was only found in the thigh muscles. Summer and Leason (1984) indicated that energy content of the diet affected chemical composition of chicken meat; high energy feeding resulted in increased fat deposition in the carcass.This consequently increased the chemically extracted fat.

2.4.1 Meat colour

The colour of lean meat is critical to the consumer decision; as it is often the only visible criterion by which a consumer can judge quality of meat (Warriss, 2000). Meat colour is determined by the proportions of the three forms of myoglobin present in the meat (Tang *et al*., 2005).Myoglobin is a molecule with a protein portion (globin) and a non-protein portion known (haeme ring), which is largely responsible for the pigmentation of meat. Within the haeme ring is an iron atom. The oxidation state of this atom governs the colour of meat (Hedrick*et al*., 1994). The development of colour in meat is known as flourishing (O'Keeffe and Hood, 1982).Meats from different species and body parts differ in colour. Meat colour is linked to other meat quality element;such as the moisture and fat contents, tenderness, etc. Tests conducted on a sensory panel have shown that lean colour is significantly related to the panel, tenderness and flavour intensity scores (Viljoen *et al*., 2002).

2.4.2 Meat tenderness

The perception of tenderness involves ease of fragmentation, mealiness, texture and the adhesion of muscle fibres during mastication (Hedrick*et al*., 1994). According to Maiti *et al*. (2008), of all the eating quality characteristics, the average consumer currently rates tenderness of meat as one of the most essential factors. Only when the tenderness of cooked meat is acceptable that judgements of flavour and juiciness can be made (Dumont, 1981).No any meat sensual characteristic has received more research study than tenderness. The overall impression of tenderness to the palate involves three aspects: firstly, the initial ease of penetration of the meat by the teeth, secondly, the ease with which the meat breaks into fragments; and thirdly, the amount of residue remaining after chewing (Weir, 1960).

The degree of tenderness can be related to those of connective tissue, myofibrils and sarcoplasmic proteins (Lawrie, 1991). Singh and Panda (1984) reported that myofibrillar components of meat contributed the toughness known as actomyosin toughness, even though the toughness in meatfrom old animals is caused by connective tissue known as background toughness,rather than by actomysin. Lowimes(2008) noted that although the purchase decision of raw meat is primarily affected by colour, the likeability of meat is markedly affected by the tenderness of the cooked product.The tenderness of meat is not always consistent or acceptable following cooking. This is due to many intrinsic properties of meat that determine tenderness,which include meat pHu, the occurrence of cold shortening and the effect of connective tissues on meat (Purchas, 2004). A significant relationship exists between tenderness and the pHu, but there are contradicting results regarding the trend of this relationship (Lowimes, 2008). The abundance of connective tissue surrounding the muscle fibres bundles and the entire muscle is also an important source of variation on the meat tenderness (Purchas, 2004). Although connective tissues are only a minor component of meat, they have structural, protective and mechanical functions.

The proteins; collagen and elastin are also of particular interest (Singh and Panda 1984; Purchas, 2004).

2.4.3 Meat flavour

Raw meat is a heterogeneous medium which contains proteins, fats, vitamins, sugars and nucleotides, which are flavour precursors in cooked meat. The interactions between these components and their degradation products during cooking are responsible for the flavour profile in meat (Oddy *et al*., 2001).The flavour of meat arises from the interaction of a host of compounds during cooking. The chemical composition of raw meat ultimately gives rise to the flavour in cooked meat (Pegg and Shahidi, 2004). This is because the volatile elements of cooked meat are what is predominantly perceived as meat flavour (Pegg and Shahidi, 2004).Flavour is mainly a combination of two sensory responses;the taste and smell. The smell profile is mainly of interest when discussing meat aroma. Aroma is perceived as the detection of volatile substances by olfactory receptors in passages at the back of the nose (Hedrick*et al*., 1994; Warriss, 2000).

2.4.4 Meat fat

Fats are present in muscle as structural components of the muscle membranes, as storage droplets of triacylglycerol between muscle fibres and as adipose tissue (marbling fat). Meat fat is important in human nutrition with PUFA and conjugated linoleic acids (CLAs) playing beneficial roles.These fats, or more precisely fatty acids, which contribute to a wide range of quality attributes of meat. For fresh meat, these are colour stability, drip loss and the development of oxidative rancidity. Finally, nutritional quality of meat depends upon the fat content of the meat and its fatty acid composition (Cascone, 2005).

2.4.5 Meat pH

The pH has been defined as the log10c, where c is the hydrogen ion concentration in mole per kg. The pH metre is used for measuring the hydrogen ion concentration in meat and other substances, which runs from 1 to 14, with 7 at the centre representing the neutral point. The lower values (1- 6) are acidic while higher values (8 – 14) are alkaline.The pH value is an important yardstick that influences shelf life, colour, water-holding capacity (WHC) and cooking yield of meat and meat products (Clarke *et al.,* 1988; El Rammouz *et al*.,2004).It is widely used as a predictor of meat technological and sensory qualities (El Rammouz *et al*., 2004). A low meat pH is mostly associated with low WHC and pale colour while high meat pH often causes a dark meat colour. Both the pale and dark meat colours are unattractive meatsto consumers. In addition, meat with dark colour(high pH)has a shorter shelf life than the normal reddish-pink colour that consumers prefer (Pearce, 2011).

Meat quality is influenced largely by the pHu (Sales and Mellett, 1996; Young *et al*., 2004). Fletcher (1999) and Van Laack *et al*. (2000) reported significant correlations between muscle pH and poultry meat quality. In chickens, normal pH value at 15 minutes post mortem ageing (pH15) is around 6.2 to 6.5 (Kijowski and Niewiarowicz, 1978; Berri *et al*., 2005), whereas normal pHu value is around 5.8 (Fletcher, 1999; Van Laack *et al*., 2000). If the pH15 value is low (below 6.0) when the muscle is still warm, the proteins are subjected to denaturation (Monin, 1988) which leads to a decreased WHC and a decolouration of the meat. Such defects have been sufficiently described in pigs(Barbut, 1996;Pietrzak *et al*., 1997; Sosnicki *et al*., 1998; Owens *et al*., 2000) and chickens (Barbut, 1997b; Van Laack *et al*., 2000).

2.5 Factors Affecting Meat Quality

2.5.1 Lipid oxidation

Lipid oxidation is a chain-reaction that damage lipids; stir up meat rancid off-flavour and odour, reducing its juiciness and tenderness, increasing meat spoilage and reducing shelf life (Delles *et al*., 2014; Hygreeva *et al*., 2014). Furthermore, according to Est´evez (2015), the susceptibility of meat to oxidative reactions involves many other endogenous (heme iron content, antioxidants and enzymes) and external factors. Pre-slaughter stress and physical damage during slaughtering, ageing (pH, temperature, shortening and tenderising techniques), processing (cooking, size-reduction processes, emulsification, deboning, addition of additives), and storage conditions (temperature, time and oxygen availability) are among the most relevant external factors that influence lipid oxidation (Min and Ahn, 2005). The endogenous factor, which involvesoxidation of lipids, is commonly described as an oxidative, oxygen dependent, deterioration of fats, notably the unsaturated fatty acids.

Lipid peroxidation is initiated by the notion of hydrogen radicals from unsaturated fatty acids, induced by light (Cascone, 2005), heat, metal ions (Kanner, 1994), or other oxidising agents. The reaction of oxygen with preformed free radicals results in accelerated lipid peroxidation (Delles *et al*., 2014), which leads to the formation of secondary by-products from PUFA such as malondialdehyde (MDA) and the potential appearance of lesser sensory scores. During the oxidation processes, it is also necessary to consider the effects of enzymatic component (autocatalysed oxidation) that operates after slaughter. In the post mortem step, endogenous antioxidant systems (for example superoxide dismutase and glutathione peroxidase) available in the cells are not active and this doesnot permit to balance free radical production (Cascone, 2005), leading to high production of MDAs. The occurrence of oxidative rancidity and spoilage in meat is most visible during processing and storage conditions (Bamidele, 2015). Evidence has shown that oxidation affects

virtually every muscleregardless of its protein and fat (lipid) contents (Velasco and Williams, 2011).

The rate and extent of meat deterioration can be reduced through various means, such as freezing, application of antioxidant (natural/synthetic) etc (Bamidele, 2015). However, the use of natural antioxidants, which are rich in bioactive compounds and also less expensive, has been considered to be more valuable to consumer health (Bamidele, 2015). Generally, antioxidants have been supplementary to commercial feeds to delay lipid oxidation and oxidative rancidity during production, processing and storage of feeds. More importantly, the current trend of formulating diets with PUFAs-rich ingredients has intensified the use of antioxidants in animal feeds (Salami *et al*., 2015).The addition of natural antioxidants had been found to reduce cholesterol levels,lower the formation and absorption of MDAs, polycyclic aromatic hydrocarbon and heterocyclic amine in cooked meat (Megan-Tempest, 2012; Kobus-Cisowska *et al*., 2014).

The meat industry has worked for reduction of meat fat, reaching important results, but the problem of lipid oxidation remains still open (Granit *et al*., 2001). Many factors can influence lipid oxidation but the influence from fat composition of meat tends to be more pronounce, which in turn depends on diet, race, weight, age, tissue of deposit, sex, hormones, etc (Rule *et al*., 1995; Enser *et al*., 1999).According to Granit *et al*.(2001), the fatty acids composition of meat affects the profile of compounds produced during lipid oxidation.The abundance of unsaturated fatty acids favour the abstraction of a hydrogen atom and the start of the oxidation process. Dietary supplementation to animal feeds and the tendency of the species to accumulate certain fatty acids in the membrane phospholipids affect the lipid composition of the membrane and consequently, its susceptibility to oxidation. PUFA of muscle membrane cells are particularly susceptible to oxidationduring

storage.The degree of unsaturation of membrane lipids is enhanced with decline of oxidative stability of muscle. Furthermore, other factors that affect the lipid oxidation of muscle and foods are exposure to light, oxygen availability, temperature conditions and microbial growth (Skibsted *et al*., 1998). Cooking process can affect lipid compounds in meat, especially the fatty acids component, changing the nutritional value of the cooked products with respect to the raw sample (Candela *et al*., 1996). These factors can make the quality of the meat and foods not acceptable for consumers, but before these conditions take place, lipid oxidation could generate toxic molecules with possible hazards for human health (Lowimes, 2008).

2.5.2 Glycogen levels

Glycogen is a polymer of glucose units with three dimensional structure; comprising of glucose units forming a helical chain that is linked by α-1,4 acetal bonds, with α-1,6 bonds present where branches occur in the chain. Structurally, glycogen is similar to amylopectin in plants, but it is much larger (up to 50,000 glucose units) and more branched (Warriss, 2000). Glycogen functions as an energy store and is readily available as a source of glucose in the form of glucose-1-phosphate. In live animals, dietary carbohydrates that are not immediately needed by the body are converted to glycogen and stored in the muscle and liver. Glycogen is initially degraded to glucose-1-phospate moieties before it is metabolised to yield ATP (Lowimes, 2008). In situations where animals have been fasted and carbohydrate concentrations in the blood are low, free fatty acids are metabolised from the fat depots of the body. However, when the breakdown rates of carbohydrates and free fatty acids are not sufficient to keep up with the demands of contracting muscles, glycogen is utilised (Lowimes, 2008). This is typically a result of an animal going through fasting (staying over a long period without food) or intense physical activity. Glycogen is also

immediately metabolised in response to an external stressor such as fear. Stress releases adrenaline into the bloodstream; which immediately triggers the rapid metabolism of glycogen to energy for contraction, for example, when the animal needs to avoid predation (Warriss, 2000). Therefore, physiological and psychological stresses lead to the depletion of glycogen stores in the muscle.Environmental factors in which the animals have been reared has also been linked to the depletion of muscle glycogen where a significant number of animals yield dark firm dried (DFD)meat during prolong stress state(Brown *et al*., 1990; Knee *et al*., 2004).

2.5.3 Water-holding capacity (WHC) of meat

Lean muscle contains approximately 75% water, commonly referred to asmoisture. Other main components include protein (approximately 20%), fat (approximately 5%), carbohydrates (approximately 1%) and vitamins and minerals (often analysed as ash, approximately 1%) (Offer and Cousins, 1992).The ability of fresh meat to retain moisture,referred to as the water-holding capacity (WHC) of meat, isone of the most important quality characteristics of raw meat products. The most water in muscle is held either within the myofibrils, between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscles bundles. Once muscle is harvested, the amount of water and location of that water in meat can change, depending on numerous factors related to the muscle tissue itself and how the product is handled (Honikel and Kim, 1986; Offer and Cousins, 1992; Honikel, 2004).Meat weight losses due to moisture removal can average as much as 1–3% in fresh retail cuts and can be as high at 10% in pale soft and exudative (PSE) meat (Melody *et al*., 2004).Meat weight losses occur as a result of dripping and during cooking (Cooking loss).Cooking loss is reduction in weight of meat; as a result of cooking (Jama *et al*., 2008).Dripping is usually associated with loss of fluid and

water from meat resulting to shrinking of muscle proteins (actin and myosin) (Yu *et al*., 2005).

Both drip and cooking losses are important quality criteria for the meat processing industry and the consumer (Offer and Trinick, 1983).In addition, both cooking and drip losses increase loss of valuable nutrients and flavour compounds in the exudates (Lawrie, 1998).It has been estimated that as much as 50% or more of pork has unacceptably high drip loss (Kauffman *et al*., 1992; Stetzer and McKeith, 2003).In addition to the loss of profitable weight, drip loss also entails the loss of a significant amount of protein (Offer and Knight, 1988a; Offer and Knight, 1988b). On average, purge can contain approximately 112 mg of protein per milliliter of fluid; mostly water-soluble, sarcoplasmic proteins (Savage *et al*., 1990). A high drip loss alters the aesthetic quality of the meat as found in PSE meat. Cooking loss in meat cuts is important for maintaining an attractive retail display of meat (Lawrie, 1991). However, high cooking losses are not only undesirable, but have a large financial implication on the meat industry. High cooking losses not only reduce the size of the meat portion but also result in reduced juiciness, loss of meat flavour and sometimes tenderness. These lead to increased loss of nutrients, deteriorate the meat's nutritional quality and lower its economic value (Jama *et al*., 2008).

2.5.4 Microbial analysis

Meat and meat products provide excellent media for growth of a variety of microflora (bacteria, yeasts and molds) some of which are pathogenic (Jay *et al*., 2005). The most common source of major micro flora of meat are skins and feathers, in addition to digestive and respiratory organs(AhoM and Hirn, 1988).Therefore, microbial quality of processed carcasses mostly depends on a healthy condition and external micro flora of an animal (AhoM and Hirn, 1988), the sterile conditions during slaughtering and processing (McCrea

et al., 2006; Northcutt and Berrange, 2006).El Nasir *et al*. (2015)reported that the microbial quality of poultry meat depends mainly on the nutritional status of the birds prior to slaughter and operational hygiene during poultry meat processing as well as storage temperature. Mead (2007)reported that microbial count found on carcasses depends on the site examined. Also as a result of variations in processing technology, large variations occur in the microbial loads of raw poultry meat (Klinger *et al*., 1980; Klinger *et al*., 1981). Since poultry meat itself offers an excellent medium for the multiplication of most bacteria, including those that are not inhibited by low temperatures preservation, storage of processed poultry meat is vital and is considered as means of inhibiting the multiplication of the initial load of bacteria (Blankenship, 1986). Chicken meat is not only highly susceptible to spoilage, but also often implicated in the spread of food-borne illnesses (Selvan *et al*., 2007; Adu-Gyamfi *et al*., 2012) to consumers. This is because, during the various stages of slaughter and processing, all potential edible tissues are subjected to contamination from a variety of sources within and outside the animal (Alvarez-Astorga *et al*., 2002; Kozacinski *et al*., 2006), from the environment, equipment and operators (Mead, 1989). Several efforts to reduce the microbial load of chicken at various stages of production have generally been ineffective (Adu-Gyamfi *et al*., 2012). Attempts made to decontaminate chicken meat by the adding of chemicals to the processing water had only limited success (Sheldon and Brown, 1986; Frels *et al*., 1988), because of residual effect of toxic synthetic chemicals.

El Nasir *et al*. (2015) further stated that chicken thighs are of main importance in investigating the contamination during poultry slaughtering process. In his study, the highest bacterial count was seen in the thigh cuts, which is similar to results reported by Rahman (1998). Study conducted by Selvan *et al*. (2007), revealed that chicken products

recorded the lowest total viable count and anaerobic count as compared to beef, mutton and pork products. According to the World Health Organisation (WHO 1986; 1989), the elimination of pathogenic microorganisms in poultry meat depends largely on the correct application of processing technologies such as pasteurization, irradiation, cooking, freezing and pickling at the industrial, retail and domestic levels. However, some of these processing techniques are not realistic considering the nature of our industrial transition from analogue to digital phase especially in a developing country like Nigeria.

2.6 Concept of Cholesterol in Meat

Cholesterol is carbon compound of sterol type that is present in most body tissues. It is an important constituent of cell membranes and precursor of other steroid compounds. However, high concentration of cholesterol in the body may predispose the body to atherosclerosis (i.e the deposition of cholesterol in the arterial vessels). Cholesterol is transported in the blood by different carriers (Adeniyi *et al*., 2016). The two major blood cholesterol carriers are low density lipoprotein (LDL) and high density lipoprotein (HDL).The LDL carried cholesterol is known as "bad" cholesterol, because it deliver the blood cholesterol throughout the body, depositing it as tile in the arterial walls resulting to a condition known as atherosclerosis. On the other hand, HDL cholesterol is known as the 'good' cholesterol because it transports cholesterol from the body tissues back to the liver which turns it to bile and is excreted via the gastrointestinal tract (Adeniyi *et al*., 2016). It is needed for good health, hence, a moderate intake is not harmful but problem arises when the LDL cholesterol levels become elevated and the HDL cholesterol becomes too low. When cholesterol is consumed in excess it elevates total cholesterol (TC) to a high level which may result in atherosclerosis, hence, many people always desire to consume less

cholesterol in their diets (Adeniyi *et al*., 2016). It is of interest to note that the body is capable of producing the cholesterol that it needs, hence, the extra consumed from foods may predispose the body to some ill health states or conditions. The higher the level of LDL cholesterol in the body the greater the chances of developing heart disease (Varbo *et al*., 2013), while the higher the level of HDL cholesterol in the blood the lower the chance of developing heart disease (Burillo *et al*., 2012).

2.6.1 Impact of plant products on cholesterol level in chicken

Several researchershave applied different measures to reduce the cholesterol contents in poultry egg, serum and meat (Salma *et al*., 2011; Kumar *et al*., 2012; Mikulski *et al*., 2012; Shi *et al*., 2012; Singh *et al*., 2013; Vidal *et al*., 2013; Laudadio *et al*., 2014; Cayan and Erener, 2015; Sanda, 2015;Vivian *et al*., 2015). Salma *et al*.(2011) reported that diet containing 0.04% *Rhodobacter capsulatus* fed to laying hens for sixty (60) days resulted in reduced cholesterol content of egg yolk, serum and hepatic cholesterol levels, but increased excreta cholesterol. On the other hand, consumption of diet containing 0.25% dry ginger and 0.3% garlic for four weeks reduced egg yolk cholesterol content by 24.8% in quail with a simultaneous reduction in serum total cholesterol (TC), triglyceride (TAG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and liver cholesterols, but the diet had led to a significant increasein the plasma high density lipoprotein (HDL) cholesterol level (Singh *et al*., 2013).Vivian *et al*. (2015) reported that dietary supplementation of banana (*Musa Paradisiaca*) leaf powder at the level of 50 g/kg feed and 50 ml/L of drinking water to broiler birds was observed to significantly reduce the concentration of serum cholesterol and LDL cholesterol.Laudadio *et al*. (2014) reported that serum and egg-yolk TC concentration decreased significantly in laying hens when fed diet substituted with low-fibre alfalfa meal as plant protein source.

Sanda (2015) reported that feeding of laying chickens with water leaf mucilage, up to 200 ml/L of drinking water, was observed to significantly reduce TC content of egg yolk. Moreover, supplementation of laying birds with Atorvastin (at 0.03%), Niacin (at 375 ppm) and EDTA (at 0.5%) was reported to reduce egg yolk cholesterol content by up to 35% in White Leghorn layer birds (Kumar *et al*., 2012). Vidal *et al*. (2013) also reported that feeding of laying hens with diet containing up to 25% cashew nut meal reduced the cholesterol content of egg yolk, but increased monounsaturated/ saturated fatty acid ratio of the yolk in chicken eggs. Feeding of Lohmann Brown laying chickens with feed fortified with olive leaf powder (at 3%) was observed to reduce the cholesterol content of the egg yolk by 10%, increased the intensity of the yellow colour of the yolk, but had no effect on feed intake, egg weight and egg yield (Cayan and Erener, 2015). Also replacing soybean meal with sunflower seed meal was found to reduce egg yolk cholesterol of Rugao laying hens after six (6) weeks (Shi *et al*., 2012). Feeding of 222 HyLine Brown laying hens for twenty-four (24) weeks on diet containing probiotic *Pediococcus acidilactici* reduced the egg yolk cholesterol by 10% independent of the dose of the probiotic in the feed. There was also reduction in the number of broken eggs and eggs without shell with a related increase in egg weight and egg shell thickness (Mikulski *et al*., 2012).

2.7 Sensory Evaluation of Meat

According to Stone and Sidel (2004), sensory evaluation is the scientific method used to suggest, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing. It is a scientific discipline that analyses and measures human responses to the composition of foodrelated to appearance, touch, odour, texture, temperature and taste. According to Institute of Food Technology, sensory evaluation is a scientific method used to suggest measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Anonymous, 2005).

In schools, sensory evaluation provides an ideal opportunity for students to evaluate and give feedback on their dishes. According to Ribah (2012), sensory evaluation is a very useful tool in quality assessment of processed meat products. It makes use of the various human senses to evaluate the general acceptability and quality attributes of the products. It uses human panelists and their senses of sight, smell, touch and hearing to measure the sensory characteristic and acceptability of meat products as well as many other materials.

The sensory quality of meat may be evaluated in a more objective manner through instrumental or organoleptic methods used by scientists as measurement tools (Terra *et al*., 2009). Vaclavik and Christian (2008) described sensory testing that, in the simplest way, the meat processor, possibly assisted by staff, will test a product's colour, smell, taste and texture upon manufacture. In a more sophisticated approach a team of trained panelists can be used in order to make the results as objective as possible. For this reason, it is useful to have an appropriate testing room available with lights, temperature and seating arrangements with individual testing compartments so as not to distract the members of the panel. As an ideal arrangement, the panel is composed of ten well trained panelists. If ten

panelists are not available, a smaller panel can also produce good results provided the panelists are knowledgeable at sensory testing. It is obvious that for reliable results, the panelists need relevant instructions and some experience of the food sector. Only people with good sensory capability should be chosen in order to find out differences in colour, texture, flavour and taste. All panelists must use proven and identical test methods in order to make their results comparable. Each panelist involved in such tests is given a score sheet, where they mark their finding. Score sheets of the team of panelist are evaluated and a test result for each individual product is produced based on multiple observations.

2.8 Typesof Sensory Evaluation

2.8.1 Difference tests

These are based on comparison of samples to test for similarity or difference between samples using trained panels. The taste panels should be able to detect and describe sensory aspects of a product, differentiate and rate the intensity of each attribute and define the degree to which each attribute is present. Panel size may vary between 5-100 judges. For mass products such as beer, soft drinks, confectionary where small differences can be important, large panels are usually necessary (Geoff, 2004; Stone and Sidel, 2004).

2.8.2 Descriptive tests

This is concerned with providing description of the sensory qualities of food. The purpose of descriptive analysis is to obtain detailed description of aroma, flavour and real texture of foods for a range of purposes. It is used both to obtain qualitative descriptors of the product and to obtain quantitative evaluations of product (Geoff, 2004; Stone and Sidel, 2004).

2.8.3 Affective tests

Affective test is also known as hedonic or consumer test. It is used to assess consumer response to products; it is concerned with acceptability of a product or whether one product is preferred over another. Affective tests may be used for a variety of purposes including product maintenance, product improvement, new development, assessment of market potential and support for advertising claims (Geoff, 2004; Stone and Sidel, 2004). Affective tests can be quantitative or qualitative, depending on purpose. Quantitative test may be divided into preference tests and acceptance tests. Whichever type of test is used, care needs to be taken to ensure that the sample of testers is representative of the target population expected to use the product (Geoff, 2004; Stone and Sidel, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The feeding trial was conducted at the poultry production unit of Sokoto State Veterinary Centre, located at Aliyu Jedo Road, in Sokoto Metropolis. Sokoto State is within the savannah agro-ecological zone located between latitude 13.157, longitude 5.2457 111 N 13^{o} 3'25", E 5^{o} 14'45", which is on about 350m above sea level. The rainy season starts in mid-May to early June and reaches peak in August. Dry season starts in mid-October and ends in late April. The hottest months are March and April while the coldest months are December to February, characterised by dry harmattan winds (SERC, 2012). The area has an average annual temperature of 30.26^{o}C with average rainfall of 26.55mm and average annual humidity of 48.54% in the year 2012 (SERC, 2012; Ahmed and Egwu, 2014).

Livestock production in the study area is usually closely integrated with crop production. The main livestock production system is the village system under which a number of husbandry practices such as free range, seasonal confinement and herding may be recognized (FDLPCS, 1992). Poultry production practice adopted for exotic breeds is intensive management system; battery cage housing for layer birds and deep litter housing for broiler birds. Local poultry breeds are mostly managed under semi intensiveand extensive management system.

3.2 Methodology

This study was conducted in three phases: The first phase was evaluation of phytochemical composition of the test ingredients and proximate composition of the experiment diets. The second phase was a feeding trial with 210 spent layer birds at the age of 115 weeks fed

diets containing supplemental levels of basil, mint and pawpaw leaves and their combinations which lasted for 21 days. The third phase was evaluation of performance characteristics, meat quality and lipid profile of spent layer birds on the test ingredients.

3.3 Treatments and Experimental Designs

Both the feeding trial and meat quality assessment comprised of seven treatments each.The treatments consisted of a broiler finisher diet(control = T1)supplemented with six different plant additives at 2% leveleach. The treatments were described below:

T1 = Broiler finisher diet without additive (control).

T2 =Broiler finisher diet + 2% pawpaw leaf powder (PLP)

T3 =Broiler finisher diet+ 2% basil leaf powder (BLP)

T4 =Broiler finisher diet+ 2% mint leaf powder (MLP)

T5 =Broiler finisher diet+ 1% % BLP and 1% PLPmixture

T6 =Broiler finisher diet+ 1% MLP and 1% PLPmixture

T7 =Broiler finisher diet+ 1% BLP and 1% MLP mixture.

3.3.1 Sources of test ingredients and other feedstuffs

The test ingredients (basil, mint and pawpaw leaves) were sourced from the Sokoto main vegetable market (*Ramin Kura*) within Sokoto metropolis.The plant leaves specimens were identified by a botanist in the Botany unit (Department of Biological Sciences), Usmanu Danfodiyo University, Sokoto (UDUS).Already processed feedstuffs for compounding the broiler finisher diet (maize, groundnut cake, blood meal, bone meal, wheat offal, salt, limestone,methionine, lysine and premix) were sourced from the Feed-mill section of the Sokoto Technology Incubation Centre (STIC), Sokoto.

3.3.2 Formulation of treatmentdiets

The seven treatment diets used in this experiment were formulated to satisfy the 2900 Kcal/kgMEand 20% CPrequiredby the broiler finisher (Table 3.1) as recommendations by the NRC (1994).

Table 3.1 Gross and chemical compositions of experimental diets

	Treatment Diets						
Ingredients (%)	T1	T2	T3	T4	T5	T6	T7
Maize	56.00	56.00	56.00	56.00	56.00	56.00	56.00
Wheat offal	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Groundnut cake	15.50	15.50	15.50	15.50	15.50	15.50	15.50
Blood meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bonemeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
BLP	0.00	0.00	2.00	0.00	1.00	0.00	1.00
MLP	0.00	0.00	0.00	2.00	0.00	1.00	1.00
PLP	0.00	2.00	0.00	0.00	1.00	1.00	0.00
Calculated Chemical Composition							
ME(Kcal/kg)	2900.00	2920.49	2912.87	2913.75	2916.68	2917.12	2913.31
CP(%)	20.00	20.64	20.50	20.48	20.57	20.56	20.49
CF(%)	4.87	4.93	4.91	4.90	4.92	4.91	4.91
EE (%)	4.10	4.12	4.12	4.11	4.12	4.11	4.12

BLP- Basil leaf powder, MLP- Mint leaf powder, PLP- Pawpaw leaf powder, ME- Metabolisable energy, CP- Crude protein, CF- crude fibre, EE – ether extract.

Two hundred and ten (210) spent layer birds were divided into seven groups of 30 birds each and allotted to the seven dietary treatments.Each treatment group was replicated into three with 10 birds each. The 7 x 3 = 21 treatment combinations were laid out in completely randomised design (CRD).

3.4 Experimental Birds and their Management

The 210 spent layer birds at 115 weeks old used for this experiment, were purchased from the Labana Farms Limited, Aliero, in Aliero Local Government Area of Kebbi State. A week to arrival of the birds, a pen with battery cagesystem was swept and the battery cage thoroughly washed and disinfected. On arrival, the birds were housed in the penand fed broiler finisher diet and offered drinking water containing anti-stress (glucose), to calm them for transit stress, for seven days to enable them adapt to the new environment. They were subsequently allocated randomly to the treatment diets (broiler finisher diet + 2% test ingredients) for 14 days.

3.5 Phytochemical and Proximate Compositions Analyses

3.5.1 Phytochemical analysesof test ingredients

Phytochemical analyses of aqueous extracts of basil, mint and pawpaw leaves were conducted at the Biochemistry Laboratory of Usmanu Danfodiyo University, Sokoto, Nigeria. The Phytochemical compounds evaluated include alkaloids, cardiac glycosides, flavonoids, phenols, saponin, tannins and terpenoids.

3.5.1.1 Preparation sample

The basil, mint and pawpaw leaves used for this experiment were washed, air-dried andthen oven-dried at temperature of 50°C to obtain moisture content of 10% as recommended by Hagerman (1988). The dried leaves were ground and sieved using 3 mmlaboratory mesh.

The fine particles (powder)obtained from each leaf sample was packed and used to prepare an aqueous extract of the samples.

Aqueous crude extracts of the basil, mint and pawpaw leafpowders were prepared by first suspending 25 g of each of the plants powder in 250ml of distilled water and then stirring the aqueous suspension on water bathat 40^{o}C for 30 minutes. At the end of this process, the aqueous suspensions were filtered using Whatman number one filter paper. The filtrateswere then centrifuged at 500 rpmfor 15 minutes.The supernatants were decanted and stored in sterile bottles at 4^{o}C as prescribed by Solomon *et al*. (2013). The procedures used for evaluation of the various phytochemical compounds are as described below:

3.5.1.2 Wagner's reagent test for alkaloids

2 mleach of the basil, mint and pawpaw leaves aqueous crude extracts weretaken and mixedseparately with 2 drops of hydrochloric acid and filtered. The filtrates werethen treated with Wagner's reagent (iodine in potassium iodide)and formation of brown/reddish-brown precipitate indicated the presence of alkaloids (Mittal *et al*., 1962; Velmurugan *et al*., 2010).

3.5.1.3 Keller-killani test for cardiac glycosides

2 mleach of the basil, mint and pawpaw leaves aqueous crude extracts were taken and mixed with 2 ml of glacial acetic acid containing 2 drops of 5% iron (III) chloridesolution and concentrated tetraoxosulphate (VI) acid. Formations of a brown ring at the junction of the two liquid layers indicated the presence of cardiac glycosides (Mittal *et al*., 1962; Velmurugan *et al*., 2010).

3.5.1.4 Test for tannins

2 mleach of the basil, mint and pawpaw leaves aqueouscrude extracts were taken and mixed with 2 ml of bromine solution.Decolouration of the bromine solution in the mixture indicatedthe presence of tannins (Mittal *et al*., 1962; Velmurugan *et al*., 2010).

3.5.1.5 Test for flavonoids

2 mleach of the basil, mint and pawpaw leaves aqueouscrude extracts were taken and mixed with 2 drops of 10% of ammonium hydroxide solution. The formations of an intense yellow colour in the mixture indicated the presence of flavonoids (Victor and Chidi, 2009; Velmurugan *et al*., 2010).

3.5.1.6 Salkowski test for terpenoids

2 mleach of the basil, mint and pawpaw leaves aqueouscrude extracts were taken and mixed with 2 ml of chloroform followed by a 3 mltetraoxosulphate (VI) acid.Grey colouration formed in the mixture indicated presence of terpenoids (Victor and Chidi, 2009; Velmurugan *et al*., 2010).

3.5.1.7 Test for saponins

2 mleach of the basil, mint and pawpaw leaves aqueouscrude extracts were taken and mixed with 5 ml of distilled water in a test tube. The suspension was mixed with 2 drops of olive oil then shaken vigorously and allowed to stand for 10 minutes. Presence offoam at the top layerindicated the presence of saponins(Mittal *et al*., 1962; Velmurugan *et al*., 2010).

3.5.1.8 Test for phenols

2 mleach of the basil, mint and pawpaw leaves aqueouscrude extracts were taken and mixed with 2 ml of 5% iron (III) chloride solution.Formations of dark green colour in the mixture indicated presence of phenolic compounds (Mittal *et al*., 1962; Velmurugan *et al*., 2010).

3.5.2 Proximate composition analyses of the experimental diets

Samples were taken from the seven treatment diets for evaluation of proximate composition. The samples were ground and thoroughly mixed for proximate composition analyses. Dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE),ash and nitrogen free extract (NFE) contents were determined according to the procedures described by the Association of Official Analytical Chemist(AOAC)(AOAC, 2007).

3.5.2.1 Determination of dry matter (DM) content

10 g each of the seven feed samples was weighed in a Petri dishof known weight and transferred into an oven set at temperature of 100^{o}C.The feed samples were then heated for 24 hours to obtain constant weights of the samples. This removes the moisture contents(MC) and the %MC was calculated using the formula below:

$$\%MC = \frac{W_1 - W_2}{W_{1-}W_0} \times 100$$

Where; W_0= Weight of empty dish

W_1= Weight of dish plus sample

W_2= Weight of dish plus oven dried sample

Then, % DM = 100 - % MC

3.5.2.2 Determinationof crude protein (CP) content

The crude protein contentin a feed sample is usually estimated from the percent nitrogen content (%N) of the feed. The %N content in the diet samples was determined using the Kjeldhal method described by AOAC (2007), which is carried out in three stages; digestion, distillation and titration.The crude protein content was determined using the equation as follows;

$$\%\ CP = \frac{(A - B) \times N \times 14.01 \times F}{Mg\ of\ distilled\ sample} \times 100$$

Where A= ml of acid used for titrating the sample

B= ml of acid used for titrating blank

N= normality of acid used for titration

F= protein factor (6.25)

3.5.2.3 Determination ether extract (EE) content

The ether extract content in the feed samples was determined by extraction of fat from the sample with petroleum ether and thimble using the soxhlet apparatus.2 g of each prepared sample and 200 ml of petroleum ether was placed into soxhlet apparatus. The heating mantle was turned on a temperature of 60^{o}C for 5 hour. A mixture of ether and fat was then obtained and weighed. The ether + fat mixture was then separated by distillation without thimble. At the end of the distillation, the flask containing the fat was removed and oven dried at 70^{o}C for 12 hours, cooled in the desiccators and weighed again. The dried material in the flask represents the EE. The %EE for each treatment diet was then calculated using the equation below;

$$\%EE = \frac{W_2 - W_0}{W_1} \times 100$$

Where; W_0 = weight of the empty flask (g)

W_1 = weight of the sample (g)

W_2 = weight of flask plus fat (g)

3.5.2.4 Determination of crude fibre (CF) content

The crude fibre content of the feed samples was determined from the residues left after extractionof the fat (or EE) for each treatment sample. The residue left in each treatment sample was weighed and then oven dried overnight at 70^{o}C, cooled in desiccators and re-

weighed. The amount of dried material recovered represents the CF. The %CF for each of the treatment was calculated using the formula below;

$$\%CF = \frac{W_1 - W_2}{W_0} \times 100$$

Where; W_0 = weight of crucible plus residues before ashing

W_1 = weight of crucible plus residues ash after ashing

W_2 = initial weight of the residues

3.5.2.5 Determination of Ash (mineral) content

2 g of the feed sample for each treatment diet was weighed into a porcelain crucible and transferred into the muffle furnace fixed at 550°C and left for about 4 hour. After this time, it had turned to white ash. The crucible and its content were cooled in desiccators and weighed (AOAC, 2007). The percentage ash content was calculated from the formula below:

$$\%Ash = \frac{W_1 - W_2}{W_0} \times 100$$

Where; W_0 = weight of sample before ashing

W_1 = weight of sample plus crucible before ashing

W_2 = weight of crucible plus ash (after ashing)

3.5.2.6 Determination of nitrogen-free extract (NFE) content

The nitrogen-free extract(NFE) content was estimated as residual component of the feed samples and its value was determined by calculation, using the formula below;

$$\%\ NFE = 100 - (\%CP + \%CF + \%EE + ash)$$

3.6 Data Collection

3.6.1 Feeding trial

Data collected during the feeding trial include initial body weight,feed intake, finalbody weight, weight gain and feed conversion ratio.

Initial and final body weights: After allocation of the birds to various treatments and prior to commencement of the feeding trial, thebirds on each treatment were weighed and the live weight obtained was recorded as initial body weight.At the end of the feeding trial, the birds on each treatment were also re-weighed after a 12 hour fasting as recommended by Olomu (2011)andthe live weightobtained for each treatment was recorded as the final body weight.

Feed intake: Daily feed intake was calculated by subtracting the quantity of feed left over from the quantity of feed given the previous day.

Weight gain: Weight gain was calculated by subtracting the initial body weight of the birds from each treatment from their finalbody weight.

Feed conversion ratio (FCR): The FCR was determined by as ratio of the quantity of the feed consumed to the body weight gained, and was calculated using the equation below;

$$\text{Feedconversion ratio} = \frac{\text{feed intake}}{\text{weight gain}}$$

3.6.2 Estimation of carcass yield and primal parts

Three birds were randomly selected from each treatmentfor carcass and primal parts yield estimations. The birds were weighed using electronic sensitive balanceand then humanely slaughtered,dressed and eviscerated to obtain the hot carcass weight (dressed weight).The

birds dressing percentage for each treatment was calculated as ratio of the dressed carcass weight to the live weight multiply by 100 as in the equation below.

$$\text{Dressing \%} = \frac{\text{dressed carcass weight}}{\text{live weight}} \times 100$$

Primal cuts of breasts, drumsticks, thighs, giblets and visceralof the birds for each treatment were also weight and expressed as a percentage of the live weight.

3.6.3 Determinationof physical and chemical properties of muscles and meat

The physical and chemical properties of muscles and meat were carried out in the Biochemistry laboratory in the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto.Three birds were newlyand randomly selected from each treatmentand were humanely slaughtered,dressed and eviscerated for determination of physical and chemical properties of muscles and meat from the spent layers. The dressed carcasses of the three birdsfrom each treatment were splited through the backbone and keel to produce two (2) halves of approximately equal weight.The two halves were subjected to post mortemageing in a chiller at 4^{o}C, for 0 and 24 hour post mortem ageing. At completion of the each ageing period, muscles and meat from breast were collected for determination of pH, drip loss, cooking loss,glycogenandmalondialdehyde(MDA) contents.

3.6.3.1 Determinationof pH

The pH values of the muscles and meat storedat 0 and 24hour post mortem ageingwere measured by using a digital pH metre.The pH metre electrode was calibrated by using one standard solution of pH 7.00 (Mettler Toledo) at ambient temperature. The pH determination was carried out as described by (Koniecko, 1979); by immersing the pH metre glass electrode into the sample and the readings on the pH metre were recorded. The

pH metre calibration was repeated for everyreading to check if the pH had a deviation of more than 0.01 units.

3.6.3.2 Determinationof evaporative loss

The muscles and meat samples stored at 0 and 24hour post mortem ageing were weighed and placed in the polyethylene bags and then tied to prevent surface evaporative loss. The muscles and meat samples were then stored in the Chiller at 4ºC for 24 hour. The samples were then removed from the Chiller and out of the polythene bags and were blotted using tissue paper and were reweighed (Honikel, 1998). The percent evaporative loss was calculated using the formula below;

$$\%\ \text{evaporative loss} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: w_1 =weight ofsample before chilling.

w_2 = weight ofsample after chilling.

3.6.3.3 Determination ofcooking loss

The muscles and meat samples storedat 0 and 24hourpost mortem ageing were weighed and packed and tied in polyethylene bags and then boiled in hot water at 100°C for 25 minutes. The boiled samples were thenremoved, cooledand dried from fluids usingtissue paper and reweighed. Cooking loss was estimated as loss in weight by the muscles and meat during cooking; expressed as a percentage (Honikel, 1998).The percent cooking loss was calculated using the formula below;

$$\%\ \text{Cooking loss} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: w_1 = weight of sample before cooking;

w_2 = weight of sample after cooking

3.6.3.4 Determination ofglycogen content

Glycogen content of muscles and meat stored at 0 and 24hourpost mortem ageingwere determined based on a coloured reaction that occurs when a dilute solution of glucose is heated with concentrated sulphuric acid(H_2SO_4).Since glycogen is hydrolysed by hot concentrated H_2SO_4 to glucose, the reaction can be used for the determination of glycogen (Dalrymple and Hamm, 1973).One g of muscles and meat samples were thoroughly mincedin mortar mixedwith 5ml deproteined solution in a centrifuge tubesthen marked with a glass cap and then placed in a boiling water bath for 15 minutes. The tubes were removed and cooled under cold tap water bath. The mixture was then centrifuged for 5 minutes at 3000 rpm. One ml of clear supernatant was added to 3 ml of concentrated sulphuric acid in test tubes. The mixture was heated in a boiling water bath for 6 minutes, cooled and the intensity of the pink colour formed was measured spectrophotometrically at 520 nm. The resulting glucose was determined using hexokinase and glucose-6-phosphate dehydrogenase as described by Bergmeyer *et al*. (1974). After subtraction of the originally free glucose, the glycogen content was expressed in g per kg of the sample.

3.6.3.5 Determination ofmalondialdehyde (MDA) content

Musclesand meat samples stored at 0 and 24hourspost mortem ageingwere analysed for MDA content using the colorimetric reaction with thiobarbituric acid (TBA) (Buege and Aust, 1978). One g of muscles and meat samples were thoroughly ground with mortar and mixed with 10 ml of 15% TCA for 15 minutes. The resultant homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant fraction was collected and subjected to MDA assay. The reaction mixture contained 1 ml of extract and 2 ml of TBA reagent. The mixture was heated for 15 minutes in a boiling water bath to form a precipitate. The mixture was then removed and cooled.The resultant precipitate was removedby filtering and centrifuged at 3000 rpm for 10 minutes, and a pink-colouredsupernatant was obtained.

The absorbance of the pink-coloured supernatant was read at 531 nm using spectrophometer and converted to moles of MDA by using extinction coefficient. MDA contents were expressed as mg/kg of fresh weight.

3.6.4 Microbial analysis ofmuscles and meat

Three birds were randomly selected from each treatment for microbial analysis of muscles and meat. The birds were humanely slaughtered,dressed and eviscerated. The dressed carcasses were splitedto produce four(4) quartersof approximately equal weight and stored in a chiller at 4^{o}C.. The twelve quarters of muscle and meat samples for each treatment weresubjected to 0, 6, 12 and 24 hours post mortem ageingwith three replicate per treatment. At completion of each period, muscles and meat from each quarter were collected for microbial load count and species identification. The results obtained were then recorded.

Microbial analysis was carried out at Microbiology laboratory in the Department of Microbiology Usmanu Danfodiyo University, Sokoto.The microbial analysis was conducted using aseptic technique by serial dilution following the procedure of Adams and Moss (2007). Sterilisation: All glass wares were washed with detergent rinsed with water and sterilised using hot air oven at 160^{o}C for 1 hour. While all the liquid media were sterilized in an autoclave at 121^{o}C for 15 minutes.

Nutrient agar medium: Twenty-eight (28) g of nutrient agar powder was weighed and dissolved into conical flask containing 1000 ml of distilled water, after plugging with non-absorbent cotton wool and cover with aluminium foil. This was heated and agitated gently for about 10 minutes in order to dissolve the nutrient agar which was later autoclave at 121^{o}C for 15 minutes in order to achieve sterility. The agar formed was allowed to cool to

about 45^{o}C, and then dispensed into sterile petri dishes. The agar was then left to solidify and was refrigerated at 4^{o}C for further use (Willey *et al*., 2011).

Serial dilution: One 1 g each of muscles and meat samples were weighed and dissolved in a test tube containing 9 ml of sterile distilled water (10^{-1}) dilution. This was shaken to obtain a good suspension. The suspension was then serially diluted to 7 tubes (10^{-7}). This process of transfer from preceding tube continued till 1:7000 dilutions is achieved using a fresh siring tip for each dilution. Furthermore, 10^{-4} and 10^{-7}test tubes were used for inoculating media. Colony count was performed by using colony count metre as described byWilley *et al*. (2011).

$$Bacterial\ load = Total\ num. of\ colonies\ in\ a\ plate\ \times reciprocal\ of\ the\ dilution\ factor$$

3.6.4.1 Bacterial specie identification

Subculture for pure culture isolation: Using a sterile wire loop of the correct size dip it into the enrichment culture and pick a single colony and inoculate into a small area of a plate containing fresh nutrient agar and spread by using a sterile wire loop, which was incubated for 24 hour and observed the growth. The pure isolates of each of the colony was obtained and transferred into a sterile slant bottles containing fresh nutrient agar and refrigerated at 4^{o}C for further use(Willey *et al*., 2011).

Gram staining: Gram staining was carried out according to Willey *et al*. (2011)method. Smear of bacterial isolates were made on clean glass slide using drop of water with sterile wire loop. It was then allowed to air dry and then passed over a flame in order to fix the smear. After fixation the smear was covered with gentian violet for 60 seconds and washed. Iodine was then poured to cover the smear, allowed for 60 seconds and then washed. Ethyl alcohol (ethanol) was used to decolourised the smear and washed immediately with the distilled water, then follow by the application of safranin and left for 60 seconds, and later

washed with distilled water. Back of the slide was cleaned with cotton and allowed to air dry. The slide was examined under electrical microscope using oil immersion x100 objectives

3.6.4.2 Biochemical identification of the isolated bacterial

The biochemical test was carried out according to the description of Willey *et al*. (2011).

3.6.4.2.1 Triple sugar iron

The triple sugar iron was put in a slants test tube and was inoculated with the isolates using a sterile transfer needle.Using the needle the butt was stabbed then the needle was withdrawn and the slant test tube was streaked. The test tubes were incubated at 37ºC for 24 hours, which was examined for gas production, hydrogen sulphide production, glucose production, lactose production, sucrose production and motility (Willey *et al*., 2011).

3.6.4.2.2 Urease production

Slants of urease medium in universal bottles were inoculated with loopfull of isolates by streaking. These were incubated for 4 days at 37ºC with daily examination.Change of colouration of the media from brown to red indicates presence of urease(Willey *et al*., 2011).

3.6.4.2.3 Methyl red production

To prepare glucose phosphate medium in a test tube, a loopfull of isolates was inoculated and incubated for 2 days at 37ºC. Drops of methyl red solution were added to the 2 days old culture, shacked and examined. Appearance of red colour at the surface of the reagent layer showed positive methyl red production(Willey *et al*., 2011).

3.6.4.2.4 Indole production

A loopfull of the isolates was inoculated in a sterile nutrient broth at 37^{o}C for 48 hours. After incubation, 0.5 ml of Kovacs reagent was added and shacked. This was examined after one minute.A red colour in the reagent layer indicates positive indole production (Willey *et al*., 2011).

3.6.4.2.5 Citrate production

To a sterile simons citrate medium a loopfull of 24 hours culture isolate was inoculated aseptically, at 37^{o}C for 24 hours after which it was examined for turbidity daily for a period of 3 days. Turbidity indicated citrate utilization (Willey *et al*., 2011).

3.6.4.2.6 Hydrogen sulphide production

This test detects the ability of bacterial species to produced hydrogen sulphide, e.g. by reduction of sulphur from the metabolism of sulphur containing amino acids to hydrogen sulphide. A speck of each isolate was inoculated into triple sugar iron agar and incubated at 37^{o}C for 24 hours. Evolution on blackening of the medium indicates positive hydrogen sulphide (Willey *et al*., 2011).

3.6.4.2.7 Motility test

Motility test was carried out according to Willey *et al*. (2011), motility can sometime be inferred from the way an organism grew on solid media. Motile species tend to spread outward from the inoculated area as organism swim in the layer of surface moisture. A bit of each isolate was stab onto triple sugar iron agar and incubated at 37^{o}C for 24 hours. Motility observe is by spread of the organisms outward from the stab area.

3.6.5 Sensory evaluation

Sensory evaluation was carried outusing consumer sensory panel, which constitutedof both undergraduate and postgraduate students **(**x males and y females**)** of various Departments in

the Usmanu Danfodiyo University, Sokoto, Nigeria. The panelists were trained on the criteria for the sensory evaluation of meat.

Meat samples for each treatment were packed in polyethylene bags and placed in boiling water (100°C)for 25 minutes.The cooked meat samples were placed in separate coded disposable plates and presented to the panelists for evaluation. The Panelists were instructed to use water for cleansing mouth between samples to reduce impact between samples. Each panelist was asked to evaluate the aroma, tenderness, colour and overall acceptability of the meat samples. A nine-point hedonic scale was used to score each of these attributes;1= extremely dislike, 2 = to 9, like extremely as presented in appendix II (Stone and Sidel, 2004)

3.6.6 Determination of serumlipid profile

Serum lipid profile were analysed with Spectrophotomer (model: AE-350, By ERMA INC) using Randox Cholesterol Kit Enzymatic Endpoint method according to the procedure described by Bhagavan (2002) in appendix 1.

3.7 Data Analysis

All data generated were checked for normality using PROC UNIVARIATE of SPSS. All data expressed in percentage were transformed to ARCSIN before ANOVA. Data on growth performance,carcass yield and primal parts, serum lipid profile and sensory evaluation were analysed using One-way ANOVA. Data on muscles and meat pH, evaporative loss, cooking loss, MDA concentration,glycogen concentration and bacteriaload were analyzed using GLM univariate analysis of SPSS statistical software. Means were separated by multiple comparisons using Duncan'sMultiple Range Test (DMRT) at 5% level of significance($P<0.05$).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Phytochemical Assay of Test Ingredients

The assay conducted revealed the following bioactive compounds; alkaloids, flavonoids, cardiac glycosides, phenols,saponins, tannins and terpenoids (Table 4.1). These bioactive compoundswere found in varying quantities while others were not detected in one test ingredient or the other. BLP contains higher amount of cardiac glycosides and flavonoids compared to MLP and PLP, MLP contains higher amount of tannins and terpenoids compared to BLP and PLP while PLP contains higher amount of alkaloids and saponins compared to BLP and MLP.

Table 4.1: Proportions (mg/kg) of phytochemical components in the test ingredients

Component	Ingredient		
	BLP	MLP	PLP
Alkaloids	0.75	0.34	0.80
Cardiac glycosides	0.60	0.45	0.54
Flavonoids	1.04	0.67	0.93
Phenols	0.09	-	-
Saponins	-	0.52	0.64
Tannins	0.075	1.60	-
Terpenoids	0.024	0.34	-

- = Not detected

Several studies conducted by Osuna-Torres *et al*. (2005); Bihari *et al*. (2011) andKumar*et al*. (2013) affirm the presence of bioactive substances in BLP, MLP and PLP. However,these herbs possess different concentrations of bioactive compounds which could be due to varietal differences of the plants.The phytochemical assay of BLP is in line with the work of Bihari *et al*. (2011) who reported the phytochemical screening of basil (*Ocimum. basilicum*) revealthe presence of cardiac glycosides, flavonoids, tannins and

terpenoids.Similarly, the following secondary metabolites; flavonoids, saponins, tannins and terpenoids were identified in MLP by Kumar *et al*. (2013).Osuna-Torres *et al*. (2005) also reported the presences alkaloids (carpain, carpasemine), cardiac glycosides and flavonoids in pawpaw leaves.

4.2 Performance Characteristics of Spent Layers

There were (P<0.05)differencesin average daily feed intake and average daily weight gain as presented in Table 4.3. The birds in treatments 6 and 7 had significantly higher feed intake than those in treatments 3 and 4 but similar with those in treatments 1, 2 and 5. The control birds had significantly higher weight gain than treatment 3 but similar with other treatments (2, 4, 5, 6 and 7) birds.

Table 4.2: Performance (g) characteristics of spent layer according to treatment

Treatment	Ave. daily feed Intake/ bird/g	Initial body Weight/g	Final body Weight/g	Ave. daily gain /bird/g	FCR
Overall	92.40	1711.93	1807.49	6.83	13.00
1	94.24^{ab}	1705.67	1863.56	11.28^{a}	8.58
2	94.06^{ab}	1683.33	1793.63	7.88^{ab}	19.44
3	85.67^{b}	1796.45	1835.22	2.77^{b}	-4.81
4	83.73^{b}	1596.10	1708.77	8.05^{ab}	12.54
5	88.55^{ab}	1796.55	1886.03	6.39^{ab}	17.49
6	101.14^{a}	1748.39	1823.63	5.37^{ab}	21.08
7	99.39^{a}	1657.00	1741.61	6.04^{ab}	16.69
SEM	3.84	74.32	61.33	2.15	9.77

abcd= means bearing different superscripts within the same column differ (P<0.05)

The high average daily feed intake obtained in treatments 6and 7 birds, could be due to synergistic effect of combinations (MLP + PLP) in treatment 6 and (BLP + MLP) in treatment 7supplemented to the diets that influences the intake of the birds. The high feed intake could also be due to the presence of flavonoids in the test ingredients, known to

improve feed palatability and slowing peristaltic movements of feed in the guts of birds (Kass *et al*., 1980; Xu *et al*., 2006). The low feed intake observed in treatments 3 and 4 could be due to higher anti-nutritional compounds tannins and saponins present in the test ingredients as disclose by phytochemical assay.

The high average daily feed intake of 101.84 g per bird per day obtained is lower than the value of 119.13 g per bird per day obtained by Nworgu (2016) who supplement fresh basil leaf in the diet of growing pullets in Ibadan. The wide variation in feed intake may probablydue tovariation in ambient temperature of the locations where the experiments are conducted. The moisture content of the test ingredient may have a diluting effect on the anti-nutritional factors present in the feed, which may cause high feed intake. The disparity in feed composition in terms of energy and protein, strain and age of the experimental stocks could have effect on the feed intake. This is obvious because monogastric eat to satisfy their energy requirement. High feed intake of 101.84 g per bird per day obtained is lower than 120.69 g per bird per day reported by Unigwe *et al*. (2014) who fed broiler chickens with diets compounded withsun-dried pawpaw leaf meal. The wide difference in feed intake may probablydue to cooler ambient temperature which tends to increase intake of feed. The difference in breeds of bird used in conducting the experiments could also account for the discrepancy in feed intake. The nutrient requirements of different breeds of bird may also account for the variation in feed intake.

The superior value recorded in average daily gain observed in control birds could be due to non-inclusion of supplements in the diets which are known to have anti-nutritional compounds that hinder optimum utilisation of nutrients in the feed.

The highest value obtained for average daily weight gain of 11.28 g per bird per day is superior to the value obtained by Nworgu (2016) of 9.35 g per bird per day. The slight

variation observed could be as a result of difference in nutritional composition of experimental diets, where rich and quality of diet gives better weight gain than low quality diets.

4.3 Yield of Carcass and Primal Parts

There were significant differences in all the yield components observed as shown in Table 4.4. The birds in treatment 3 had ($P<0.05$) higher carcass yield than birds in treatments 1, 5, 6 and 7 but similar with birds in treatments 2 and 4. Treatments 1 and 2 birds yielded significantly lower breast yield than other treatments birds. Thigh yield indicated birds in treatment 2 had higher ($p<0.05$) yield than birds in treatments 4 and 7 but similar with birds in treatments 1, 3, 5 and 6. Yield of drumstick followed different trend with treatment 6 birds had higher ($P<0.05$) yield than treatment 4 birds but similar with all other treatments birds. Birds in treatment 4 had significantly higher yield of giblet than birds in treatments 3, 5, 6 and 7 but similar with birds in treatments 1 and 2. The control and treatment 4 birds had higher ($p<0.05$) viscera yield than treatments 3 and 5 birds but similar with other treatments birds.

Table 4.3: Meat yield indices (%) according to treatments

Treatment	Yield component					
	Carcass	Breast	Thigh	D.stick	Giblet	Viscera
Overall	57.13	28.13	17.09	13.18	9.99	19.66
1	53.75^{b}	26.13^{bc}	17.28^{ab}	13.12^{ab}	10.42^{ab}	21.56^{a}
2	58.01^{ab}	25.52^{c}	18.44^{a}	13.44^{ab}	10.69^{ab}	20.13^{ab}
3	62.53^{a}	29.39^{ab}	16.91^{ab}	12.73^{ab}	7.94^{c}	16.09^{c}
4	58.14^{ab}	28.49^{abc}	15.83^{b}	12.35^{b}	12.52^{a}	21.59^{a}
5	57.12^{b}	30.76^{a}	17.31^{ab}	13.11^{ab}	9.36^{bc}	17.96^{bc}
6	55.33^{b}	27.39^{abc}	17.39^{ab}	14.26^{a}	9.58^{bc}	20.14^{ab}
7	55.03^{b}	29.20^{ab}	16.46^{b}	13.20^{ab}	9.48^{bc}	20.18^{ab}
SEM	1.37	1.11	0.57	0.49	0.74	0.83

abc= means bearing different superscripts within the same column differ ($P<0.05$)

The high carcass yield observed for birds in treatment supplemented with basil may be explained in terms of high flavonoids contents, which are known to stimulate digestive secretions, which will make for greater feed utilisation leading to increased growth of carcass yield components (Zhu *et al*., 2006). Furthermore, high flavonoids contents of basil serve as natural antioxidants known to increase antioxidative effect, therefore deterring oxidation and degradation of fat. Fat being a carcass component will accumulate which transform to higher carcass yield (Zhu *et al*., 2006; Bamidele, 2015). The same also apply for higher yield of primal parts (breast, drumstick and thigh).

The high giblet and viscera yield of birds in treatment supplemented with mint might be due to high tannins contents in the leaves known to affect optimum nutrient utilisation in poultry (Ahmed *et al*., 1991; Manssori and Acamovic, 2007). According Calislar (2017), poultry are very sensitive to tannins. High amounts of tannins lead to low performances in poultry, such as reduced appetite, reduced feed intake and poor nutrient absorption. The

high weight of viscera could be describe in relation to low feed utilisation, thus high weight of viscera.

The high carcass yield of birds obtained (62.53%) is inferior to the value of 70% obtained by Nworgu (2016)who supplement fresh basil leaf in the diets of growing pullets.The differences could be due to variation in diets, strain and age of the birds used in the conduct of the experiments.

The findings of this study is in consonance with the work of Odoemelam *et al*. (2013) who reported that inclusion of basil leaf at 1.00% level in broiler diets generally improved body weight gain, dressing percentage and promoted higher dressed weight and carcass quality.

4.4 Serum Lipid Components

The treatments differ ($p<0.05$) in all lipid components except HDL (Table 4.5). The control birds had ($P<0.05$) higher total cholesterol compared with birds in treatments 3, 4 and 7 but similar with other treatments. The birds on control diet had significantly higher LDL concentration than birds in all other treatments. Treatment 1 birds had higher ($P<0.05$) TAG than other treatments except treatment 2 birds. The serum very low density lipoprotein were significantly higher in treatments 1 and 2 birds than all other treatments birds.

Table 4.4: Serum lipid components (mg/dl) according to treatments

Treatment	Lipid component				
	TC	HDL	LDL	TAG	VLDL
Overall	193.09	42.76	116.29	184.38	161.67
1	204.33^{a}	37.00	136.67^{a}	202.00^{a}	119.00^{a}
2	194.33^{ab}	44.00	117.00^{b}	191.67^{ab}	119.00^{a}
3	189.33^{b}	44.33	109.67^{b}	182.33^{bc}	177.33^{b}
4	191.67^{b}	44.00	112.33^{b}	177.33^{bc}	176.33^{b}
5	193.67^{ab}	42.00	110.00^{b}	184.33^{b}	180.00^{b}
6	193.00^{ab}	44.00	115.00^{b}	172.00^{c}	180.00^{b}
7	188.33^{b}	44.00	113.33^{b}	181.00^{bc}	180.00^{b}
SEM	3.44	3.32	3.15	4.43	2.56

abc = means bearing different superscripts within the same column differ ($P<0.05$)

The control birds had higher total cholesterol than the birds fed supplements; it implies that the supplements had secondary metabolites that affect the enzymes which stimulate lessening synthesis or increases tissue absorption of TC in the serum.Flavonoids a constituent of the test ingredients was reported to cause decline in cholesterol levels by Megan-Tempest, (2012)and Kobus-Cisowska *et al*. (2014). The bioactive substances in the test ingredients had no effect on high density lipoprotein.

The serum LDL was higher for birds on control diet than all other treatments birds. This suggests that these supplements treated had secondary metabolites that affect the enzymes which stimulate decline synthesis or increases tissue absorption of LDL. Wang *et al*. (2005) reported that flavonoids make for decrease of low density lipoprotein in serum.

Triglyceride concentration was lower for birds on supplemental diets than the control. This may not be unconnected to the supplements in the diets. This suggests the bioactive substances in the test ingredient either reduces the synthesis from fat in feed or reduces the

synthesis of triglyceride in the liver. Very low density lipoprotein comprises of LDL and triglyceride, therefore, the lower concentration of VLDL observed for birds on supplemental diets may not be unconnected with the decline of TAG and LDL due to the supplements.

The lower value obtained of 188.33, 109.67, 37 mg/dl for TC, LDL and HDL in this study are closer to the value of 2.46, 1.70 and 0.44 mg/dl^{-1} acquired by Ouyang *et al*.(2016) who evaluated alfalfa flavonoids on broiler performance, meat quality and gene expression in China. The slightvariation could be attributed to differences of test ingredients,breeds and age of the birds and location of the studies.

The findings of this study is in line with the report of Ouyang*et al*. (2016) who reportedflavonoids in the diet of broiler decrease the TC, LDL and HDLlevelintheserum of birds.

4.5 Chemical Properties According to Treatment

There were differences ($P<0.05$) between treatments in all chemical properties tested. Also 24 hour ageing of meat triggered significant increases in MDA and pH and decrease in glycogen concentration (Table 4.6). The MDA concentration was higher in control meat ($P<0.05$) than the meat of other treatments for treatment factor. The meat MDA concentration also differs significantly among treatments with supplements. The muscles and meat glycogen concentration revealed treatment 2 had significantly higher value than the meat of other treatments for treatment factor. Glycogen concentration of birds fed supplements also showed difference ($p<0.05$). The pH levels of muscles and meat indicated control had higher ($P<0.05$) value than meat of the other treatments for treatment factor. The meat pH level of birds fed supplements also varies significantly.

Table 4.5: Chemical properties according to treatment

Factor		MDA (mg/kg)	Glycogen (g/kg)	pH
Overall		0.451	2.960	5.991
Treatment				
	1	0.607^{a}	2.458^{e}	6.048^{a}
	2	0.361^{d}	3.556^{a}	5.924^{f}
	3	0.431^{c}	2.802^{d}	5.944^{e}
	4	0.457^{b}	3.008^{c}	6.028^{b}
	5	0.448^{b}	2.998^{c}	5.984^{d}
	6	0.423^{c}	3.082^{b}	5.998^{cd}
	7	0.432^{c}	2.815^{d}	6.009^{c}
SEM		0.004	0.012	0.005
PMA				
	0	0.117^{b}	3.951^{a}	5.718^{b}
	24	0.785^{a}	1.969^{b}	6.263^{a}
SEM		0.002	0.006	0.003
Interaction		*	*	*

abcdef = means bearing different superscripts along the column within a subset differ (P<0.05).
*= (P<0.05)

4.5.1 MDA concentration

The lower MDA concentration observed in meat of birds on supplemental diets may be explain in terms of antioxidative effects of the secondary metabolites in the test ingredients. The meat obtained from birds fed PLP supplement had lower MDA concentration compared to meat of other treatments birds. It implies that PLP have more antioxidative effect or it has substance that favour increase assimilation antioxidants into meat tissue. Addition of natural antioxidants had been found to lessen the formation and absorption of MDA in fresh and cooked meat (Megan-Tempest, 2012; Kobus-Cisowska *et al*., 2014). High concentration of MDA observed at 24 hour ageing could be explained in respect to

long period of ageing. Lipid oxidation is time dependent reaction, the longer the period of ageing the higher the production of MDA.

The low value obtained of 0.431 (mg/kg) MDA in this studyis superior to the value of 9.3 (µmol/kg) MDA obtained by Lima *et al*. (2016) who fed alcoholic extracts of *barbatimão* and *pacari* to broiler diet as supplement for 41 days, in Brasil. The wide variation in the concentration of MDA in the samples evaluated could be due todifferences in age, breed, different test ingredients, state of test ingredient administration, ageing period, part of muscles and meat analyse.

The findings of this study affirm that natural antioxidants had been found to delay the formation and absorption of MDA in fresh and cooked meat (Megan-Tempest, 2012; Kobus-Cisowska, *et al*., 2014;Lima *et al*., 2016).

4.5.2 Glycogen concentration

The high glycogen concentration observed for meat of birds fed supplements could be due the high energy in the diets, as shown in appendix 1. The high glycogen concentration observed at 0 hour ageing may be due to low depletion of glycogen to produce lactic acid during anaerobic metabolism in muscles for conversion of muscles to meat.

4.5.3 pH level

The high pH level obtained in meat tissue of control birds might have endangered due to low glycogen concentration in the meat. This could be due to low glycogen reserve in the meat to produce lactic acid that lower the pH during metabolic conversion of muscles to meat. The high pH level observed at 24 hour ageing might be due to depletion of glycogen to produce lactic acid during anaerobic metabolism in muscles for conversion of muscles to meat.

The low pH level of 5.984 arehigher than the pH level of 5.8 obtained by Fletcher, (1999) and Van Laack *et al*. (2000) in chicken meat at 15 minutes ageing and lower than the pH levels of 6.2 – 6.5 obtained by Berri *et al*.(2005). The variation could be due to difference in the samples analysed, handling process before and after slaughter, type of test ingredients treated and time of measurement pH level.

4.6 Physical Properties According to Treatment

There were significant differences in all physical properties between treatments observed. Also 24 hour ageing of meat showed increases in evaporative and cooking losses as presented in Table 4.7. The percent evaporative loss observed in treatments 1 and 6 birds had ($P<0.05$) higher value than the other treatments for treatment factor. The percent cooking loss revealed meat of control birds had significantly higher value than treatments 2, 3 and 7 but similar with treatments 4, 5 and 6 birds for treatment factor.

Table 4.6: Physical (%) properties according to treatment

Factor		Evaporating Loss	Cooking Loss
Overall		1.092	16.216
Treatment			
	1	1.263[a]	16.772[a]
	2	0.989[c]	15.931[b]
	3	0.998[c]	15.837[b]
	4	1.069[b]	16.261[ab]
	5	1.015[c]	16.169[ab]
	6	1.072[b]	16.502[ab]
	7	1.235[a]	16.037[b]
SEM		0.013	0.221
PMA			
	0	0.457[b]	12.211[b]
	24	1.726[a]	20.220[a]
SEM		0.007	0.118
Interaction		*	*

abcd= Means bearing different superscripts along the column within a subset differ (P<0.05).
*= (P<0.05)

The lower drip and cooking losses observed in muscles and meat of birds on supplemental diets may be due to bioactive substances in the test ingredients. The muscles and meat obtained from birds fed BLP supplement had lower percent drip and cooking losses compared to meat of other treatments. It implies that BLP have secondary metabolites that favour decrease drip and cooking losses in muscles meat tissue. The high drip and cooking losses observed at 24 hour ageing may be due to several factors such as shortening of the sarcomere (Honikel *et al*., 1968), the degree of distortion of fat and water translocation (Ramsbottom and Koonz, 1939), increased enzyme activity etc. (Strange, 1987).These findings were in close agreementSonale*et al*. (2014) who reported increase in drip loss with prolonged frozen storage period.

4.7 Microbial Evaluationof Spent Layer Muscles and Meat

4.7.1 Bacteria loadsof spent layer muscles and meat

There is significant difference in bacteria load observed between treatments for treatment factor (Table 4.7.1.)The meat from control birds had (P<0.05)higher bacteria loads than the treatment 5, but similar with the bacteria loads of other treatments.

Table 4.7.1: Bacteria loads according to post mortem ageing and treatments

Factor		Bacteria Count
Overall		3.39×10^6
Treatment		
	1	6.67×10^{6a}
	2	3.93×10^{6ab}
	3	3.30×10^{6ab}
	4	2.81×10^{6ab}
	5	1.50×10^{6b}
	6	2.17×10^{6ab}
	7	3.32×10^{6ab}
SEM		1.51×10^6
PMA		
	0	2.43×10^6
	6	3.28×10^6
	12	4.08×10^6
	24	3.76×10^6
SEM		1.14×10^6
Interaction		*

abcd= Means bearing different superscripts along the column within a subset differ (P<0.05).
*= (P<0.05)

The lower bacteria load obtained for meat of birds in treatment 5 might due to synergistic and anti-bacterial effect of the test ingredients basil and pawpaw. This could be explain in terms of bacteriostatic effects of the secondary metabolites present in the meat known to reduce bacteria load. Anibijuwon and Udeze (2009) and Okunola *et al*. (2012) reported pawpaw leaf have shown antimicrobial activity against both gram-negative and gram-

positive bacteria which indicates the plant is a potential source for production of drugs with a broad spectrum of activity.Basil havealso shown a very high antimicrobial properties due to its aromatic compounds contents (Gutierrez *et al*., 2008).

The low bacterial load of 1.50×10^6obtained in this study is close to the values of 2.9 (log10 CFU/g) obtained by Najeeb *et al*. (2015) who assessed the efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block.

4.7.2 Frequency of occurrences of bacteria species on muscle and meat

The frequency of occurrences of bacteria species on muscles and meat indicated high occurrence of *Escherichia coli*and low occurrence of*Streptococcus morbillorum*and *Streptococcus zooepidemicus* as presented in Table 4.10. Thebacteria that occurred include *Escherichia coli, Enterococcus faecalis, Pseudomonas fluorescens, Staphylococcus aureus, Salmonella typhi, Streptococcus morbillorum* and *Streptococcus zooepidemicus*. The following *Staphylococcus aureus,Salmonella typhi* and*Escherichia coli* bacteria species obtained were found to be pathogenic bacteria.

Table 4.7.2: Frequency of occurrences and percentages of bacteria species

Bacteria Isolate	Frequency	Percentage (%)
Escherichia coli	54	20.22
Enterococcus faecalis	37	13.86
Pseudomonas fluorescens	35	13.11
Staphylococcus aureus	56	20.97
Salmonella typhi	43	16.10
Streptococcus morbillorum	21	7.87
Streptococcus zooepidemicus	21	7.87
Total	267	100.00

4.8 Sensory Evaluation of Spent Layer Meat

There were significantdifferences in aroma and tenderness among the treatment means (Table 4.8). The meat aroma scores of treatments 4, 6 and 7 were higher ($P<0.05$) than the scores of treatments 1 and 3, but similar with treatments 2 and 5 meat. Treatment 2 meat had significantly higher scores than all other treatments meat for tenderness.

Table 4.8: Sensory evaluation of spent layer meat according to treatments

Treatment	Aroma	Tenderness	Colour	Acceptability
Overall	5.62	4.12	7.21	7.37
1	4.70^{c}	2.63^{d}	7.37	7.56
2	4.89^{bc}	5.89^{a}	7.59	7.33
3	5.52^{abc}	4.59^{b}	7.11	7.33
4	6.33^{a}	4.74^{b}	7.41	7.74
5	5.70^{ab}	4.37^{b}	6.89	7.11
6	5.93^{a}	3.44^{c}	7.29	7.41
7	6.26^{a}	3.15^{cd}	6.78	7.07
SEM	0.29	0.26	0.27	0.24

abcd= means bearing different superscripts within the same column differ ($P<0.05$)

Thehigh rating scores for aroma of meat obtained in treatment 4 might be due to synergistic effects secondary metabolites present in the supplements. This could be attributed in terms of higher amount of terpenoids as revealed by phytochemical assay, which may be responsible for the aroma of meat. Terpenoids are widely used directly as flavouring compounds in food industries(Caputi and Aprea, 2011). The finding of this study agree with work by Navid *et al*. (2011) who concluded that dietary supplementation of 2% papaya leave meal in spent layers for a few days before slaughter, improved meat quality in terms of meat flavour/ aroma.

The high rating scores for tenderness of meat observed in treatment 2 could be as results of supplementation of PLP in the diet, known to contain tendering agent papain. Abdulla *et al*. (2013) reported that application of pawpaw leaves marinades one hour before cooking was enough for meat tenderisation. The findings of this study supportthe report by Navid *et al*.

(2011) who maintained that supplementation of 2% pawpaw leave meal (PLM) with vitamin D_3 and the combination in spent layer diet two weeks before slaughter improves the meat of spent layer meat tenderness.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The research compared meat quality of spent layer fed basil, mint and pawpaw leaves powder. A total of two hundred and ten spent layers (210) of 115 weeks' old were randomly assigned to seven (7) dietary treatments in a completely randomised design. The birds were kept for three (3) weeks and fed compounded broiler finisher diets with 2 % level of supplements. Data collected include phytochemical assay, performance characteristics, yield of carcass and primal parts, serum lipid components, physico-chemical properties, microbial load and sensory evaluation of muscles and meat. Data obtained were then analysed using inferential statistics.

The result of phytochemical assay indicated the presence of bioactive substances in basil, mint and pawpaw leaves at different proportions. The result of growth performance revealed significant differencein average daily feed intake and average daily gain perbird perday with treatment 6 had the highest valuecompared with other treatments while treatment 1 had the highest value compared with other treatments for feed intake and daily gain respectively. All the yield of carcass and primal parts showed significant difference. The birds in treatment 3 had the highest carcass yield compared with other treatments. All serum lipid components showed ($p<0.05$) difference except HDL. The control birds had higher TC, TAG, LDL and VLDL compared with birds in other treatments.

There were difference ($p<0.05$) in all physico-chemical properties observed. Also 24 hour ageing of meat triggered significant increase in MDA concentration and pH level with decrease in glycogen concentration. Treatment 1 had higher MDA concentration and pH level with a corresponding lower glycogen concentrationcompared to other treatments. The

percent evaporative loss had higher value in treatments 1 and 6 compared to other treatments. The control had higher percent cooking loss compared to other treatments.

There is significant difference in bacteria load observed between treatments for treatment factor. Treatment 1 had higherbacteria load compared to other treatments. Sensory evaluation showed significant difference in aroma and tenderness.

5.2 Conclusion

The research on the meat quality assessment of spent layers supplemented with basil, mint and pawpaw leaves conclude that; basil, mint and pawpaw leaves which are economically cheaper, viable and health wise safer than synthetic chemicals and antibiotics can be used successfully in the diets of finishing spent layer. They contain varying proportions of bioactive compounds capable of improving performance characteristics, physico-chemical properties, sensory properties, serum lipid profile and reduce bacteria load of spent layer meat and muscles without adverse effect on performance.

5.3 Recommendations

Based on the findings of this study, it is recommended that, poultry farmers in the semi-arid zone are encouraged to utilise these herbs for better performance and meat quality of spent layer to avoid toxic effects of synthetic chemicals and antibiotics for better health of consumers. Basil (*dun duya*), mint (na'a na'a) and pawpaw (*gwanda*) leaves at 2 % level of supplementation should be included in the diet of finishing spent layers to improve meat quality.

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APPENDICES

Appendix I: Proximate composition of experimental diets

Treatment	DM (%)	CP (%)	CF (%)	EE (%)	Ash (%)	NFE (%)	ME(Kcal/kg)
1	90.90	17.45	4.10	3.47	2.70	55.88	2730.53
2	90.54	18.10	4.32	3.50	2.91	58.96	2800.36
3	90.30	18.00	4.21	3.51	2.72	57.72	2790.55
4	90.29	17.78	4.12	3.47	2.70	56.16	2740.12
5	90.42	18.06	4.21	3.50	2.75	57.88	2800.10
6	90.35	17.90	4.28	3.49	2.73	56.28	2779.02
7	90.32	17.50	4.30	3.50	2.71	56.16	2760.25

AppendixII: Lipid profile estimation procedure

Estimate of total cholesterol enzymatic method principle

Cholesterol Ester + H_2O $\xrightarrow{\text{cholesterol Esterase}}$ Cholesterol + Fatty Acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholesterol + H_2O_2

$2H_2O_2$ + Phenol + 4 Aminoantipyrine $\xrightarrow{\text{Perioxidase}}$ Red quinone + $4H_2O$

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red quinone) which is measured at 500 nm

Reagent composition

Reagent 1 (Enzymes/ Chromogen)

Cholesterol Esterase	≥ 200 µ/l
Cholesterol Oxidase	≥ 250 µ/l
Peroxidase	≥ 1000 µ/l
4-Aminoantipyrine	0.5 mmol/l

Reagent 1A (buffer)

Pipes Buffer, pH 6.90	590 mmol/l
Phenol	24 mmol/l
Sodium Cholate	0.5 mmol/l

Standard (cholesterol 200 mg/dl)

Cholesterol	2 g/l

Working Reagent

Working reagent is constituted by mixing reagent 1 with reagent 1A swirling gently for uniform mixing.

Assay Procedure

The following were pipetted into clean, dry test tubes tabulated as blank (b) standard (S) and Test (T) as mentioned below:

Pipette into Test Tube	Blank (B)	Standard (s)	Test (T)
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled Water	10 µl		
Standard		10 µl	
Serum Sample			10 µl

The contents were mixed well and incubated at 37ºC for 5 min. after zeroing the instrument with blank, the absorbance of standard followed by the test sample was measured at 500 nm.

Calculations

$$Cholesterol\ (mg/dl) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Conc.Standard\ (200\ mg/dl$$

Normal Values

Normal values are	150-200 mg/dl (4-6 mmol/l)
Desirable	<200 mg/dl
Borderline	+200-239 mg/dl
High	≥ 240 mg/dl

Quality Control

Normal and abnormal control serum were employed

Estimation of HDL cholesterol [74,76,77,78,79] enzymatic method principle

Phosphotungstate method

Chylomicrons, VLDL, LDL fractions in serum or plasma were separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation the cholesterol in HDL fraction, which remains in the supernatant was assayed with enzymatic cholesterol method using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen, 4-Amonoantipyrine/phenol.

$$Serum = \frac{Phosphotungstate}{Mg++} \; HDL + LDL, VLDL, Chylomicrons \; (Supernatant)(precipitate).$$

Reagent Composition

Reagent 1 (Enzymes / Chromogen)	
Cholesterol Esterase	200 µ/l
Cholesterol Oxidase	250 µ/l
Peroxidase	1000 µ/l
4-Aminoantipyrine	0.5 mmol/l
Reagent 1A (buffer)	
Pipes buffer, pH 6.90	50 mmol/l
Phenol	24 mmol/l
Sodium Cholate	0.5 mmol/l
Reagent 2 (Precipitating Reagent)	
Phosphotungstic acid	2,4 nmol/l
Magnesium Chloride	39 nmol/l
Standard (HDL cholesterol)	50 mg/dl
Cholesterol	0.5 mg/dl

Working Reagent

Working reagent is constituted by mixing reagent 1 with reagent 1A swirling gently for uniform mixing.

Assay Procedure

Precipitation

0.5 ml precipitating reagent was added to 0.2 ml serum in a centrifuged tube mix and centrifuge at 2000 rpm for 5 minutes the supernatant was used for the estimation.

HDL Cholesterol Estimation enzymatic method principle

The following were pipetted into clean, dry test tubes tabulated as blank (b), standard (S) and Test (T) as mentioned below:

Pipette into Test Tube	Blank (B)	Standard (s)	Test (T)
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled Water	10 µl		
Standard		10 µl	
Serum Sample			10 µl

The contents were mixed well and incubated at 37ºC for 5 min. The absorbance of standard and test against the blank was measured at 500 nm.

Calculations

$$HDL\ Cholesterol = \frac{Absorbance\ of\ test}{Absorbance\ of\ Standard} \times Conc.of\ Stanard\ (200\ mg/dl)$$

Quality Control

Normal and abnormal control serum are employed.

Normal values

Male: 30-70 mg/dl

Female: 30-90 mg/dl

Estimation of Triglycerides [74,75]

Triglycerides are hydrolyzed by lipase to glycerol and free fatty acids. ATP phosphorylates glycerol in the presence of glycerol kinase (GK) to glycerol-3 phosphate which was oxidised by the enzyme glycerol-3 phosphate oxidase with 4 Aminoantipyrine and N-Ethyl-N_sulfoprophyl-N-anisidine (ADPS) in the presence of the enzyme peroxidase to produce a red coloured complex. The intensity of the colour was measured by using 546 nm fitter and was directly proportional to the concentration of triglycerides present is serum.

Triglycerides + H_2O $\xrightarrow{\text{Lipoprotein Lipase}}$ Glycerol + Fatty Acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3 Phosphate +ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dehydroxyacetone phosphate +H2O2

$2H_2O_2$ +4 Aminantipyrine + ADPS $\xrightarrow{\text{Peroxidase}}$ Red quinone + 4 H_2O

GPO = Glycerol-3-Phosphate oxidase

ADPS = N-Ethyl-N_sulfoprophyl-N-anisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the triglycerides concentration in the sample and is measured at 546 nm.

Reagents

Reagent 1 (Enzymes/ Chromogen)

Lipoprotein lipase	≥ 1100 µl
Glyceride kinase	≥ 800 µl
Glyceride-3-phosphate oxidase	≥ 5000 µl
Peroxidase	≥ 3500 µl
4-Aminoantipyrine	0.5 mmol/l

ATP	0.3 mmol/l
Reagent 1A (buffer)	
Pipes Buffer, pH 7.50	50 mmol/l
ADPS	1 mmol/l
Magnesium	15 mmol/l
Standard (Triglycerides 200 mg/dl)	
Glycerol (Triglyceride Equivalent)	2 g/l

Working Reagent

Working reagent is constituted by mixing reagent 1 with reagent 1A swirling gently for uniform mixing.

Assay Procedure

The following were pipetted into clean, dry test tubes labelled as blank (B), standard (S) and Test (T) as mentioned below.

Pipette into Test Tube	Blank (B)	Standard (s)	Test (T)
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled Water	10 µl		
Standard		10 µl	
Serum Sample			10 µl

The contents were mixed well and incubated at 37ºC for 5 mins. The absorbance of the standard (S) and test (T) against the blank (B) was measured at 546nm.

Calculation

$$Serum\ Triglycerides\left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ Standard} \times Conc.of\ Standard$$

Normal value < 160 mg/dl

Estimation of LDL cholesterol and VLDL cholesterol enzymatic method principle

Serum LDL was calculated by Freidwald's formula (Freidwald *et al*., 1972). After the estimation of total cholesterol, triglycerides and HDL cholesterol, the values of LDL cholesterol and VLDL-cholesterol were calculated.

Appendix III: Hedonic Scale for Sensory Evaluation

Instruction: You are presented with seven coded samples of meat, please taste the sample and indicate (X) how well you liked or disliked for each of the samples characteristics. Which of the samples did you like best and how much did you like the sample overall.

Please rinse your mouth with water after every tasted sample (between samples).

Name of panelist_____

Gender _____

Age _____

Date _____

Like extremely……..9

Like very much…….8

Like moderately……7

Like slightly…….6

Neither like/ dislike……..5

Dislike slightly……...4

Dislike moderately…….3

Dislike very much …….2

Dislike extremely……...1

	Samples						
Code	588	972	437	156	729	341	225
Attributes							
Aroma							
Tenderness							
Colour							
Overall Acceptance							

Appendix IV: Anova Table for Performance Parametre

Parametre	Source	SS	df	MS
Feed intake	Model	180085.913[a]	7	25726.559
	TRT	180085.913	7	25726.559
	Error	619.305	14	44.236
Initial weight	Model	61643354.579[a]	7	8806193.511
	TRT	61643354.579	7	8806193.511
	Error	231991.413	14	16570.815
Final weight	Model	68681356.306[a]	7	9811622.329
	TRT	68681356.306	7	9811622.329
	Error	158006.994	14	11286.214
Weight gain	Model	1103.822[a]	7	157.689
	TRT	1103.822	7	157.689
	Error	194.367	14	13.883
FCR	Model	4980.930[a]	7	711.561
	TRT	4980.930	7	711.561
	Error	4004.585	14	286.042

Appendix V: Anova Table for Yield of Carcass

Parametre	Source	SS	df	MS
Yield of carcass	Model	68687.581[a]	7	9812.512
	Trt	68687.581	7	9812.512
	Error	79.052	14	5.647
Yield of breast	Model	16677.602[a]	7	2382.515
	Trt	16677.602	7	2382.515
	Error	51.630	14	3.688
Yield of thigh	Model	6143.687[a]	7	877.670
	Trt	6143.687	7	877.670
	Error	13.462	14	.962
Drumstick	Model	3651.493[a]	7	521.642
	Trt	3651.493	7	521.642
	Error	10.349	14	.739
Yield of giblet	Model	2136.009[a]	7	305.144
	Trt	2136.009	7	305.144
	Error	22.795	14	1.628
Yield of viscera	Model	8189.818[a]	7	1169.974
	Trt	8189.818	7	1169.974
	Error	29.116	14	2.080

Appendix VI: Anova Table for Serum Lipid Component

Parametre	Source	SS	df	MS
TC	Model	811575.000[a]	7	115939.286
	Trt	811575.000	7	115939.286
	Error	288.000	14	20.571
HDL	Model	35755.000[a]	7	5107.857
	Trt	35755.000	7	5107.857
	Error	94.000	14	6.714
LDL	Model	298244.667[a]	7	42606.381
	Trt	298244.667	7	42606.381
	Error	61.333	14	4.381
TAG	Model	739953.333[a]	7	105707.619
	Trt	739953.333	7	105707.619
	Error	782.667	14	55.905
VLDL	Model	692114.000[a]	7	98873.429
	Trt	692114.000	7	98873.429
	Error	422.000	14	30.143

Appendix VII: Anova Table for Chemical Properties

Parametre	Source	SS	df	MS
MDA	Model	13.496[a]	14	.964
	Trt	.203	6	.034
	PMA	4.678	1	4.678
	Trt * PMA	.065	6	.011
	Error	.003	28	.000
Glycogen	Model	413.305[a]	14	29.522
	Trt	4.029	6	.672
	PMA	41.217	1	41.217
	Trt * PMA	.072	6	.012
	Error	.024	28	.001
pH level	Model	1510.571[a]	14	107.898
	Trt	.070	6	.012
	PMA	3.121	1	3.121
	Trt * PMA	.056	6	.009
	Error	.005	28	.000

Appendix VIII: Anova Table for Physical Properties

Parametre	Source	SS	df	MS
Cooking loss	Model	11722.242[a]	14	837.303
	Trt	3.912	6	.652
	PMA	673.512	1	673.512
	Trt * PMA	1.088	6	.181
	Error	8.240	28	.294
Evaporative loss	Model	67.742[a]	14	4.839
	Trt	.456	6	.076
	PMA	16.903	1	16.903
	Trt * PMA	.341	6	.057
	Error	.027	28	.001

Appendix IX: Anova Table for Bacterial Load

Parametre	Source	SS	df	MS
Bacteria load	Model	1300769465020575.000[a]	28	46456052322163.400
	Trt	197912751322751.060	6	32985458553791.844
	PMA	32533180482069.332	3	10844393494023.111
	Trt * PMA	104414514991181.610	18	5800806388398.979
	Error	1545195720164609.000	56	27592780717225.164

Appendix X: Anova Table for Sensory Characteristics

Parametre	Source	SS	df	MS
Aroma	Model	2010.765[a]	7	287.252
	Trt	2010.765	7	287.252
	Error	41.901	56	.748
Tenderness	Model	1134.321[a]	7	162.046
	Trt	1134.321	7	162.046
	Error	34.346	56	.613
Colour	Model	3276.346[a]	7	468.049
	Trt	3276.346	7	468.049
	Error	36.099	56	.645
Acceptance	Model	3420.370[a]	7	488.624
	Trt	3420.370	7	488.624
	Error	29.185	56	.521

Appendix XI: Table for Biochemical Test

SN	GS	Bacteria Isolate	Cat.	Ure.	Ind.	Cit.	MRD	VP	Glu.	Lac.	Suc.	Mot.	Gas.	H_2S	STH
1	+ve	S. aureus	+	+	-	-	-	+	+	+	+	-	-	-	-
2	-ve	P. fluorescens	+	+	-	+	-	-	+	-	+	+	-	-	-
3	-ve	E. coli	+	-	+	-	+	-	+	+	+	+	+	-	+
4	-ve	S. typhi	+	-	-	+	-	-	+	-	-	+	-	+	+
5	-ve	E. coli	+	-	+	-	+	-	+	+	+	+	+	-	+
6	+ve	Strep. Zooepidemicus	+	-	+	+	-	-	+	+	-	-	+	+	+
7	+ve	Strep. Morbillorum	+	-	-	+	+	+	-	-	-	-	+	-	-