EFFECT OF DIETARY INCLUSION OF GINGER MEAL (*Zingiber Officinale*) ON BROILER PERFORMANCE, SERUM LIPID PROFILE AND CARCASS QUALITY

BY

AGU, EKENE CLETUS,

B. AGRIC. TECH. (FUTO)

20124760358

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CERTIFICATION

I certify that this work "**Effect of Dietary Inclusion of Ginger Meal on Broiler Performance, Serum Lipid Profile and Carcass Quality**" was carried out by Agu, Ekene Cletus (20124760358) in partial fulfilment for the award of the degree of Master of Science in Animal Products Technology in the Department of Animal Science and Technology of the Federal University of Technology, Owerri.

..... 21-03-2016

Prof. N J. Okeudo — **Date**
(Principal Supervisor)

..... 21-03-2016

Dr. V.M.O. Okoro — **Date**
(Co-Supervisor)

..... 01. 04. 2016

Dr. E. B. Etuk — **Date**
Ag. HOD, Dept. of Animal Science and Technology

..... 04/04/16

Prof. B.O. Esonu — **Date**
Dean, School of Agric. and Agric. Technology

....

Engr. Prof. (Mrs.) K.B. Oyoh — **Date**
Dean, Postgraduate School

..... 1-4-16

Prof. N.M. Anigbogu — **Date**
External Examiner

DEDICATION

This work is dedicated to the Almighty God, whose favours, guidance and directions saw me through in this academic pursuit. This is because how I started and ended this programme is a miracle.

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ABSTRACT

This study was conducted to evaluate the effect of dietary inclusion of ginger meal (*Zingiber officinale*) on broiler performance, serum lipid profile and carcass quality. One hundred and eighty (180) day old broiler chicks (Cobb strain) were used, and divided randomly into 4 treatment groups, comprising 45 birds each and further subdivided into 3 replicates (15 birds/replicate). Each group was fed one of 4 formulated diets containing ginger meal at levels of 0%, 0.2%, 0.4% and 0.6%, at starter and finisher stages, for 8 weeks in a completely randomized design. At the termination of the experiment, 20 birds (5 birds per treatment) were selected, sacrificed and used for analyses of serum lipid profile, haematological constitution and post mortem microbial load on the meat. Results showed no significant ($P > 0.05$) effects of dietary ginger meal inclusion on live-weight, daily weight gain, daily feed intake and feed conversion ratio. Cholesterol, triglycerides and low density lipoprotein (LDL) were not significantly affected ($P > 0.05$) while high density lipoprotein (HDL) was significantly higher ($P < 0.05$) in the 0.4% ginger meal dietary treatment group. No significant effect ($P > 0.05$) was found on the ether extract of broiler meat associated with ginger meal inclusion in the diet. The haemoglobin, packed cell volume, mean cell haemoglobin, total white blood cell and neutrophil were significantly increased ($P < 0.05$), but the lymphocyte was significantly reduced. Where the red blood cell, mean cell volume, mean cell haemoglobin concentration and platelet were not significantly affected ($P > 0.05$) by the dietary ginger meal inclusion. Heavy bacteria growth of Salmonella species were observed in the broiler meat left after 10 hours of slaughter in all groups, including the control. Significant difference on organ proportion was observed only in the neck at 0.4% ginger meal level ($P < 0.05$). The 0.4% ginger meal treated group was significantly higher in dressing percentage ($P < 0.05$). Meat juiciness, flavour and hedonic score were not affected by ginger meal dietary treatment; however, meat tenderness was significantly higher at 0.4% and 0.6% ginger meal levels ($P < 0.05$) when compared to the control. Dietary inclusion of ginger meal in broiler diets within the levels reported in this study had positive effects on lipoprotein level and organoleptic quality of meat. It is therefore concluded and recommended that the dietary inclusion of ginger meal at 0.4% or 0.6% levels in broiler diets should be encouraged and adopted by poultry producers since it significantly increased broilers' serum high density lipoprotein (HDL) level, dressing percentage, carcass weight and meat tenderness.

Keywords: ginger meal, broiler, cholesterol, lipid profile, meat quality.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Animal protein is essential in human nutrition due to its balanced amino acid profile and relatively high level of availability (Tewe, 1997). Shortage of vital nutrients has been reported in most parts of Africa (Agunbiade *et al.*, 2000). Available evidence suggests that, poultry production is the fastest means of bridging the animal protein deficit in Africa (Oluyemi & Roberts, 1988). This is because, poultry has a short generation interval, high rate of reproduction, and characterized by relatively high efficiency of nutrient transformation into quality animal protein (Akinfala *et al*., 1999).

The chicken is one of the most important animal protein sources for most Nigerians. The growth of broiler chicken is relatively faster with a short production span compared with other meat-producing animals. At the age of 5 to 6 weeks, broiler can reach slaughter weight of 1.5 to 2.0 kg (Herawati & Marjuki, 2011). In terms of volume of production, broilers can be raised intensively in large numbers from hundreds or thousands to hundreds of thousands per farm per year. Thus, broiler production is capable of meeting the demand for meat which is expected to increase continuously, because of the rapidly increasing human population and the requirement for high quality food.

Animal products, especially meat and eggs are major sources of protein in human diets. The increasing concern of consumers to the nutritional value of food has awakened the poultry industry to the fact that "all men are grass" (Kalavathy *et al*., 2003). Consequently, the industry is assuming responsibility towards alleviating the health challenges associated with the consumption of poultry products. In order to achieve this, several measures are constantly

being tested and adopted. These measures are spread across the two fundamentals of animal husbandry, namely breeding and nutrition. Since animal product quality is critically dependent on nutritional regimen, a dietary intervention is crucial to the improvement of poultry products. The competition between humans and domestic fowl for some feedstuffs greatly hampers the production of quality meat and eggs.

Recently, research has become more focused on the use of naturally occurring phytobiotics in replacing the chemically based feed additives (Herawati & Marjuki, 2011). Some phytogenic feed additives have been successfully incorporated into the feeding standard of poultry birds without any deleterious effect or toxic residues (Oyekunle & Owonikoko, 2002). There has been a resurgence of interest in improving the physicochemical and sensory properties of meat, as well as its storage life. Raw and cooked meat quality is of immense consideration to consumers in present scenario. To the consumer, appearance is the major criterion for purchase, selection and initial evaluation of meat quality. Eating quality (texture, flavour, and juiciness) are other main product traits assessed by consumers (Jahan *et al*., 2005). Other quality aspects of meat include food safety, sensory quality, animal welfare and sustainability of production.

In pursuit of improved chicken healthiness and in order to fulfil consumer expectations in relation to food quality, poultry producers are at present applying natural feeding supplements, mainly herbs (Gardzielewska *et al*., 2003). The positive effects of herbal supplements on broiler performance, carcass quality and quality traits of meat have been demonstrated (Schleicher *et al*., 1998). There are several naturally existing herbs (medicinal plants) which could be used in preventing the accumulation of lipids including neutral fats and cholesterol. Prominent among these plants is ginger (*Zingiber officinale*). Ginger has gained prominence due to its wide range of properties not only in reducing lipids but, in many

other ways where the utmost aim is to improve the nutritive value of the animal products (meat, milk or egg) (Bamidele & Adejumo, 2012).

1.2 Problem Statement

The problems that necessitated this research study are of twofold. Firstly, there has been an increasing health concern being associated with the consumption of meat due to its fat distribution. It is known that the body acquires some cholesterol through diet but about two-third is manufactured in the liver. Hepatic production is stimulated by dietary saturated fat. Saturated fats are found predominantly in animal products, such as meat and dairy products, and are highly associated with higher cholesterol levels. When cholesterol levels rise in the blood, they have dangerous consequences, depending on the type of cholesterol. Low density lipoprotein (LDL) particles are the type of cholesterol referred sometimes as "*bad cholesterol"* because they can transport their content of fat molecules into artery walls, attract macrophages, and thus drive atherosclerosis (Good vs. Bad cholesterol, 2014). In contrast, high density lipoprotein (HDL) particles are often called "*good cholesterol* or *healthy cholesterol"* because they remove fat molecules from macrophages in the wall of arteries (Good vs. Bad Cholesterol, 2014). Hence, the need to produce more HDL meat than LDL meat.

Secondly, cold preservation of meat is being hampered due to erratic power supply in the country which results in immense deteriorative losses and product quality depreciation. This necessitates the development of alternative and easily implementable strategies for prolonging the shelf-life of animal products.

1.3 Research Objectives

1. To investigate the effect of dietary inclusion of ginger meal on production performance, serum lipid profile and carcass quality of broiler.
2. To determine at what level the ginger meal would be added in broiler diets.

1.4 Justification of Study

It has been shown that ginger acts as a hypolipidemic agent in cholesterol-fed rabbits (Bhandari *et al*. 1998, Sharma *et al.* 1996). A pure constituent from ginger (E-8 beta, 17 epoxylabd-12-ene-15, 16-dial) was shown to inhibit cholesterol biosynthesis in homogenated rat liver (Tanabe *et al*. 1993). Studies in the past confirm that the growth of gram positive and gram negative foodborne bacteria, yeast and mould can be inhibited by garlic, onion, cinnamon, cloves, thyme, sage and other spices (Smith-Palmer *et al*., 1998). Effects of the presence of these spices/herbs can be seen in food products such as pickles, bread, rice and meat products. Ginger contains the compound caprylic acid, which has potent antifungal properties (Ernst & Pittler, 2000).

Reduction of total fat and cholesterol contents and alteration of the lipid profile to a more unsaturated kind are known strategies for improving the quality of meat health-wise. Achieving this through dietary inclusion of naturally occurring herbs would be a cheap and safe way, and amenable to increased adoption by poultry farmers.

1.5 Scope of Study

The scope of this research work encompassed different areas of animal and food industry such as animal products, animal nutrition, animal health and food science. It included investigation of innovative ways of producing high quality products. Nutritionally, it

investigated the dietary inclusion of herbs, and studied its effect on broiler performance. The safety of the products was investigated as well as its organoleptic quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN, DISTRIBUTION AND DESCRIPTION OF GINGER

2.1.1 Botanical Classification

Ginger or ginger root is the rhizome of the plant *Zingiber officinale* and is consumed as a delicacy, medicine, or spice (Ginger, 2009). It lends its name to its family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom, and galangal. The full classification of ginger is shown below:

Domain	Eukaryote	Has eukaryotic cells, cells move, they make their own food
Kingdom	Plantae	Consists of multicellular organisms that are formed from eukaryotic cells. Organisms in the Plantae kingdom receive their nutrients through photosynthesis. The eukaryotic cells form tissues and have cell walls made of cellulose.
Phylum	Magnoliophyta	This phylum is considered to be an Angiosperm or "flowering plant". An angiosperm is a plant that bears fruit, has seeds, and is vascular
Class	Liliopsida	The Liliopsida class is a monocot or monocotyledon. Monocots have a single cotyledon, floral parts in multiples of threes, and are herbaceous.
Order	Zingiberales	Has flowers composed of colourful specialized leaves, has large leaves with parallel veins, and a long stalk
Family	Zingiberaceae	Aromatic herb
Genus	Zingiber Mill	Ginger
Species	Zingiber officinale	Garden Ginger

Source: USDA, 2011

2.1.2 Global Production of Ginger

Ginger is indigenous to southern China and India, from whence it spread to the Spice Islands and other parts of Asia, and subsequently to West Africa and to the Caribbean (Spices, 2014). Ginger appeared in Europe, via India, in the 1st century CE as a result of the lucrative spice trade (Medical News, 2014). The country of origin is not however known with certainty, but it may have been in India. The antiquity of the use of ginger in India is supported by the fact that the living plant and the dried rhizome are known by different names. The species were known in Germany and France in the 9th century and England in the 10th century (Anonymous, 1970). By the thirteenth and fourteen centuries, it was nearly as common in trade as peppers (Purseglove *et al*, 1981). Preserved ginger from China was imported into Europe as early as the middle ages (Anand, 1982; Purseglove, 1975).

The area under cultivation in India is 107.54 thousand ha and the total production in the country is 385.33 thousand tons (FAO, 2004). The total production of ginger in the world is 1683.00 thousand tons with the total acreage of 310.43 thousand ha (FAO, 2004). China, India, Nepal and Thailand are the major producers of ginger in the world, having production of 396.60 thousand tons, 385.33 thousand tons, 210.79 thousand tons and 172.68 thousand tons respectively (FAO, 2004). India and Indonesia have the largest area under cultivation (FAO, 2004). The area, production and productivity of ginger in the world are shown in Table (2.1). The production of ginger in Nigeria started in 1927 (NRCRI, 2005). It is grown extensively, mainly in the North Central area of Nigeria in Kaduna, Nasarawa, Benue, Niger and Gombe with Kaduna as the major producer.

Table (2.1): Area, Production and Productivity of Ginger in the World

Country	Area (000' ha)	Production (000'tons)	Productivity (tons/ha)
China	36.10	396.60	10.99
India	107.54	385.33	3.58
Nepal	18.04	210.79	11.68
Thailand	10.25	172.68	16.85
Nigeria	52.33	162.22	3.10
Indonesia	60.47	109.02	1.80
Bangladesh	9.07	74.84	8.26
Philippines	3.97	27.10	6.83
Republic of Korea	2.09	24.97	11.98
Sri Lanka	2.07	12.05	5.82
Other Countries	8.51	107.39	12.62
Total	**310.43**	**1683.00**	**5.42**

Source: FAO, 2004

Globally there is an increasing trend in ginger production. For instance, from 1999 to 2007, production increased from 952,222 tons to 1,387,445 tons. In like manner, area under cultivation has increased globally from 762,313 acres (34927.2 Ha) in 1999 to 1,060,818 acres (424,327.2 Ha) in 2007 (FAO, 2004). Available statistics show that ginger production in Nigeria is predominately extensive (Zab Zuro Ginger, 2011).

2.1.3 Ginger: Botany and Cultivation

Ginger is a monocotyledonous plant, a rhizome, although it is often called "ginger root". The rhizome is not a true root as is the case with cassava (Manihot esculenta) but a modified underground stem similar to yams (Dioscorea spp) which produce stem tubers (Zab Zuro Ginger, 2011). It is differentiated from the root by the presence of nodes, buds and scales. Ginger is a perennial herb but treated as an annual crop under cultivation. The plant is about 35-100 cm tall with heavily branched rhizomes called "races" or "hands" and branches called "fingers". Flowering is not common but when they occur, inflorescence arises directly from the root stock. The lance-shaped leaves are bright green, 15 - 20 cm (6-8 in) long, with a prominent longitudinal rib, enclosing conical clusters of small yellow-green flowers marked with purple speckles (Zab Zuro Ginger, 2011).

Ginger grows in the tropics and sub tropics. It can also grow in places that experience dry season but put under irrigation. It requires a mean annual rainfall of 1000 mm that is well distributed over a period of 6 months and supplementary irrigation where rainfall is less than 800 mm per annum. It can also do well in the rain forest region of the country where rainfall is about 2000 mm (NRCRI, 2005) and in altitudes ranging from 800-1,500 m above sea level. Temperature requirement is between 230 - 250 ^{0}C (Zab Zuro Ginger, 2011). Though it grows on a wide range of soils, lateritic loams are preferred for higher yields.

Ginger is planted on raised beds which could be 1.0 m wide and any convenient length. Irrigated ginger crop should be planted on ridges. The crop is propagated vegetatively through rhizomes which are cut into pieces, each having at least a good "bud" or growing point and weighing about 5 g. About 1.25 tonnes of ginger will plant up to one hectare. The planting is done in shallow holes 4 - 5 cm deep made on the seed beds at a distance of 20 cm x 20 cm apart. Under disease free conditions, it is possible to recover the "seed rhizomes" (mother ginger or planting material) at the time of harvest. Ginger is planted in the month of March/April or May/June (Zab Zuro Ginger, 2011). In the rain forest zones it is planted in March while in the savannah zone of Nigeria in the month of May/June (N.R.C.R.I, 2005).

Fertilizer can be inorganic or organic. Broadcasting method of fertilizer application is recommended in order to save time and labour. Compound fertilizer (NPK) 15:15:15 should be applied at the rate of 300 kg/ha soon after planting, and at 12 weeks after planting 120 kg/ha of urea should be applied (Zab Zuro Ginger, 2011). Nitrogen is the most important nutrient for ginger. The quantity of fertilizer to be applied to medium and high fertility soils should be 1/3 to 1/2 of the dosage recommended for low fertility soil (Asumugha *et al*, 2006). In the case of organic fertilizer, well rotten farm yard manure or compost at the rate of 25 - 30 tons/ha may be applied at planting (Zab Zuro Ginger, 2011).

About 10 months after planting, ginger is usually ready for harvesting. It requires at least 32 weeks of field life before harvesting. When the leaves turn yellow or when they start drying up or showing drying back, the rhizomes are ready for harvesting. Harvesting of ginger starts from October and normally continues until April/May (Zab Zuro Ginger, 2011). This largely depends on the market situation as ginger can be left on the ground (not harvested) for 2 years. They should then be dug out with hoes, machetes, spades or even with the digging iron

rod or stick and collected. The roots are then removed. Yields of up to 20 - 30 tonnes per hectare are possible under improved cultural management (Zab Zuro Ginger, 2011).

After harvesting, ginger should be cleaned and stored. Storage of small quantities of rhizome can been done in covered pits or in baskets covered with wet sawdust. This has been experimented at National Root Crop Research Institute, Umudike (NRCRI, 2005). Larger quantities are best stored in heaps under tree shade and covered with dry grass or inside well ventilated huts. The heaps should be cleaned periodically to remove rotted rhizomes. Sometimes farmers leave ginger in soil and harvest as needed (Zab Zuro Ginger, 2011).

2.2 FORMS OF GINGER

Ginger is available in a variety of forms including fresh, dried, pickled, preserved, crystallized (or candied) and powdered or ground (Zab Zuro Ginger, 2011). Ginger is most commonly processed into a dried form.

Fresh ginger or Whole Raw Roots: Fresh ginger comes in young and mature forms. Young ginger has mild flavour and has a pale, thin skin that does not require peeling. The skin of mature ginger on the other hand is tough, which requires peeling to get to the fibrous flesh. It is either chopped or grated before use.

Dried ginger: Dried ginger is available in both whole fingers and slices. Dried roots are sold either with the root skin left on or with the skin peeled off. To prepare dried skinless ginger, the roots are boiled in water, killing the rhizomes. Peeling, scraping or slicing is also done to remove the skin. Before use it is soaked in recipe liquid. Dried ginger may be further processed to produce oil.

Ginger oils: Ginger oil is extracted by steam distillation from the root of the plant. It is used for its antiseptic, aphrodisiac, laxative, stimulant and tonic properties and is often blended with other essential oils to produce many different mixtures for many different ailments. Ginger oil has a spicy and peppery aroma.

Crystallized ginger: Ginger is cooked in sugar syrup, then air dried and coated in sugar to obtain candied ginger.

Pickled ginger: The root of the ginger is sliced paper-thin and pickled in a sweet vinegar solution. This pickle often accompanies sushi and is served to refresh the palate between courses. It is coloured pink or red.

Ground or Powdered Ginger: Ground or powdered ginger is the buff-coloured, ground spice made from dried rhizome. It is used in various recipes.

Preserved ginger: Preserved ginger or stem ginger is made from fresh young rhizome, peeled and sliced, then cooked in a heavy sugar–salt mixture. The ginger pieces and syrup are canned together. They are soft and pulpy, but extremely hot and spicy. It is used both in confection and desserts.

2.3 HERBAL AND INDUSTRIAL USES OF GINGER

Ginger has been used in folk medicine to treat minor gastrointestinal problems such as gas or stomach cramps (Indian Food, 2012). The juice of fresh ginger has been used in folk medicine as a remedy for burns and minor skin irritation. The uses of ginger is not restricted to its raw form of usage, but the oil extracted from ginger has also been proved to be curative in many diseases and is also been used in herbal therapy to ward off pains and other problems (Indian Food, 2012). In recent times, some spas use the ginger oil to bring refreshing effect to

the mind and body. Even ginger tea is recommended together with honey to get relief from cough and cold, and improve digestion (Indian Food, 2012). Sometimes powdered dried ginger root is made into capsules to be taken as a supplement.

Ginger produces a hot, fragrant kitchen spice (McGee, 2004). Young ginger rhizomes are juicy and fleshy with a very mild taste. They are often pickled in vinegar or sherry as a snack or just cooked as an ingredient in many dishes. They can also be steeped in boiling water to make ginger tea, to which honey is often added; sliced orange or lemon fruit may also be added. Ginger can also be made into candy, or ginger wine which has been made commercially since 1740 (Plain Ginger Tea, 2012).

The juice from old ginger roots is extremely potent (Baliga *et al.*, 2012) and is often used as a spice in Indian recipes, and is a quintessential ingredient of Chinese, Korean, Japanese, Vietnamese and many South Asian cuisines for flavouring dishes such as seafood or goat meat and vegetarian cuisine (McGee, 2004).

Ginger acts as a useful food preservative (Baliga *et al.*, 2011). Fresh ginger can be substituted for ground ginger at a ratio of 6 to 1, although the flavours of fresh and dried ginger are somewhat different. Powdered dry ginger root is typically used as flavouring for recipes such as gingerbread, cookies, crackers and cakes, ginger ale, and ginger beer.

Candied ginger, or crystallized ginger, is the root cooked in sugar until soft, and is a type of confectionery. Fresh ginger may be peeled before eating. For longer-term storage, the ginger can be placed in a plastic bag and refrigerated or frozen.

2.4 GINGER AND ITS BIOACTIVE COMPONENTS

In fresh and dried ginger varieties, at least 115 constituents have been identified by a variety of analytical processes. The major constituents of fresh ginger are the gingerols which have been found slightly reduced in dry ginger, whereas the concentrations of shogaols, which are the major gingerol dehydration products, are more abundant in dry ginger than in fresh ginger (Jolad *et al*. 2005). At least 31 gingerol-related compounds have been identified from the methanolic crude extracts of fresh ginger rhizome (Jiang, Solyom *et al*. 2005). Ginger has been fractionated into at least 14 bioactive compounds, including [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]-shogaol, [6]-shogaol, 1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol (Koh *et al.* 2009). The proportion of each individual component in a sample of ginger depends on country of origin, commercial processor, and whether the ginger is fresh, dried, or processed (Schwertner *et al*., 2006). Of the bioactive pungent components of Jamaican ginger, including [6]-, [8]-, and [10]-gingerols and [6]-shogaol, [6]-gingerol appears to be the most abundant pungent bioactive compound in most of the oleoresin samples studied (Bailey-Shaw *et al*. 2008). Although phylogenetic analysis has shown that all ginger samples from widely different geographical origins are genetically indistinguishable, metabolic profiling showed some quantitative differences in the contents of [6]-, [8]-, and [10]-gingerols (Jiang *et al*. 2006). An examination of the concentrations of [6]-, [8]-, and [10]-gingerols and [6]-shogaol in 10 different ginger-root dietary supplements purchased randomly from a variety of pharmacies and health food stores yielded some disconcerting results (Schwertner *et al*., 2006). Perhaps not surprisingly, the content of these active components was found to vary extensively from none or very minute amounts to several milligrams per gram. In addition, the suggested serving size ranged from about 250 mg to 4.8 g/day (Schwertner *et al*., 2006).

The basis for the wide range of dosing is not clear. These studies suggest that ginger contains a variety of bioactive compounds and standardization of contents is critically lacking.

2.5 GINGER AND ITS HEALTH EFFECTS

The gastrointestinal tract appeared to be accumulated by ginger and its metabolites, and for this reason the consistent observations of ginger exerting many of its effects in this area are not surprising (Ann & Zigang, 2011). Ginger has been reported to exert a variety of powerful therapeutic and preventive effects and has been used for thousands of years for the treatment of hundreds of ailments from colds to cancer. Like many medicinal herbs, much of the information has been handed down by word of mouth with little controlled scientific evidence to support the numerous claims. However, in the last few years, more organized scientific investigations have focused on the mechanisms and targets of ginger and its various components (Ann & Zigang, 2011).

2.5.1 Antioxidant Properties of Ginger

The presence of oxidative stress is associated with numerous diseases and a common mechanism often put forth to explain the actions and health benefits of ginger is associated with its antioxidant properties (Aeschbach *et al*., 1994; Ahmad *et al*., 2001). Ginger was reported to decrease age-related oxidative stress markers (Topic *et al*., 2002) and was suggested to guard against ethanol-induced hepatotoxicity by suppressing oxidative consequences in rats treated with ethanol (Mallikarjuna *et al*., 2008). Ginger root contains a very high level (3.85 mmol/100 g) of total antioxidants, surpassed only by pomegranate and some types of berries (Halvorsen *et al*., 2002). Reports have shown that ginger compounds effectively inhibited superoxide production (Krishnakantha & Lokesh, 1993). Other reports indicated that ginger suppressed lipid peroxidation and protected the levels of reduced

glutathione (GSH; Reddy & Lokesh 1992; Ahmed *et al*., 2000; Shobana & Naidu 2000; Ahmed *et al*., 2008; El-Sharaky *et al*., 2009).

Reactive nitrogen species, such as nitric oxide (NO) influence signal transduction and cause DNA damage, which contributes to disease processes. Nitric oxide is produced by inducible nitric oxide synthase (iNOS), which is stimulated in response to various stresses. [6]-gingerol was reported to dose-dependently inhibit NO production and reduce iNOS in lipopolysaccharide (LPS)-stimulated mouse macrophages (Ippoushi *et al*., 2003). [6]-gingerol also effectively suppressed peroxynitritemediated oxidative damage (Ippoushi *et al*., 2003). Ippoushi *et al*. (2003) later proposed that [6]-gingerol and peroxynitrite form a symmetric dimer with [6]-gingerol covalently linked at the aromatic ring of peroxynitrite, attenuating peroxynitrite-induced oxidation and nitration reactions (Ippoushi *et al*., 2005). [6]-shogaol, 1-dehydro-[10]-gingerdione, and [10]-gingerdione also decreased LPS-induced NO production, and [6]-shogaol and 1-dehydro-[10]-gingerdione were reported to effectively reduce iNOS expression (Koh *et al*., 2009). In the bromobenzene induced hepatotoxicity model, orally given ginger extract (100 mg/kg body weight) normalized NO levels and total and reduced glutathione levels, and also decreased the level of lipid peroxidation (El-Sharaky *et al*., 2009). Ginger consumption has also been reported to decrease lipid peroxidation and normalize the activities of superoxide dismutase and catalase, as well as glutathione and glutathione peroxidase, glutathione reductase, and glutathione-S-transferase in rats (Ahmed *et al*., 2008).

Ginger extract has been reported to exert radio protective effects in mice exposed to gamma radiation (Jagetia *et al*., 2003), and the effect was associated with decreased lipid peroxidation and protection of GSH levels (Jagetia *et al*., 2004). [6]-gingerol pre-treatment also decreased oxidative stress induced by ultraviolet B and activated caspase-3, -8, -9, and FAS expression (Kim *et al*., 2007).

2.5.2 Anti-Inflammatory Effects of Ginger

The ability of ginger to decrease inflammation, swelling and pain is among many health claims attributed to it. A dried ginger extract, [6]-gingerol (Young *et al*., 2005) and a dried gingerol-enriched extract (Minghetti *et al*., 2007) were each reported to exhibit analgesic and potent anti-inflammatory effects. Earlier animal studies suggest that rat hind limbs perfused with [6]-gingerol showed increased heat production that was associated with increased oxygen consumption and lactate efflux (Eldershaw *et al*., 1992). The thermogenesis was at least partly associated with vasoconstriction independent of adrenergic receptors or secondary catecholamine release. In contrast, larger doses of ginger components inhibited oxygen consumption, which was attributed to disruption of mitochondrial function (Eldershaw *et al*., 1992). These results were supported in a later study in which rats that were given a single intraperitoneal injection of [6]-gingerol (2.5 or 25 mg/kg) exhibited a rapid, marked drop in body temperature and a significant decrease in metabolic rate (Ueki *et al*., 2008).

Results suggest that ginger may exhibit anti-inflammatory effects through the modulation of calcium levels mediated through transient receptor potential vanilloid subtype 1 (TRPV1), which is a heat-and pain-sensitive receptor that can interact with [6]-gingerol (Dedov *et al*., 2002). [6]-gingerol has been reported to induce a substantial rise in intracellular calcium levels in Madin-Darby canine kidney renal tubular cells by stimulating both extracellular calcium influx and thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor)-sensitive intracellular calcium release (Chen *et al*., 2008). The gingerols are known to be transient receptor potential vanilloid subtype 1 (TRPV1) agonists (Dedov *et al*., 2002), and the [6, 8,10]-gingerols and [6,8,10]-shogaols can increase the intracellular calcium concentration in TRPV1-expressing HEK293 cells through TRPV1 (Iwasaki *et al*. 2006). Shogaols appear to be more potent than the gingerols, and most of the compounds cause aversive or nociceptive

responses mediated by TRPV1 when applied to the eye or following subcutaneous injection to the hind paw, respectively (Iwasaki *et al*., 2006). In this case, most of the ginger compounds also promoted adrenal catecholamine secretion, which influences energy consumption (Iwasaki *et al*., 2006).

Ginger has been suggested to be effective against osteoarthritis and rheumatism (Reginster *et al*., 2000). However, inconsistencies in clinical studies have led to debate regarding the effectiveness and safety of ginger for treatment of arthritis (Marcus & Suarez-Almazor, 2001). An earlier study showed that ginger oil (33 mg/kg), administered orally to rats for 26 days, caused a significant repression of paw and joint swelling associated with severe chronic adjuvant arthritis (Sharma *et al*., 1994). More recently, the effectiveness of a crude ginger extract was compared with a fraction containing only gingerols and derivatives to inhibit joint swelling in the streptococcal cell wall-induced arthritis animal model of rheumatoid arthritis (Funk *et al*., 2009). Results indicated that although both extracts could prevent joint inflammation, the crude dichloromethane extract, which also contained essential oils and more polar compounds, was more effective (when normalized to gingerol content) in preventing both joint inflammation and destruction (Funk *et al*., 2009). In humans, one study showed no difference between placebo and ginger in patients with osteoarthritis of the hip or knee (Bliddal *et al*., 2000). In contrast, patients suffering from osteoarthritis of the knee showed a consistently greater response to treatment with ginger extract compared with the control group (Altman & Marcussen, 2001). In addition, relief from pain and swelling was reported in patients suffering from rheumatoid arthritis, osteoarthritis, or general muscular discomfort when using powdered ginger as a dietary supplement for 3 months to 2 years (Srivastava & Mustafa, 1992). Besides pain relief from arthritis, results of a double-blind comparative clinical trial indicated that ginger (250-mg capsules) was as effective as the nonsteroidal anti-inflammatory drugs mefenamic acid (250 mg) and ibuprofen (400 mg) in

relieving pain in women with primary dysmenorrhea (Ozgoli *et al*., 2009b). In contrast, consumption of 2 g of ginger before 30 minutes of cycling exercise (60 % VO_2) had no effect on quadriceps muscle pain, rating of perceived exertion, work rate, heart rate or oxygen uptake (Black & O'connor, 2008).

The majority of scientific evidence does seem to suggest that ginger and its various components have anti-inflammatory effects both *in vitro* and *ex vivo*. However, the data supporting ginger as an effective anti-inflammatory agent in humans *in vivo* are still contradictory and incomplete (Ann & Zigang, 2011)

2.5.3 Ginger as an Antinausea Agent

The utilization of ginger in alleviating symptoms of nausea and vomiting is probably the most common and well-established use of ginger throughout history. The benefits and dangers of herbal treatment of liver and gastrointestinal distress have been reviewed (Langmead & Rampton, 2001), and several controlled studies have reported that ginger is generally effective as an antiemetic (Borrelli *et al*., 2005; Bryer, 2005; Mahesh *et al*., 2005; Chaiyakunapruk *et al*., 2006; Thompson & Potter, 2006; Quimby, 2007). The effectiveness of ginger as an antiemetic has been attributed to its carminative effect, which helps to break up and expel intestinal gas. This suggestion was supported by the results of a randomized, double-blind trial in which healthy volunteers reported that ginger effectively accelerated gastric emptying and stimulated antral contractions (Wu *et al*., 2008). Previously, [6]-gingesulfonic acid, isolated from ginger root, was showed to be effective against HCl/ethanol-induced gastric lesions in rats (Yoshikawa *et al*., 1992). This compound showed weaker pungency but more potent antiulcer activity than [6]-gingerol or [6]-shogaol (Yoshikawa *et al*., 1994).

Ginger root is commonly recommended for prevention of sea-sickness (Schmid *et al*., 1994) and is found to be superior to dimenhydrinate (Dramamine) or placebo against symptoms of motion sickness (Mowrey & Clayson, 1982). A follow-up study also indicated that 1 g of ginger might be effective in reducing the subjective severity of seasickness in naval cadets on the high seas (Grontved *et al*., 1988). On the other hand, additional research studies showed no benefits of using ginger for treating motion sickness (Wood *et al*., 1988; Stewart *et al*., 1991), and at least one group reported that patients receiving ginger extract for treating osteoarthritis experienced more, although mild, gastrointestinal adverse events compared to a placebo-treated group (Altman & Marcussen, 2001). The exact antiemetic mechanism of ginger is not clear, although some evidence suggests that it inhibits serotonin receptors and exerts its antiemetic effects directly on the gastrointestinal system and in the central nervous system (DerMarderosian & Beutler, 2006). Although the antiemetic effects of ginger are the most well-studied effects of this condiment and have been reviewed extensively, the effectiveness and safety of ginger for treating nausea and vomiting have been questioned in the past because the findings reported were often contradictory (Wilkinson, 2000b). At the same time, ginger continues to be recommended for alleviating nausea and vomiting associated with pregnancy, chemotherapy, and certain surgical procedures (Aikins Murphy, 1998).

2.5.4 Anticarcinogenic Activities of Ginger

Focus on the cancer-preventive and potential cancer therapeutic applications of ginger and its various components is now of great interest by numerous research groups. Several aspects of the chemo-preventive effects of numerous phytochemical dietary and medicinal substances, including ginger, have been reviewed previously (Surh *et al*., 1998; Surh, 1999, 2002; Bode and Dong, 2004; Shukla & Singh, 2007; Aggarwal *et al*., 2008). Studies focused on the

anticancer activities of various forms of ginger, from a crude or partially purified extract to gingerols (especially [6]-gingerol) shogaols (especially [6]-shogaol) and zerumbone (a sesquiterpene compound derived from ginger) and a number of minor components and metabolites have been carried out. The effectiveness of ginger in preventing or suppressing cancer growth has been examined in a variety of cancer types, including lymphoma, hepatoma, colorectal cancer, breast cancer, skin cancer, liver cancer and bladder cancer (Aggarwal *et al*., 2008).

The anticancer activities of [6]-gingerol and zerumbone have been associated with their antioxidant activities. Several ginger components were reported to have effective anticancer promoter activity based on their ability to inhibit tissue-type plasminogen activator (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) in Raji cells (Vimala *et al*., 1999; Kapadia *et al*., 2002). [6]-gingerol was reported to suppress the reactive oxygen species-potentiated invasive capacity of ascites hepatoma AH109A cells by reducing peroxide levels (Yagihashi *et al*., 2008). Others have reported that zerumbone decreases TPA-induced hydrogen peroxide formation and edema corresponding to enhanced levels of various antioxidant enzymes (Murakami *et al*., 2004). These types of changes have been linked with lower 7,12-dimethylbenz[a]anthracene (DMBA)-initiated/TPA-promoted tumor incidence, number of tumors per mouse, and tumor volume (Murakami *et al*., 2004).

Earlier studies suggest that gingerol is an effective inhibitor of azoxymethane-induced intestinal carcinogenesis in rats (Yoshimi *et al*., 1992). Ginger supplementation (50 mg/kg BW) was reported to suppress the number of tumors as well as the incidence of 1, 2-dimethylhydrazine (DMH)-induced colon cancer (Manju & Nalini, 2005). The effect was attributed to decreased oxidative damage associated with enhanced catalase, superoxide dismutase, glutathione peroxidase, and glutathione transferase activities as well as increased

GSH (Manju & Nalini, 2005). This group later reported that administration of ginger to DMH-treated rats significantly decreased the incidence and number of tumors as well as the activity of microbial enzymes, β-glucuronidase, and mucinase (Manju & Nalini, 2006). Finally, Wistar rats that were fed a ginger extract (1% mixed in diet) exhibited significantly lower multiplicity of urothelial lesions (hyperplasia and neoplasia) than untreated groups (Ihlaseh *et al*., 2006).

2.5.5. Cardiovascular and Other Disease-Preventive Effects of Ginger

In addition to its effects in relation to cancer, some evidence supports a protective role for ginger in cardiovascular function and a number of other disease conditions. Ginger has gained interest for its potential to treat various aspects of cardiovascular disease, and the *in vitro* and animal data supporting the anti-inflammatory, antioxidant, antiplatelet, hypotensive, and hypolipidemic effects of this condiment have been reviewed (Nicoll & Henein, 2009). However, human trials are less convincing and more investigations are needed (Nicoll & Henein, 2009). Caution when taking ginger and other herbal extracts has been suggested because of an apparent association of ginger with reported incidences of increased risk of bleeding following surgery (Chang & Whitaker, 2001; Pribitkin & Boger, 2001) or if taken with anticoagulant drugs such as warfarin (Heck *et al*., 2000). However, the data are not conclusive (Vaes & Chyka, 2000). At least one study indicates that ginger has no effect on blood pressure, heart rate, or coagulation parameters and does not interact with anticoagulant drugs such as warfarin (Weidner & Sigwart, 2000). These findings were supported in a later study in which ginger was reported to have no effect on clotting status or the pharmacokinetics or pharmacodynamics of warfarin in healthy subjects (Jiang, Williams *et al*., 2005). An aqueous ginger extract was reported to induce a dose-dependent decrease in arterial blood pressure in a variety of animal models (Ghayur & Gilani, 2005a,b).

At least one group found that administration or consumption of standardized ginger extract decreased aortic atherosclerotic lesion areas, plasma triglycerides and cholesterol, low-density lipoprotein (LDL)-associated lipid peroxides, and LDL aggregation in mice (Fuhrman *et al*., 2000). In rabbits that were fed a high-cholesterol diet, administration of ginger extract resulted in a significant antihyperlipidemic effect and a lower degree of atherosclerosis compared to the group that was fed cholesterol alone (Bhandari *et al*., 1998). Importantly, ginger powder (3 g/day in 1-g capsule 3xd) significantly lowered lipid levels in volunteer patients in a double-blind, controlled clinical trial study (Alizadeh-Navaei *et al*., 2008). Triglyceride and cholesterol were substantially decreased as was LDL levels compared to placebo group. Notably, the high-density lipoprotein (HDL) level of the ginger group was higher than that of the placebo group, whereas the very-low-density lipoprotein (VLDL) level of the placebo group was higher than that of the ginger group (Alizadeh-Navaei *et al*., 2008). Dried ginger powder (0.1 g/kg BW, per oral administration [p.o.] for 75 days) significantly lowered (50 %) the development of atheroma in the aorta and coronary arteries of rabbits that were fed cholesterol (Verma *et al*., 2004). This effect was associated with decreased lipid peroxidation and increased fibrinolytic activity with ginger, but blood lipid levels were not different from control animals (Verma *et al*., 2004). Another compound isolated from ginger, (E)-8 β, 17-epoxylabd-12-ene-15, 16-dial, was reported to inhibit cholesterol biosynthesis (Tanabe *et al*., 1993), and ginger meal (1 %) decreased serum cholesterol levels significantly (Dias *et al*., 2006). Ginger was also reported to slightly reduce retinoid-binding protein mRNA expression levels in liver and visceral fat in male rats that were fed cholesterol to induce hyperlipidemia (Matsuda *et al*., 2009). These results hint that ginger consumption might improve lipid metabolism (Matsuda *et al*., 2009).

Antiplatelet therapy is an effective approach for preventing coronary heart disease. Ginger components are suggested as a potential new class of platelet-activation inhibitors without the

potential side effects of aspirin, which is most commonly used in this approach. In a comparison of gingerols and analogues with aspirin, ginger compounds were found to be less potent compared to aspirin in inhibiting arachidonic acid-induced platelet release and aggregation and COX activity (Koo *et al*., 2001). However, several analogues had a significant inhibitory effect, suggesting that further development of more potent gingerol analogues might have value as an alternative to aspirin therapy in preventing ischemic heart disease (Koo *et al*., 2001). Consumption of ginger (5 g) inhibited platelet aggregation induced in men who consumed 100 g of butter daily for 7 days (Verma *et al*., 1993), and a later study showed that ginger enhanced fibrinolytic activity (Verma & Bordia, 2001). An evaluation of the antiplatelet activity of 20 pungent constituents of ginger revealed that [8]-paradol was the most potent COX-1 inhibitor and antiplatelet aggregation agent (Nurtjahja-Tjendraputra *et al*., 2003). [8]-gingerol and [8]-shogaol were also found to be effective antiplatelet aggregation agents (Nurtjahja-Tjendraputra *et al*., 2003).

Ginger is also used to treat asthma, diabetes, and other conditions. Components of ginger rhizomes are reported to contain potent compounds capable of suppressing allergic reactions and might be useful for the treatment and prevention of allergic diseases (Chen *et al*., 2009). Ghayur *et al*., (2008) reported that a ginger extract inhibits airway contraction and associated calcium signalling, possibly by blocking plasma membrane calcium channels. In a mouse model of Th2-mediated pulmonary inflammation, an intraperitoneal injection of a ginger extract mainly comprised of gingerols markedly decreased the recruitment of eosinophil to the lungs in ovalbumin-sensitized mice and also suppressed the Th2 cell-driven response to allergen (Ahui *et al*., 2008).

Dried ginger may have beneficial effects in treating dementia, including Alzheimer's disease (Ghayur *et al*., 2008). Ulcerative colitis is a chronically recurrent inflammatory bowel disease

of unknown origin, and in rats, ginger extract alleviated the symptoms of acetic acid-induced ulcerative colitis (El-Abhar *et al*., 2008).

2.6 SAFETY, EFFICACY AND CONTRAINDICATIONS OF GINGER

The U.S. Food and Drug Administration (FDA) recognizes ginger as a food additive that is "generally recognized as safe." However, and notably, in 1930, thousands of Americans were poisoned and paralyzed by an illicit extract of Jamaican ginger (Jake) that was used to circumvent prohibition laws. The extract had been adulterated with a neurotoxic organophosphate compound, triorthocresyl phosphate (TOCP) (Crandall, 1931; Morgan & Penovich, 1978). The extract was banned in 1931.

Early studies suggest that ginger extract increased the mutagenesis ability of 2(2-furyl)-3(5-nitro-2-fury) acryl amide (AF2) or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG), and [6]-gingerol was determined to be an active mutagen (Nakamura & Yamamoto, 1982). A later study suggests that [6]-shogaol is much less mutagenic than [6]-gingerol and that the active part of [6]-gingerol is the aliphatic chain moiety containing a hydroxyl group (Nakamura & Yamamoto, 1983). Though, these studies have not been confirmed nor repeated, and no recent evidence suggests that ginger or its components are mutagenic.

Weidner & Sigwart, (2001) reported that oral administration of a ginger extract (1000 mg/kg) was well tolerated by pregnant rats, and it exerted no adverse effects on the mothers or in the development of foetuses. This result is somewhat in contrast to an earlier study, in which administration of ginger tea to pregnant rats resulted in twice the loss of embryos but heavier surviving foetuses compared to untreated controls (Wilkinson, 2000a). Ginger rhizome extract (0.5-10.0 g/kg) administered intraperitoneally to mice was reported to have no

clastogenic effects compared to ginger oil, which produced some chromosomal irregularities (Mukhopadhyay and Mukherjee, 2000).

Most recently, male and female rats that were fed ginger powder (500, 1000, or 2000 mg/kg BW) by gavage for 35 days did not exhibit any overall mortalities or abnormalities in behaviour, growth, or food and water consumption (Rong *et al*., 2009). No overt organ abnormalities were observed and haematological and blood biochemical parameters in treated and untreated control animals were similar. The only real difference observed was a slightly decreased absolute and relative weight of the testes only at the highest dose (2000 mg/kg; Rong *et al*., 2009). Observational studies in humans suggest no evidence of teratogenicity from treatments for early pregnancy nausea that included ginger (Jewell & Young, 2003). These results were confirmed in a similar trial showing that administration of ginger beginning at the first trimester of pregnancy did not appear to increase the rates of major malformations above the baseline rate of 1-3 % (Portnoi *et al*., 2003). Overall, these data indicate that ginger consumption appears to be very safe with very limited side effects.

2.7 APPLICATION OF PHYTOBIOTICS IN POULTRY PRODUCTION

Phytobiotics, also called phytogenic feed additives (PFA) can be defined as plant derived products added to feed in order to improve performance of livestock through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut (Kamel, 2001; Balunas & Kinghorn, 2005; Athanasiadou *et al*., 2007) . They originate from leaves, roots, tubers or fruits of herbs, spices and other plants. They may be available in solid, dried, and ground forms, or as extracts. In simple terms, phytobiotics are products of plant origin, and preparations such as thyme, ginger, oregano, turmeric and garlic are gaining interest among researchers and poultry producers.

The effects of FPA on production performance have shown promising results. Cabuk *et al*. (2006) measured production parameters in chickens grown on feed supplemented with a mixture of essential oils of oregano, bay leaf, sage, anise and citrus. This mixture significantly improved feed conversion. Lippens *et al*. (2005) assessed the effectiveness of a mixture composed of cinnamon, oregano, thyme, cayenne pepper, citrus extracts and of another mixture of plant extracts and organic acids in comparison with the avilamicine in chicken feed. Birds supplemented with plant extracts gained more body weight than the other groups. The increase in body weight was due to increase in feed intake. Efficiency of Feed conversion in birds on plant extracts was 0.4% lower than the avilamicine group and 2.9% lower than the organic acids group. Fenugreek seeds supplementation improved significantly feed conversion in broiler chickens which might be related to morphological changes in the gastrointestinal tissues (Srinivasan, 2006; Alloui *et al*., 2012; Mamoun *et al*., 2014). The inclusion of anise seeds at a level of 0.5 - 0.75 g/kg in corn-soybean-meal based diet and fed to broilers during 6 weeks, improved body weight gain. In contrast, a higher inclusion level of (1.5 g/kg diet) reduced growth performance (Soltan *et al*., 2008).

Garlic (*Allium sativum*), thyme (*Thymus vulgarus*) and conflower (*Echinacea purpurea*) as feed supplements have recently been reported to exert a wide range of beneficial effects on weight gain, feed conversion and egg production and quality of broilers and laying hens (Rahimi *et al*., 2011; Khan *et al*., 2012). Bolukbasi & Erhan (2007) studied the effect of dietary supplementation with thyme on performance of laying hens and *E. coli* concentrations in their faeces. Thyme addition to diet at the level of 0.1 - 0.5% resulted in an improvement in feed conversion and egg production associated to a decline of *E. coli* concentration in faeces. Effect of PFA on egg quality traits, such as yolk composition, shell thickness or Haugh unit rating were reported in a few studies only, although the majority of reports did not identify substantial effects (Nichol & Steiner, 2008; Shing *et al*., 2009; Navid *et al*., 2013).

2.8 MEAT QUALITY AND ITS FACTORS

Meat quality is objectively defined as the sum of all quality factors of meat (Hofmann, 1994). The quality factors may be presented in groups that are closely related and determine a defined component of meat quality (Table 2.3). The quality factors related to visual appeal (colour, water holding capacity and fatness) and palatability (tenderness, juiciness, flavour and aroma) are regarded as the key factors that determine consumers' initial and continued interest in the meat (Chambers IV & Bowers, 1993; Issanchou, 1996). These factors may be evaluated directly or indirectly by various physical, biochemical, histological and sensory analyses.

2.8.1 Meat Colour

Colour is one of the most important factors in consumers' selection and decision to purchase meat and meat products (Hedrick *et al*., 1994). It is considered to be the single most influential criterion in this process (Kropf, 1980). For that reason an assessment of colour is included in some carcass classification systems (Page *et al*., 2001).

The characteristic colour of meat is a function of its pigment content and light scattering properties (MacDougall, 1982, Ledward, 1992). Myoglobin is the basic pigment in fresh meat and its content varies with production factors such as species, animal age, sex, feeding system, exercise, type of muscle and muscular activity (Ledward, 1992; Varnam & Sutherland, 1995). Myoglobin's physio-chemical state; i.e. purple reduced myoglobin, red oxymyoglobin and brown metmyoglobin, determines the colour of fresh meat (Varnam & Sutherland, 1995; Lawrie, 1998). Formation of the desirable oxymyoglobin is enhanced by conditions that increase oxygen solubility, such as low temperature, low pH, high oxygen tension, and low enzyme activity (MacDougall, 1982; Ledward, 1992).

Table 2.2: The major components and factors of meat quality

Component	Meat quality factors
Yield and gross composition	Ratio of fat to lean
	Muscle size and shape
Appearance and technical characteristics	Colour and water holding capacity of lean
	Fat texture and colour
	Marbling (intramuscular fat)
	Chemical composition of lean
Palatability	Texture and tenderness
	Juiciness
	Flavour
	Aroma
Wholesomeness	Nutritional quality
	Chemical safety
	Microbial safety
	Acceptable animal husbandry

Adapted from Hofmann (1994)

Meat pH has a great effect on colour development through its effect on the physical state of muscle proteins (Abril *et al*., 2001). At high ultimate pH (pHu > 6.0), myofibres hold a lot of water and swells consequently (Offer & Trinick, 1983). At such high myofibrillar volume, incident light is not able to penetrate considerable depth and be absorbed by myoglobin before it is scattered (MacDougall, 1982). Therefore, the meat appears translucent and dark. Furthermore, enzymes that use up oxygen are more active resulting in less oxygenation of the surface myoglobin and a dark colour (Price & Schweigert, 1987; Ledward, 1992). At normal pHu (~5.5), the myofibres hold less water, and oxygen utilising enzymes are less active. The meat appears brighter and glossier (Ledward, 1992). At the other extreme, low pH meat is pale (MacDougall, 1982; Ledward, 1992). This is due to reduced myofibrillar volume (Offer & Trinick, 1983) as well as the denaturation of denatured myosin and sarcoplasmic proteins which increase the refractive properties of the meat (MacDougall, 1982; Offer & Trinick, 1983). Consequently, more incidents light is scattered resulting to a pale colouration.

Besides its strong relationship with pH, meat colour is also highly correlated with water holding capacity, evidently because the changes in myofibrillar lattice with pH also affect the ability of the muscle to retain water (Orcutt *et al*., 1984; Purchas, 1990; Watanabe *et al*., 1996). Meat colour is reported to be related to tenderness (Purchas, 1990; Jeremiah *et al*., 1991; Watanabe *et al*., 1996; Wulf *et al*., 1997) and carcass fatness (Tatum *et al*., 1982; Page *et al*., 2001).

Meat colour is objectively defined often in terms of the CIELAB co-ordinates, L*, a* and b* (Warriss, 2000). L* is the lightness component, indicating the black-whiteness of the meat. Its values range from 0 (all light absorbed) to 100 (all light reflected); a* spans from -60^0 (green) to $+60^0$ (red) and b* spans from -60^0 (blue) to $+60^0$ (yellow) (Young *et al*., 1999). Other

parameters may be calculated from these basic three, such as hue angle [$\tan^{-1}$ (b*/a*)], which describes the fundamental colour of a substance; and chroma [$\sqrt{}$ ($a^{*2}+b^{*2}$)], which describes the vividness of the colour. Hunter a* and chroma have been observed to be strongly related to visual colour scores (Eargerman *et al*., 1978).

2.8.2 Water Holding Capacity

The ability of meat to retain its natural water content is termed water holding capacity (WHC, Hamm, 1986). Most of the water is held in the interfilament spaces within the myofilament lattice. The amount held depends on the volume of the interfilament spaces (Offer & Trinick, 1983) which in turn is determined by pH, sarcomere length (degree of contraction), ionic strength, osmotic pressure and whether the muscle is in pre- or post-rigor (Offer & Trinick, 1983).

Water holding capacity is high at high muscle pH and in fact water is not readily lost from meat that is cut soon after slaughter (Offer & Trinick, 1983). This is because at high pH, the net negative charge of myofilaments results in strong repulsive electrostatic forces between the filaments, which push the filaments apart, swells up the lattice and hence increases the space where water is lodged. As the pH declines, the negative charge and hence the repulsive force of the filaments is gradually lost to a point when the filaments have no net charge at the isoelectric point of actin and myosin (about pH 5.0, Hamm, 1986). The myofilaments relax, thus shrink the interfilament space and in so doing expel the water. The expelled water accumulates in the space between the muscle fibres and the endomysium and is driven to the cut surfaces by the pressure of the endomysium (Offer & Knight, 1988). Water holding capacity of meat is at its lowest at pH 5.0 and any alteration of pH in the range 5.0 to 6.5 has a great influence on WHC (Hamm, 1986). The WHC of meat is closely correlated to meat colour in that both factors are largely determined by the effect of pH on the myofilament

lattice structure (Offer & Knight, 1988). Water holding capacity also increases with increase in intramuscular fat content, probably because the fat loosens up the myofibre microstructure and allows more water to be entrained (Lawrie, 1998). It is for this reason that good quality meat loses less water during cooking besides the fact that it has less water and more fat (Lawrie, 1998).

Cooking losses occur through a similar mechanism to drip loss. The denaturation of the myosin at 40^{o} to 53ºC causes a transversal shrinkage in the myofibres and a slow loss of water from the myofibres (Bendall & Restall, 1983). At 60 ºC, the collagen of the basement membrane shrinks, resulting in rapid fluid loss from the myofibres (Bendall & Restall, 1983). Above 64 ºC collagen of the perimysium and endomysium network shrinks (Sims & Bailey, 1981; Bendall & Restall, 1983; Bailey, 1984) and thus exerts more pressure on the aqueous solution leading to a rapid loss of volume of the cooked meat.

In addition to its effect on the aesthetic appeal of the meat, WHC affects the technological value of the meat; how well it can be processed into other products. An example is that although it is aesthetically unappealing, dark cutting meat is perfectly acceptable for a number of manufacturing purposes because of its high WHC (Hofmann, 1994). Water holding capacity of fresh meat is best determined by gravity and suction methods that do not destroy the tissue or denature the proteins (Hofmann, 1994, Honikel, 1998). Cooking loss is recommended for heated meat (Hofmann, 1994).

2.8.3 Fat in Meat

Fat is present in meat as a structural component of muscle membranes and as storage droplets between muscle fibres. The latter constitute what is perceived as marbling (Varnam & Sutherland, 1995). Marbling affects consumers' visual appreciation and their perception of

the eating quality of the meat, and hence their decision of whether or not to buy the meat (Issanchou, 1996; Brewer *et al*., 2001). Increased marbling is associated with good eating quality (Dolezal *et al*., 1982; Fernandez *et al*., 1999). Dolezal *et al*. (1982) demonstrated this phenomenon in that juiciness, tenderness and flavour desirability increased with increase in beef marbling score. In a similar line of research, Fernandez *et al*. (1999) reported an increase in ratings for pork flavour, tenderness and juiciness with increase in intramuscular fat content.

Although it may be associated with good eating quality, excessive marbling is unacceptable to consumers (Brewer *et al*., 2001). The extent of marbling and the colour of fat in red meat modify consumers' perception of the meat colour (Varnam & Sutherland, 1995). Fat is implicated in the oxidative stability of meat and hence its shelf life (Gray *et al*., 1996; Morrissey *et al*., 1998; Enser, 2001). The oxidative stability of meat is dependent on the balance between oxidative substrates (e.g. the polyunsaturated fatty acids or the phospholipids); pro-oxidants (e.g. haem proteins such as myoglobin, haemoglobin and cytochromes) and anti-oxidants e.g. vitamin E (Morrisey *et al*., 1998). Once the balance is upset, oxidative deterioration occurs and results in adverse changes in colour, flavour, texture, nutritive value and possibly the production of toxic compounds (Kanner, 1994; Gray *et al*., 1996).

The interest in fat in relation to consumer health lies in its content of essential fatty acids (EFAs), polyunsaturated/saturated fatty acids ratio, n-3/n-6 ratio, conjugated linoleic acid (CLA) and cholesterol. Linoleic acid is one of the most abundant polyunsaturated fatty acids (PUFA), particularly in animals raised on grain based diets (Wood & Enser, 1997; Fisher *et al*., 2000). Conjugated linoleic acid is associated with several health enhancing properties such as anti-carcinogenesis, anti-artherogenesis, anti-diabetes, immunomodulation and shifting the partitioning of energy towards protein instead of fat deposition (Cannella &

Giusti, 2000; Stanley & Hunter, 2001). Linoleic acid and other PUFA of the n-6 series have a desirable hypocholesterolaemic effect of reducing low-density lipoprotein (LDL)-cholesterol (Wiseman, 1997). N-3 PUFA (particularly eicosapentanoic acid and docosahexanoic acid) are similarly desirable because of their antithrombogenic effect and their association with low mortality from cardiovascular diseases (Wiseman, 1997). On the other hand saturated fatty acids, especially lauric (12:0) and myristic (14:0) acids increase total blood LDL- and high density lipoprotein (HDL)-cholesterol as well as the LDL: HDL ratio (Khosla & Hayes, 1994). These conditions are conducive to cardiovascular diseases (Wiseman, 1997).

The fat content of meat is assessed in several ways. In industry, the traditional online methods are the visual scoring of carcass subcutaneous fat cover or measuring fat depth at specified points on the carcass, usually along the mid-section (Fisher & De Boer, 1994). In the laboratory, intramuscular and subcutaneous fat are traditional determined by dissections of a side or three rib sample (Miller *et al*., 1988; Fisher & De Boer, 1994). Intramuscular fat is determined by extraction with an organic solvent such as light petroleum (Boccard *et al*., 1981).

2.8.4 Meat Juiciness

Lawrie (1998) noted that juiciness in cooked meat has two organoleptic components. First is the impression of wetness during initial chewing, which is due to the rapid release of meat fluids. Second is the sustained juiciness resulting from the stimulatory effect of fat on salivation. The latter component explains why, for example, meat from young animals gives an initial impression of juiciness but ultimately a dry sensation due to the relative absence of fat (Lawrie, 1998). By the same token good quality meat is juicier than poor quality meat because the former has a higher intramuscular fat content.

Juiciness is related to WHC and marbling. In conjunction with tenderness, it accounts for the overall eating quality and consumers may confuse the two factors when making assessments or comparisons (Varnam & Sutherland, 1995). In meat research, juiciness is usually determined by sensory evaluation or inferred from measures of water in meat, such as WHC and cooking losses (Meilgaard *et al*., 1991).

2.8.5 Meat Flavour and Aroma

Aroma is a result of the sensory of certain volatile substances by the olfactory organs (Lawrie, 1998). The flavour of meat is attributed to a complex mixture of compounds produced by heating the heterogeneous system containing its precursors (MacLeod & Seyyedain-Ardebili, 1981). It is composed of volatile compounds that give rise to the odour properties; non-volatile or water soluble compounds with taste/tactile properties and, potentiators and synergists of flavour (MacLeod & Seyyedain-Ardebili, 1981). The water-soluble fraction of meat provides the basic meaty flavour and aroma while fat provides the species characteristic flavour and aroma, albeit in interaction with the former (Mottram & Edwards, 1983; Moody, 1983; Melton, 1990).

Phospholipids have been specifically implicated in flavour development. The phospholipids appear to provide sufficient lipids for flavour and aroma development while the triacylglycerides seem not to be essential (Mottram & Edwards, 1983) such that there may be no change in flavour with increase in carcass fatness (i.e. increase in triacylglycerides). In fact the effect of the phospholipid fraction may be diluted by the higher concentration of triacylglycerides in fat animals resulting in a weaker aroma and flavour of fatter meat (Fisher *et al*., 2000). This is so because the phospholipid content in fat is fairly constant while that of triacylglycerides increases with increase in fatness. PUFA of the n-6 and n-3 series produce different flavours (Kemp *et al*., 1981; Larick & Turner, 1989; Fisher *et al*., 2000). Fisher *et*

al. (2000) suggested that it is variation in the absolute concentrations as well as the relative proportions of the different fatty acids that lead to different flavour profiles.

Fat influences the flavour of meat in two ways. One is through oxidation, principally of unsaturated fatty acids (UFA), which yields carbonyl compounds that at one level of concentration produce desirable flavours and at another, undesirable flavours (Moody, 1983). Secondly fat serves as a depot for fat-soluble compounds that volatilise upon heating and strongly affect flavour. Many of the flavour compounds are produced during cooking as a result of reactions such as the Maillard reaction, Strecker degradation, lipid peroxidation and their interactions (Moody, 1983).

2.8.6 Meat Tenderness

Meat tenderness is rated as the most important attribute of eating quality and is the factor that determines consumers continued interest in meat (Issanchou, 1996; Boleman *et al*., 1997). Tenderness is defined as the ease of mastication, which involves the initial ease of penetration by the teeth, the ease with which the meat breaks into fragments and the amount of residue remaining after mastication (Lawrie, 1998). The two major determinants of meat tenderness are the content and state of the connective tissue and the structure and state of the myofibrils (Dutson *et al*., 1976). The two components are modified to some extent by intramuscular fat and the sarcoplasmic proteins (Lawrie, 1998).

Connective tissue toughness is often referred to as background toughness because the tissue hardly changes during the standard lengths of meat storage post-mortem (McCormick, 1994). Connective tissue accounts for less than 10% of the total variance in meat tenderness (Harper, 1999). Its contribution to toughness is believed to be a product of the state of connective tissues in the perimysium, which constitutes some 90% of the intramuscular connective tissue

(Light *et al*., 1985). Collagen is the predominant protein of perimysial and endomysial connective tissues, constituting some 1.6 to 14.1% of the dry matter weight of muscle (Purslow, 1999). Collagen characteristics, mainly the content and solubility, are thus the basis for the determination of connective tissue contribution to meat toughness.

Myofibrillar contribution to meat tenderness depends on the extent of shortening during rigor development and proteolysis during conditioning (Warriss, 2000). Thus it is determined by the conditions during rigor development and post-mortem tenderisation. The two may be modified by the pre-and post-slaughter effects on the animals or carcasses. In general, intrinsic pre-slaughter factors (such as species, breed, sex and age) affect tenderness by determining the amount and properties of connective tissue (Lawrie, 1998). They may however, have an effect on tenderness in instances where they determine carcass conformation and fatness, and hence the degree of insulation against cold shortening. Carcass fat performs an important insulatory role in this effect. Of the extrinsic factors, pre-slaughter stress is of greatest concern in the meat industry. Stress effects are mediated through pre-slaughter depletion of glycogen resulting in high pH meat. Meat toughness increases between pHu 5.5 and 6 and is maximum between pH 5.8 and 6.3 (Jeremiah *et al*., 1991; Purchas and Aungsupakorn, 1993; Devine *et al*., 1996). According to Yu & Lee (1986), meat is tender at both pHu extreme because, at low pHu, the acidic proteases are active while at the higher end, the neutral calpains are active. The range 5.8 to 6.3 is outside that of the two enzyme systems, and hence there is minimum degradation of muscle proteins. Sarcomere shortening is the cause of toughening early post-mortem (Smulders *et al*., 1990; Koohmaraie, 1996). Wheeler and Koohmaraie (1994) demonstrated this phenomenon with lamb longissimus thoracic lumbranusis whose sarcomere length decreased from 2.24 mm at death to 1.69 mm 24 hours post-mortem. Koohmaraie *et al*., (1996) further showed that when sarcomeres were restrained

from shortening, no toughening occurred. These findings concur with Marsh & Leet (1966) who reported that most tender meat has sarcomere length of 2.0 to 2.5 mm, meat of intermediate tenderness 1.7 to 2.0 mm and tough meat 1.5 to 1.7 mm. The degree of sarcomere shortening depends on the rates of chilling and post-mortem glycolysis. It is high in slow glycolysing muscles especially if they are subjected to rapid chilling, and low in fast glycolysing muscle (Marsh *et al*., 1987; Smulders *et al*., 1990; O'Halloran *et al*., 1997b). Therefore any animal and post-slaughter environmental factors that affect the rate of post-mortem chilling and glycolysis will have an impact on the degree of sarcomere contraction.

2.9 ACTIONS OF PHYTOBIOTICS ON MEAT QUALITY

Phytogenics improve the microbial carcass hygiene and the preservation quality, in relation with their antimicrobial and antioxidant properties (Botsoglu *et al*., 2002b; Ruberto *et al*., 2002; Aksit *et al*., 2006). According to the European Food Safety Authority (EFSA), this alternative should be considered as one of the most effective ways to reduce the microbial contamination of food and to control the spread of foodborne diseases within the human population through the food chain (European Commission, 2003). Addition of plants extracts in foods and / or systems used on carcass surfaces decreases the bacterial contamination of poultry products (Gülmez *et al*., 2006). Oils extracted from plants (such as oregano, rosemary, sage) also have a positive effect (Young *et al*., 2003; Govaris *et al*., 2007).

Meat quality can be improved by incorporating natural antioxidants to animal diets, spreading these compounds onto the meat surface, or using active packaging. Among the positive effects of natural antioxidants on meat characteristics are retarding lipid oxidation (Djenane *et al*., 2002; 2003; Fasseas *et al*., 2007; Camo *et al*., 2008), decreasing colour loss, and hindering microbial growth (Djenane *et al*., 2002; 2003; Camo *et al*., 2008; Zinoviadou *et al*., 2009). Some authors have reported that natural antioxidants have no effect on sensory

characteristics of meat. Chaves *et al*. (2008) did not detect any effect of essential oil compounds (carvacrol and cinnamaldehyde) added to the diet of growing lambs, on the sensory characteristics of sirloins. The same was observed in pork where different essential oils were included in the diet of pigs (Janz *et al*., 2007). The only evidence of the effect of natural antioxidants on inhibiting off-odour formation and discoloration of meat is active packaging (Djenane *et al*., 2003; Nerín *et al*., 2006; Camo *et al*., 2008).

Some plant extracts have a mitigating effect on lipid oxidation by reducing 2-thiobarbituric acid (TBA) or malondialdehyde (MDA) formation on different types of meats during refrigeration storage (Valeria & Pamela, 2011). Tanabe *et al*. (2002) reported that adding methanolic extracts of 22 selected herbs and spices decreased lipid oxidation in pork with sansho (*Zanthoxylum piperitum* L.) extracts, sage, and ginger (*Zingiber officinale* Rosc.) having the greatest effect. Oregano and sage essential oils added to beef and pork meat (Fasseas *et al*., 2007), and a rosemary and vitamin C solution sprayed onto the surface (Djenane *et al*., 2003) reduced oxidation during refrigeration. Dietary incorporation of oregano, rosemary, and sage essential oils retarded lipid oxidation (MDA formation) in meat during refrigerated and frozen storage (Lopez-Bote *et al.*, 1998; Botsoglou *et al*., 2002b; 2003a; 2003b; Simitzis *et al.*, 2008). Botsoglou *et al*. (2003a) reported that α-tocopheryl acetate supplementation was more effective than dietary incorporation of oregano essential oil to extend lipid stability of chicken meat in frozen storage. There is a synergistic effect between dietary essential oil and α-tocopheryl acetate supplementation in retarding lipid oxidation in raw and cooked turkey during refrigeration (Botsoglou *et al*., 2003b). Lopez-Bote *et al.* (1998) observed an additional effect on meat cooked at 70 °C, which came from broilers fed on diets containing rosemary and sage extracts, during refrigerated storage for up to 4 days. The strongest effect of dietary oregano essential oil supplementation to reduce lipid

oxidation has been found at levels of 100 mg/kg feed in chicken meat (Botsoglou *et al*., 2002b; Botsoglou *et al*., 2003a) and 200 mg/kg feed in turkey meat (Botsoglou *et al*., 2003b). Janz *et al*. (2007) noted a reduction of lipid oxidation in oregano essential oil fed to pigs when compared with other essential oil dietary inclusion treatments (rosemary, garlic *Allium sativum* L., or ginger). Lower MDA formation in dietary oregano essential oil treatments are probably the result of the presence of oregano antioxidant compounds, which might be absorbed into the circulatory system after ingestion, distributed, and retained in muscle and other tissues (Botsoglou *et al*., 2003b; Simitzis *et al.*, 2008).

Some reports demonstrated that natural antioxidants can retard meat colour loss by extending the red colour (a*) and delaying metmyoglobin (MMG) formation (Valeria & Pamela, 2011). One example of dietary natural antioxidants affecting meat colour is the higher colour parameters (a* and yellowness b*) of meat from lambs fed with oregano essential oil supplementation (1 mL oregano essential oil kg^{-1}) (Simitzis *et al.*, 2008). Another example is the reduction of MMG formation and intense red colour obtained in fresh beef steaks whose surface was sprayed with a rosemary and ascorbic acid solution during refrigeration (Djenane *et al*., 2003). Carpenter *et al.* (2007) noted that the colour parameters (lightness L*, b*, and a*) of raw pork patties did not vary by adding grape seed and bearberry extracts. The same results were obtained for fresh chicken breast meat (Chouliara *et al*., 2007).

Some authors reported that plant extracts with antimicrobial properties can be used to increase refrigerated meat shelf-life (Skandamis *et al*., 2002; Djenane *et al*., 2003; Chouliara *et al.*, 2007). Valeria & Pamela (2011) showed values of colony forming units obtained with some plant extracts applied onto the meat surface. Eugenol, clove, oregano, and thyme extracts applied on meat were reported to be effective against *L. monocytogenes*, *A.*

hydrophila, and spoilage flora at higher concentrations than those required by *in vitro* assays (Hao *et al*., 1998a; 1998b; Skandamis & Nychas, 2001).

Presently, active packaging has been intensely developed due to recent contamination outbreaks associated with meat products (Coma, 2008). The effectiveness of antimicrobial compounds might be higher when they are incorporated in an active film applied to the food surface than if directly applied to the food surface by spraying or dipping (Coma, 2008; Zinoviadou *et al*., 2009). Oregano-based films have been effective against bacterial growth in meat. For example, active films containing oregano essential oil can reduce the growth of total flora and *Pseudomonas*, thus inhibiting the growth of lactic acid bacteria in beef (Zinoviadou *et al*., 2009). Moreover, oregano-based films were effective against *Salmonella typhimurium* and *E. coli* O157:H7 inoculated in beef muscle slices (Oussalah *et al*., 2006). Thus, beef shelf-life can be increased two fold when employing active films containing 1.5% oregano oil (w/w) (Zinoviadou *et al*., 2009). Skandamis & Nychas (2001) reported that oregano essential oil compounds affected both growth and metabolic activity (glucose and lactate consumption) of meat microorganisms during storage. In addition, meat packed in active film containing natural antioxidants exhibited higher antioxidant activity than meat packaged in films without antioxidants (Nerín *et al*., 2006; Camo *et al*., 2008). Oregano based films stabilized lipid oxidation in beef muscle slices during 7 days when stored at 4 °C (Oussalah *et al*., 2004).

2.10 SENSORY EVALUATION OF MEAT QUALITY

Sensory properties of food impact on consumer appreciation of the food and determine the acceptability and quality (Chambers IV & Bowers, 1993). There have been several investigations to determine the sensory attributes that drive acceptance of food. Most have concluded that acceptability of meat can be predicted from tenderness/texture, juiciness and flavour (Horsefield & Taylor, 1976; Parrish *et al*., 1991). Studies in the United States of America have identified tenderness as the most important factor influencing the acceptability of beef (Morgan *et al*., 1991; Boleman *et al*., 1997) and that juiciness and flavour have a greater effect on consumer satisfaction as toughness increases (Miller *et al*., 2001).

Laboratory techniques have been developed to quantitatively assess the palatability attributes. While laboratory methods provide technical, precise and reliable information about a product, the results do not tell whether or not the food would be acceptable to consumers. Therefore, consumer studies are used to determine the range of quantitative values that are acceptable as well as the degree of liking or preference for a product (Muñoz & Chambers IV, 1993).

The sensory test that is carried out to determine whether or not consumers like a product is that of acceptance, which is defined as a positive attitude after the tasting experience and is directly measured on a hedonic scale (Baker *et al*., 1994). It may be determined as an overall measure or for individual sensory attributes (Meilgaard *et al*., 1991). The test may be accompanied by a food action rating test, which requires the consumers to estimate their intended frequency of consumption of the product (Penfield & Campbell, 1990). The latter test is essential because consumers tend to be realistic when they evaluate or predict actions. As such a measure of consumption intent is considered more sensitive and action-orientated than the hedonic tests (Penfield & Campbell, 1990). A complimentary test, when several

products are being evaluated, is an indication of whether or not consumers prefer one product to others. Preference determination is useful because it is possible for consumers to show a strong preference for a sample but not want to consume it frequently or to reject it for other reasons than not liking it (Penfield & Campbell, 1990).

2.11 LIPOPROTEINS AND CHOLESTEROL TRANSPORT

Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the aqueous environment of body fluids and enable their transport throughout the body of all vertebrates and insects to tissues where they are required (Jonas & Philip, 2008). Because of their clinical importance, a very high proportion of research on lipoproteins deals with their functions in humans in relation to health, and the discussion that follows has a human bias (Fielding & Fielding, 2008). Lipoproteins are synthesised mainly in the liver and intestines (Vance & Adeli, 2008). Within the circulation, these aggregates are in a state of constant flux, changing in composition and physical structure as the peripheral tissues take up the various components before the remnants return to the liver (Fielding & Fielding, 2008). They are typically composed of 80-100 proteins/particle (organized by one, two or three ApoA; more as the particles enlarge picking up and carrying more fat molecules) and transporting none to hundreds fat molecules/particle, and the fats carried include cholesterol, phospholipids, and triglycerides; amounts of each quite variable (Fielding & Fielding, 2008). Lipoproteins, in order of molecular size/density, largest to smallest, are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high density lipoprotein (HDL) (Jonas & Philip, 2008).

Chylomicrons are the least dense type of cholesterol transport molecules, and contain apolipoprotein B-48, apolipoprotein C, and apolipoprotein E in their shells (Mansbach &

Siddiqi, 2010). Chylomicrons are the transporters that carry fats from the intestine to muscle and other tissues that need fatty acids for the energy or fat production (Mansbach & Siddiqi, 2010). Cholesterol that is not used by muscles remains in more cholesterol-rich chylomicrons remnants, which are taken up from here to the bloodstream by the liver (Mansbach & Siddiqi, 2010). The liver is able to scavenge chylomicron remnants much more rapidly than LDL particles, and it seems likely that this specificity has evolved because the former are especially atherogenic (Olofsson & Borèn, 2005).

Very-Low-Density Lipoproteins (VLDLs) are the primary transport particles for delivering energy-rich but insoluble triglycerides to tissues throughout the body and contain apolipoprotein B100 and apolipoprotein E in their shells (Shelness & Ledford, 2005). VLDLs molecules are produced by the liver and contain excess triacylglycerol and cholesterol that is not required by the liver for synthesis of bile acids (Tiwarri & Siddiqi, 2012). Cholesterol is essential to provide hydrophobicity to the VLDL so that they can carry triacylglycerols efficiently in the aqueous environment of plasma (Tiwarri & Siddiqi, 2012). However, once this has been accomplished the cholesterol-rich, triacylglycerol-depleted remnant LDL by-products are potentially toxic and must be safely removed from circulation. Disease states appear to influence VLDL properties. In patients with apoB mutations, hepatic VLDL secretion is impaired and often leads to fatty liver—the excessive accumulation of fat in the liver (steatosis). The extent of steatosis is related to the size and number of VLDL particles produced within this patient population (Schonfeld *et al*., 2003).

Low-Density Lipoprotein (LDL) molecules have the highest percentage of cholesterol within them, and therefore, are the major carriers of cholesterol in the blood (Scheffer *et al*., 1997) LDL carries cholesterol from the liver to cells and each one contains approximately 1500 molecules of cholesterol ester. The shell of the LDL molecule contains just one molecule of

apolipoprotein B100, which is recognized by the LDL receptor in peripheral tissues (Segrest *et al*., 2001). When the cell has abundant cholesterol, LDL receptor synthesis is blocked so new cholesterol in the form of LDL molecules cannot be taken up. On the converse, more LDL receptors are made when the cell is deficient in cholesterol. When this system is deregulated, many LDL molecules appear in the blood without receptors on the peripheral tissue. These LDL molecules are oxidized and taken up by macrophages, which become engorged and form foam cells (Peter *et al*., 2014). LDL particles pose a risk for cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans (Stocker & Keanery, 2004). A complex set of biochemical reactions regulates the oxidation of LDL particles, chiefly stimulated by presence of necrotic cell debris and free radicals in the endothelium (Stocker & Keanery, 2004). Increasing concentrations of LDL particles are strongly associated with increasing amounts of atherosclerosis within the walls of arteries over time, eventually resulting in sudden plaque ruptures and triggering clots within the artery opening, or a narrowing or closing of the opening, *i.e.* cardiovascular disease, stroke, and other vascular disease complications (Krauss, 2010).

High-Density Lipoprotein (HDL) molecules are thought to transport cholesterol back to the liver for excretion or to other tissue that use cholesterol to synthesize hormones in a process known as reverse cholesterol transport (RCT) (Lewis & Rader, 2005). Reverse cholesterol transport is a term that comprises all the different steps in cholesterol metabolism between cholesterol efflux from macrophage foam cells and the final excretion of cholesterol into the faeces either as neutral sterols or after metabolic conversion into bile acids. Having large numbers of large HDL particles correlates with better health outcomes (Gordon *et. al*., 1989). In contrast, having smaller numbers of large HDL particles is independently associated with atheromatous disease progression in the arteries (Hausenloy & Yellon, 2008). This is because

HDL particles can transport fat molecules (including cholesterol, triglycerides, etc.) out of artery walls, reduce macrophage accumulation, and thus help prevent, even regress atherosclerosis over weeks, years, decades, thereby helping prevent cardiovascular disease, stroke(s) and other vascular disease complications body wide (Hausenloy & Yellon, 2008).

2.12 UTILIZATION OF GINGER IN POULTRY PRODUCTION

2.12.1 Feed Intake and Efficiency

Opinion in the literature is divided regarding the influence of ginger on feed intake. Tekeli *et al*. (2011) reported that 240 ppm of ginger (*Zingiber officinale)* had a positive effect on feed consumption with respect to control broilers. Nasiroleslami & Torki (2010) showed that the addition of the essential oil of ginger did not affect feed intake or feed conversion in laying hens. Zhang *et al*. (2009) did not find any significant difference in daily feed intake by feeding ginger at the rate of 5 g/kg (0.5%) when processed to different particle sizes (300, 149, 74, 37, and 8.4 μm) although numerically the feed intake was higher than the control. Zhao *et al*. (2011) found that daily feed intake and feed conversion ratio (FCR) did not differ between laying hens fed ginger in the diet (5, 10, 15 and 20 g/kg), and Akbarian *et al*. (2011) found that feeding ginger at different levels (0.25, 0.5 and 0.75%) had no effect on feed intake and FCR in laying hens. Incharoen & Yamauchi (2009) fed dried fermented ginger (1% and 5%) to White Leghorn laying hens and found that feed consumption and FCR tended to increase in ginger fed groups. Likewise, the use of 2% red ginger in the ration of broiler chickens resulted in higher feed intakes and FCRs (Herawati, 2010). Onu (2010) reported that the addition of ginger (0.25%) in the basal diet of broiler chicks resulted in improved FCR although feed intake did not change.

In some cases ginger was applied via drinking water in poultry. Javed *et al.* (2009) reported that broiler chicks dosed with aqueous extract (15 ml/l of drinking) of a mixture

of plants containing ginger improved feed intake and FCR. However, Kausar *et al*. (1999) showed that a carminative mixture containing ginger at the dose rate of 2 and 4 ml/l of drinking water increased FCR.

Some researchers have combined plant products, including ginger, in trials. Moorthy *et al*. (2009) found that feed intake did not differ at six week of age, although FCR was significantly higher in birds fed a combination of 0.2% ginger and 0.2% curry leaf powder. The differences in these reported findings may be due to the different varieties of ginger used, their processing, dose and the duration of the experiments.

Most of the researchers attributed the better performance of the broiler birds fed ginger to an improvement in palatability and the quick digestive effect of this natural product. They suggested that due to the effect of this natural product, the digestive tract would be emptied earlier and feed consumption promoted. Ginger has been found to increase secretion of gastrointestinal enzymes including lipase, disaccharidase and maltase (Zhang *et al*., 2009). According to Herawati (2010) the improved performance may be attributed to the two types of digestive enzymes in ginger; protease and lipase, which are present as part of the plants natural protective mechanisms (Zhang *et al*., 2009). Zhao *et al*. (2011) reported that ginger enhances nutrient digestion and absorption because of its positive effect on gastric secretion, enterokinesia and digestive enzyme activities.

2.12.2 Growth Performance and Body Weight Gain

Tekeli *et al*. (2011) stated that *Zingiber officinale* improved body weight gain in broiler chickens at the rate of 120, 240 and 360 ppm; however Zhang *et al*. (2009) did not find any significant difference for average daily gain in broilers by feeding ginger at the rate of 5 g/kg. Herawati (2010) found that the use of 2 % red ginger in the ration of broiler chickens produced higher body weights. Onu (2010) reported that the addition of ginger (0.25%) to the basal diet of broiler chicks resulted in higher body weights. In their work, Kausar *et al*.

(1999) showed that carminative mixture containing ginger at the dose rate of 2 and 4 ml/l of drinking water increased body weight on the 5th week of the experiment. El-Deek *et al*. (2002) observed that a diet containing 1 g/kg of ginger did not affect growth performance, whereas Farinu *et al*. (2004) reported that supplementation of ginger at the levels of 5, 10, or 15 g/kg slightly improved growth in broilers. In contrast, Al-Homidan (2005) observed reduced growth rate in starter broilers (1 to 4 weeks) when ginger was fed at the rate of 60 g/kg body weight at the 6th week of age (Moorthy *et al*., 2009) which may be due to the toxic effect of this compound (Zhang *et al.*, 2009). The different results on growth performance of broilers may be ascribed to the different doses used in the experiments.

2.12.3 Meat Quality and Carcass Traits

Poultry meat is susceptible to development of off-flavour (Gray & Pearson, 1987) and changes in texture and nutritive value (Pearson *et al*., 1983). There has been a resurgence of interests for "all natural" medicinal plants like herbal feed additives, plant extracts with growth, flavour, colour enhancing, antioxidant and antibacterial activities (Adodo, 2002; Omojasola & Awe, 2004). Herbal formulations have proved useful in increase in weight gain beyond doubt. However, there is dearth of data on efficacy of ginger especially on carcass characteristics (colour, water holding capacity and pH) and organoleptic attributes (juiciness, flavour and tenderness).

Yellow ginger was capable of affecting meat quality by different ways. Inclusion level of 0.5 % appeared to be the optimum level to achieve the best tenderization effect (Naveena, 2004). However, Janz *et al*. (2007) did not accept that meat quality was improved by barely adding ginger into diets. In a more recent study, Zhang *et al*. (2009) observed that birds fed ginger produced higher carcass weights compared to untreated birds. Dressing percentage, breast weight and leg weights increased significantly in response to an aqueous extract of a plant mixture containing ginger (Javed *et al*., 2009). Zhang *et al*. (2009) suggested that

improved carcass quality of broiler may be associated with the antioxidant effect of ginger which enhances protein and fat metabolism. Conversely, Moorthy *et al*. (2009) reported no effect of ginger supplementation on carcass characteristics including New York dressed percentage, eviscerated weight, ready to cook percentage, abdominal fat pad and giblet weight. El-deek *et al*. (2002) found that the dressing percentage did not differ between control and ginger treated broilers up to the sixth week of age. Likewise, Onu (2010) observed that the addition of ginger (0.25%) in the basal diet of broiler chicks did not result in significant differences in carcass characteristics.

2.12.4 Blood Profile

Saeid *et al*. (2010) reported that serum glucose, total cholesterol, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol decreased significantly in broilers fed with 0.4 and 0.6% aqueous ginger extract, however, high density lipoprotein (HDL) cholesterol concentration increased in these birds. Onu (2010) reported that supplementation of ginger (0.25%) in the basal diet of broiler chicks did not result in any significance difference in terms of serum total protein, albumin, globulin, urea and creatine levels. Recently, Rehman *et al*. (2011) studied the effect of dosing broilers (10 ml/L of drinking water) with an aqueous extract of a mixture of medicinal plants (garlic, berberine and aloe vera) along with ginger, which resulted in a significant decrease in serum glucose, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase concentrations. However, serum protein increased significantly in the treated group. In the same experiment, the cholesterol profile (including total cholesterol, LDL and VLDL cholesterol) decreased significantly in the treated group, while HDL cholesterol concentration increased. Zhang *et al*. (2009) reported that total protein concentration was higher at 21 days and 42 days of sampling in broilers treated with ginger powder but cholesterol concentration was reduced at these intervals. Kausar *et al*. (1999) reported that carminative mixture containing ginger at the

dose rate of 2 and 4 ml/L of drinking water did not affect serum albumin, globulin and total protein in broilers. Al-Homidan (2005) observed reduced total protein and globulin in the plasma of broiler chicks due to dietary supplementation at 60 g/kg which may be due to a toxic effect of the ginger. Farinu *et al*. (2004) reported that supplementation of ginger at the rate of 5, 10 and 15 g/kg did not affect total protein and albumin in the serum of broiler chickens. The discrepancies in these results may be due to the difference in doses used as well as experimental conditions.

The exact mechanisms through which blood metabolites are altered are not known. It was postulated that (E)-8 beta, 17-epoxyllabed-12-ene-15, 16-dial, a compound isolated from ginger, interferes with cholesterol biosynthesis in liver homogenates of hypercholesterolaemic mice causing its reduction (Tanabe *et al*., 1993). Srinivasan and Sambaiah (1991) reported that feeding rats with ginger significantly elevated the activity of hepatic cholesterol 7-alpha-hydroxylase which is a rate limiting enzyme in the biosynthesis of bile acids and stimulates the conversion of cholesterol to bile acids leading to the excretion of cholesterol from the body.

2.12.5 Egg Production and Quality

Nasiroleslami & Torki (2010) found that the addition of the essential oil of ginger increased egg shell weight and egg shell thickness in laying hens. However, feeding ginger essential oil did not affect egg index, yolk index and Haugh unit. Other researchers have not always observed the same benefits. Zhao *et al*. (2011) reported that laying hens fed with ginger at the rates of 5, 10, 15 and 20 g/kg of feed had no effect on laying rate and average egg weight, however egg mass increased significantly in supplemented groups. Akbarian *et al*. (2011) showed that feeding ginger at the rates of 0.5 and 0.75% improved egg production although egg weight did not differ between the control and treated groups. Previous work (Incharoen & Yamauchi, 2009) showed that White Leghorn laying hens fed dried fermented

ginger (1 and 5%) showed better egg production and mass in comparison to those of control birds. However, there were no significant differences in shell breaking strength, shell thickness, shell ratio, albumin ratio, yolk ratio, yolk colour and Haugh unit among the dietary treatments.

The exact mechanism through which egg laying performance is enhanced is not known. According to Zhao *et al*. (2011) the higher performance of the laying hens may be due to antioxidant, antimicrobial and other activities such as increased blood circulation and secretion of digestive enzymes and reduction in the oxidation of feed.

2.12.6 Toxicological Effects

Studies concerning the toxicological effects of using ginger as a feed supplement in poultry feed are rare. However, Herewati (2010) reported that broilers fed diets containing 0.5, 1.0 and 1.5% red ginger showed oedema, necrosis and inflammation in muscles. Ginger contains atsiri sescuiterpen oil which is potentially toxic in animals (Herewati, 2010).

All phytobiotics have toxic characteristics and the intensity of their toxicity is determined by the dose and duration of the feeding period (Herewati, 2010). Feeding such substances at higher doses causes symptoms of congestion, oedema, inflammation and necrosis (Ganiswarna, 1995).

2.12.7 Bacteria Count and Other Effects

For a long time, plants have played an important role in maintaining the health and improving the life quality of poultry. They have the potential to be used as non-antibiotic growth promoters in broilers diets (Torres-Rodrigues *et al.,* 2005). Due to antibacterial activity, some ingredients in herbal oils reduce the harmful bacteria population in the gastrointestinal tract of poultry (American Botanical Council, 2000) or stimulate the immune system (Chen *et al.,* 2003).

The surface of the gastrointestinal tract in chicken responds very quickly to alterations in

nutrient intake (Dou *et al*., 2002). The histology of the intestinal villi and epithelial cells on the apical surface is commonly affected by dietary feed components (Yamauchi *et al*., 2006). Incharoen & Yamauchi (2009) showed that the villus height, surface area, cell area and cell mitosis in the intestinal segment had higher values in ginger-fed laying hens compared to the control birds. It has been suggested that longer villi absorb a greater amount of available nutrients due to an increased surface area (Caspary, 1992). Greater villi height and more mitosis in the gut indicate that the function of intestinal villi is stimulated as a result of enhanced absorption (Langhout *et al*., 1999; Yasar & Forbes, 1999).

Kausar *et al*. (1999) reported that carminative mixture containing ginger at the dose rate of 4 ml/l of drinking water increased mean titre in primary and secondary responses against Newcastle disease, suggesting immunomodulating effects of ginger. Sudrashan *et al*. (2010) reported that essential oil isolated from ginger resulted in a significant reduction in the bacterial counts of *Staphylococcus*, *E. coli* and *Salmonella* when applied as a decontaminating agent in the ratio of 1:150, 1:250 and 1:500 to chicken meat. Zhao *et al*. (2011) found that ginger reduced the oxidation of stored feed which may be partially responsible for the improved laying performance and for the serum and egg yolk antioxidant contents.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Location

This research was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria. Owerri is situated in South-eastern part of Nigeria. The vegetation is tropical rainforest. The city lies between latitude $5^{0}10^{1}$ N and $6^{0}35^{1}$ N and longitude $6^{0}35^{1}$ E and $7^{0}28^{1}$ E (MLS, 1992). The annual rainfall is between 2000 mm and 2500 mm while the mean annual temperature is between 26 ^{0}C to 28 ^{0}C with a relative humidity of about 98 % during the wet season (Imo ADP, 1990). The annual evapotranspiration is 1450 mm and soil type is essentially sandy loam with average pH of 5.5 (MLS, 1984).

3.2 Collection and Processing of Ginger

Fresh ginger roots where procured from commercial dealers. The fresh roots were washed and sliced for effective drying. The sliced ginger roots were air dried for about 10 days in an open ventilated space away from sunlight. The air dried ginger was milled into fine particles using a local milling machine. The ground samples were stored in an air tight container and stored under room temperature (23.1 – 24.6 ^{0}C) as ginger meal that was used for this study.

3.3 Experimental Diet Formulation

The composition of the diet formulated for the starter and finisher broiler chicken are shown in Tables 3.1 and 3.2 respectively.

Table 3.1: Diet Composition of Broiler Starters Fed Ginger Meal

	Ginger meal levels (%)			
Ingredients (%)	**T1 (0)**	**T2 (0.2)**	**T3 (0.4)**	**T4 (0.6)**
Maize	51.00	50.80	50.60	50.40
Ginger Meal	0.00	0.20	0.40	0.60
Soya Bean Meal	28.00	28.00	28.00	28.00
Fish Meal	4.00	4.00	4.00	4.00
Palm Kernel Cake	5.00	5.00	5.00	5.00
Wheat Offal	8.00	8.00	8.00	8.00
Bone Meal	3.00	3.00	3.00	3.00
Vitamin/Mineral Premix[a]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated Values:				
Crude Protein	21.57	21.58	21.58	21.59
Metabolizable Energy (Kcal/kg)	2778.68	2771.83	2764.96	2758.11

[a] Contains in the following per kg: vitamin A: 2400000 IU, vitamin D: 1000000 IU, vitamin E: 16000 IU, vitamin K: 800 mg, vitamin B_6: 1000 mg, vitamin B_{12}: 6mg, niacin: 8000 mg, folic acid: 400 mg, pantothenic acid: 3000 mg, biotin: 40 mg, antioxidant: 3000 mg, cobalt: 80 mg, copper: 2000 mg, iodine: 400 mg, iron: 1200 mg, manganese: 1800 mg, selenium: 60 mg and zinc: 14000 mg.

Table 3.2: Diet Composition of Broiler Finishers Fed Ginger Meal

	Ginger meal levels (%)			
Ingredients (%)	**T1 (0)**	**T2 (0.2)**	**T3 (0.4)**	**T4 (0.6)**
Maize	56.00	55.80	55.60	55.40
Ginger Meal	0.00	0.20	0.40	0.60
Soya Bean Meal	18.00	18.00	18.00	18.00
Fish Meal	4.00	4.00	4.00	4.00
Palm Kernel Cake	10.00	10.00	10.00	10.00
Wheat Offal	8.00	8.00	8.00	8.00
Bone Meal	3.00	3.00	3.00	3.00
Vitamin/Mineral Premix[a]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated Values:				
Crude Protein	18.55	18.57	18.57	18.58
Metabolizable Energy (Kcal/kg)	2843.28	2836.43	2829.56	2822.69

[a] Contains in the following per kg: vitamin A: 2400000 IU, vitamin D: 1000000 IU, vitamin E: 16000 IU, vitamin K: 800 mg, vitamin B_6: 1000 mg, vitamin B_{12}: 6mg, niacin: 8000 mg, folic acid: 400 mg, pantothenic acid: 3000 mg, biotin: 40 mg, antioxidant: 3000 mg, cobalt: 80 mg, copper: 2000 mg, iodine: 400 mg, iron: 1200 mg, manganese: 1800 mg, selenium: 60 mg and zinc: 14000 mg.

Four experimental diets were formulated and they contained ginger meal at 0 %, 0.2 %, 0.4 % and 0.6 % levels, respectively, both at starter and finisher levels. The starter diets were fed from 0 – 4 weeks while the finisher diets were fed from 5 – 8 weeks.

3.4 Experimental Birds

One hundred and eighty (180) unsexed day old broiler chicks (Cobb strain) were used in this study. The broiler chicks were procured from a commercial distributor of day-old-chicks in Imo State.

3.5 Experimental Design

This experiment was carried out using one hundred and eighty (180) broiler chicks. The chicks were divided into four treatment groups. Each treatment comprised 45 chicks and were randomly subdivided into 3 replicates with 15 chicks per replicate. Each treatment group was assigned to each of the diets as follows: 0 % ginger meal (T1, Control), 0.2 % ginger meal (T2), 0.4 % ginger meal (T3), and 0.6 % ginger meal (T4) in a completely randomized design (CRD) experiment.

The Statistical Model is given as;

$Yij = U + Ti + Eij$

Where; Yij = Single Observation

U = Population mean

Ti = Treatment effect due to different levels of ginger

Eij = Random error.

3.6 Experimental Procedure

The chicks were reared and raised intensively in a deep litter system. The condition of housing and management were same for all the birds. Conventional vaccination and medication procedures were observed and the experimental diets measured and water was given *ad libitum* throughout the period of the experiment. The feeding trial lasted for 8 weeks. The chicks were weighed at the beginning of the experiment to obtain the initial live-weights and subsequently on weekly basis. Feed intake was obtained by difference between the quantities of feed offered and that left over the following morning. Data obtained were used to compute daily weight gain, daily feed intake and feed conversion ratio. The economy of production was estimated as feed cost/kg weight gain. At the 8^{th} week of age a total of 20 birds (5 birds from each treatment) were selected, kept in separate pens and starved for 24 hours overnight with only water given. Each bird was later weighed and tagged for identification. They were slaughtered by cutting the carotid arteries and the jugular veins and the blood samples were collected individually using sterilized bottles containing ethylene diamine-tetra acetic acid (EDTA) for analysis of haematological parameters, while other sets of blood samples were collected with sterilized bottles without anticoagulant for the determination of serum lipid profile.

3.7 Laboratory Analyses

3.7.1 Proximate Analysis of Ginger

Ginger meal and fresh ginger samples were analysed for proximate compositions (moisture, crude protein, crude fibre, ash, ether extract and nitrogen free extract) using the method of AOAC (1990).

Determination of Moisture: Moisture was determined by oven drying. Two Empty crucibles were weighed individually (W_1). Thereafter 2.083g of ginger meal and 12.743g of fresh ginger samples were accurately and separately weighed in clean and dry crucibles (W_2). The crucible samples were placed in an oven at 100 – 105 ^{0}C for about 12 hours until a constant weight was obtained. Then, the crucibles were placed in a desiccator for 30 minutes to cool, after which they were weighed (W_3). The percentage moisture content was calculated using the following formula:

$$\% \text{ Moisture} = \frac{(W_2 - W_3) \times 100}{W_2 - W_1}$$

Determination of Ash: Two clean empty crucibles were placed in a muffle furnace at 600 ^{0}C for an hour, cooled in a desiccator and then weighed (W_1). Thereafter 0.452g of ginger meal and 3.089g of fresh ginger samples were taken in the crucibles and weighed (W_2). The samples were ignited over a burner with the help of blowpipe, until charred. The crucibles were then placed in muffle furnace at 550 ^{0}C for 2 – 4 hours. The appearances of grey white ash indicated complete oxidation of all organic matter in the samples. After ashing, the crucibles were cooled in a desiccator and weighed (W_3). Then, the percentage ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{(W_2 - W_3) \times 100}{W_2 - W_1}$$

Determination of Crude Protein (CP): Determination of crude protein was carried out following the Kjeldahl method. About 0.136g of ginger meal and 0.751g of fresh ginger samples were separately taken in digestion flask. Then, the 10 – 15 ml of concentrated H_2SO_4 and 8 g of digestion mixture (K_2SO_4: $CuSO_4$ (8:1)) were added. The flasks were swirled to mix the contents thoroughly, and then placed on heater to digest till the mixture became blue

green in colour. This took 2 hours to complete. The digesta were cooled and transferred to 100 ml volumetric flasks and volumes made up by addition of distilled water. Distillations of the digesta were performed in Automatic Kjeldahl Distillation System (Markham Scientific, U.S.A.). Ten millilitres of digesta were introduced in the distillation tubes, followed by 10 ml of 0.5N NaOH which were gradually added. Distillation was continued for at least 10 minutes, and the NH_3 produced was collected in a conical flask containing 20 ml of 4 % boric acid solutions, with few drops of modified methyl red indicator. During the distillation, the yellowish colour appearance was due to NH_4OH. The distillates were then titrated against standard 0.1M HCL solution till the appearance of pink colour. A blank was run through all steps as above. Per cent crude protein of the samples was calculated using the following formula:

% Crude Protein = 6.25 x % N

$$\% N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Weight of sample} \times V}$$

Where S = Sample titration reading

B = Blank titration reading

N = Normality of HCL

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

6.25 = Correction factor

Determination of Crude Fibre (CF): Approximately, 2 g samples each of air dried and fresh ginger were weighed (W_0) and transferred to 2 porous crucibles. The crucibles were placed in Dosi-fiber unit, and valve kept in "OFF" position. After which, 150 ml of preheated

H_2SO_4 solution and some drops of foam suppressor were added to each column. The cooling circuit was then opened while the heating element (power at 90 %) was turned on. When boiling, the power was reduced at 30 % and left for 30 minutes. Valve was opened for drainage of acid, and then rinsed with distilled water thrice to completely ensure the removal of acid from samples. The same procedure was used for alkali digestion using KOH instead of H_2SO_4. The samples were dried in an oven at 150 ^{0}C for 1 hour. The residues were allowed to cool in a desiccator and weighed (W_1). The residues in crucibles were ashed in muffle furnace at 550 ^{0}C for 3 – 4 hours. The remaining ash was later cooled in a desiccator and weighed again (W_2). Calculations were done using the formula:

$$\% \text{ Crude Fibre} = \frac{(W_1 - W_2) \times 100}{W_0}$$

Where; W_0 = Weight of sample

W_1 = Weight after drying

W_2 = Weight after ashing

Determination of Ether Extract (EE): Ether extract was determined by ether extraction method using Soxhlet apparatus. Two filter papers were weighed and recorded (W_1), after which 1.168g of ginger meal and 4.645g of fresh ginger samples were put in each of the filter papers and weighed (W_2). The samples were wrapped in the filter papers and placed in an extraction tube. A clean and dry receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were turned on to start extraction. After 4 – 6 siphonings the beaker was disconnected. The extracted samples were removed from the tube, and allowed to cool for about 2 hours, then re-weighed (W_3). The percentage ether extract was calculated using the following formula:

$$\% \text{ Ether Extract} = \frac{\text{Weight of ether extract} \times 100}{\text{Weight of sample}}$$

Note: weight of sample = $W_2 - W_1$

weight of ether extract = $W_2 - W_3$

Determination of Nitrogen Free Extract (NFE): Nitrogen free extract was calculated by difference after determination of all the other fractions. The NFE was calculated using the following formula:

$$\% \text{ NFE} = [100 - (\% \text{ Moisture} + \% \text{ CP} + \% \text{ EE} + \% \text{ CF} + \% \text{ Ash})]$$

3.7.2 Blood Counts

The blood samples in EDTA bottles were used to estimate the following **haematological parameters**: Haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet, total white blood cell (TWBC), lymphocyte, neutrophils, monocyte, eosinophil and basophil levels. The method used for estimation was the **Standard Operating Procedure (SOP)** for automated full blood count using Erma PCE 210, Roller Mixer equipment (AGD Biomedicals limited, India).

Procedure: Before the start of the analysis, it was ensured that the measurement stand-by mode was displayed and that measurement mode was 'VEIN'.

A 25 ml blood collecting tube (bottle filled with well stirred sample) was pushed up against a suction probe. It was ensured that the tip of the suction probe gets into blood. With the tip of the suction probe inside the blood, the START switch (button) was pressed to start measurement. With the completion of suction, buzzer sound goes off and the probe was

drawn into the unit. The blood collecting bottle or tube was removed, after which the sucked sample was diluted inside the unit, and measurements started automatically. At the completion of measurement, the measured results and histogram appeared '0' display and suction probe came out. After printing operation was over, it became possible to measure the next sample.

These procedures were repeated for the 20 blood samples to estimate each of the parameters.

3.7.3 Lipid Profile

The serum samples were analysed for cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL).

Estimation of Cholesterol: The method employed in the cholesterol estimation was cholesterol oxidase/peroxidase method following the spectrophotometric method (Allain *et al*., 1974). Reagent A (composition: 0.5 mmol/L sodium cholate, 28 mmol/L phenol, > 0.2 U/mL cholesterol esterase, > 0.1 U/mL cholesterol oxidase, 0.8 U/mL peroxidase, 0.5 mmol/L 4-aminoantipyrine and pH 7.0) was brought to room temperature (20-25 ^{0}C). Three test tubes were provided and labelled 'blank', 'standard' and 'sample'. A 10 µL of cholesterol standard, S [composition: cholesterol 200 mg/dL (5,18 mmol/L)] was pipetted into 'standard' test tube while 10 µL of serum sample was pipetted into 'sample' test tube. 1.0 ml of reagent A was mixed in each of the three test tubes and the contents were thoroughly stirred and incubated for 10 minutes at room temperature (16-25^{o}C). After 2 hours when the colour was stable, the absorbance (A) of the standard and that of the sample were measured against the blank at 500 nm by spectrophotometry. The cholesterol concentrations in the samples were calculated using the following general formula:

$$[A_{sample} \div A_{standard}] \times C_{standard} = C_{sample}$$

Estimation of Triglycerides: Glycerol phosphate oxidase/peroxidase method was employed (Allain *et al*., 1974). Reagent A (composition: 5 mmol/L magnesium chloride, 6 mmol/L 4-chlorophenol, > 100 U/mL lipase, > 1.5 U/mL glycerol kinase, > 4 U/mL glycerol-3-phosphate oxidase, > 0.8 U/mL peroxidase, 0.75 mmol/L 4-aminoantipyrine, 0.9 mmol/L ATP and pH 7.0) was brought to room temperature (20-25 ^{0}C). Three test tubes were provided and labelled 'blank', 'standard' and 'sample'. A 10 µL of triglyceride standard, S [composition: glycerol equivalent to 200 mg/dL (2.26 mmol/L) triolein] was pipetted into 'standard' test tube while 10 µL of serum sample was pipetted into 'sample' test tube. 1.0 ml of reagent A was mixed in each of the three test tubes and the contents were thoroughly stirred and incubated for 15 minutes at room temperature (16-25^{o}C). After 2 hours when the colour was stable, the absorbance (A) of the standard and that of the sample were measured against the blank at 500 nm by spectrophotometry. The triglyceride concentrations in the samples were calculated using the following general formula:

$$[A_{sample} \div A_{standard}] \times C_{standard} = C_{sample}$$

Estimation of HDL: Phosphotungstate/mg-cholesterol oxidase/peroxidase method was employed (Allain *et al*., 1974). An aliquot of 0.2 ml of blood serum sample was mixed with 0.5 ml of reagent A (composition: 0.4 mmol/L phosphotungstate and 20 mmol/L magnesium chloride) in a centrifuge tube for precipitation. The content was thoroughly stirred and left to stand for 10 minutes at room temperature (20-25 ^{0}C). It was then centrifuged at a minimum of 4000 r.p.m. for 10 minutes. Thereafter, a supernatant was carefully collected. For colorimetry, reagent B (composition: 35 mmol/L phosphate, > 0.2 U/mL cholesterol esterase, > 0.1 U/mL cholesterol oxidase, > 1 U/mL peroxidase, 0.5 mmol/L 4-aminoantipyrine, 0.5 mmol/L sodium cholate, 4 mmol/L dichlorophenolsulfonate and pH 7.0) was brought to room

temperature (20-25 ^{0}C). Three test tubes were provided and labelled 'blank', 'standard' and 'sample'. A 50 µL each of distilled water, HDL cholesterol standard S (composition: cholesterol 15 mg/dL) and sample supernatant were pipetted in 'blank', 'standard' and 'sample' test tubes respectively. 1.0 mL of reagent B was mixed in each of the test tubes and the contents were thoroughly stirred and incubated for 30 minutes at room temperature (16-25 ^{0}C). At about 30 minutes when the colour was stable, the absorbance (A) of the standard and that of sample were measured against the blank at 500 nm by spectrophotometry. The HDL cholesterol concentrations in the samples were calculated using the following general formula:

$$[A_{sample} \div A_{standard}] \times C_{standard} \times \text{Sample dilution factor} = C_{sample}$$

Estimation of LDL: The LDL levels were estimated using the Friedewald equation (Friedewald *et al*., 1972) as stated below:

$$LDL = Cholesterol - [(Triglycerides) \div 5] - HDL$$

3.7.4 Bacterial Load

In the evening, that is 10 hours after slaughtering and dressing of the birds, about 5g sample of the meat were taken from a portion from each of the 20 drumsticks into a sterilized plastic bottle to analyse for bacterial load. The samples were inoculated in a culture plate and viewed under an optical (light) microscope with magnification 400x.

3.7.5 Ether Extract in Meat Samples

About 15 g of meat samples were each taken from the wings of the dressed carcass (20 samples in all), and cut to small pieces for the estimation of ether extract. The Soxhlet extraction method was used (AOAC, 1990).

The 20 meat samples were dried at 50 ^{0}C for 72 hours in an oven. The dried samples were weighed after drying, and then ground. Twenty (20) filter papers were weighed individually and recorded (W_1). About 2 grams of each of the meat samples were measured and placed in each of the filter papers and weighed (W_2). The samples were folded and tied in each of the filter papers, and placed in a Soxhlet extractor tube, where petroleum ether (40 – 60 ^{0}C) was poured. Water and heater were turned on, and set up left for extraction. After 4 – 6 siphoning, the samples were removed from the extractor tube, allowed to cool for about 2 hours and were re-weighed (W_3).

Percentage Ether Extract (%) = [weight of oil extracted ÷ weight of sample] × 100

Note: weight of oil extracted = $W_2 – W_3$; weight of sample = $W_2 – W_1$

3.7.6 Carcass Characteristics and Organ Weights

After slaughtering and blood sample collection, the birds were dressed and the carcass and organ weights were taken. The parameters measured includes: Head, neck, heart, shanks, liver + gall bladder, gizzard full, gizzard empty, small intestine, drumsticks and carcass weights. The dressing percentages were also determined using the below formula.

Dressing percentage (%) = [carcass weight ÷ live weight] × 100

3.7.7 Meat Colour Change

One drumstick was removed from each carcass, and was refrigerated overnight. In the morning, the drumsticks were assessed for colour quality using a 3-point rating scale: normal colour = 3, slightly abnormal = 2 and clearly abnormal = 1.

3.7.8 Organoleptic Quality Assessment

After the assessment of each of the drumsticks for colour quality, the drumsticks were thoroughly washed in water, and about 15g was cut from the same side giving a total of 20 meat samples. Each sample was immersed in a super saturated brine solution for a few seconds. Thereafter, samples were packaged individually in double layered transparent polyethylene bags, properly tied and labelled. The meat samples were boiled in water for 10 minutes, after which a sensory assessment (organoleptic quality) was conducted using a 10 man panel. Each panellist was required to masticate two different samples and score each for tenderness, juiciness, flavour and degree of likeness (hedonic score) using the 9-point Category Rating Scale (AMSA, 1978).

3.7.9 Cooking Loss

The remaining drumsticks were weighed then packaged in a double layered polyethylene bag and labelled. The meats were boiled in water for 10 minutes, then brought down and allowed to cool to room temperature. Paper towels were used to wipe each of the cooked drumsticks and were re-weighed.

The loss in weight (during cooking) expressed as a percentage of fresh weight was regarded as per cent cooking loss.

Percentage cooking loss (%) = [(fresh weight – cooked weight) ÷ fresh weight] × 100

3.8 Statistical Analysis

The data generated for each parameter were meaned and were subjected to the analysis of variance based on the completely randomized design. Significant differences amongst treatment means were determined using least significant difference. Data analysis and means

separation were performed using the R statistics program as described by R Core Team (2014).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of Ginger

The proximate compositions of the ginger (ginger meal and fresh) samples are shown in Table 4.1. The result showed that the crude protein and crude fibre values of air dried ginger were 13.19 % and 5.95 % respectively. These values are comparatively higher than corresponding values in maize (Aduku, 1993); while the fresh ginger has values lower in crude protein and crude fibre than that of maize. Information is scarce in literature on the proximate composition of ginger. However, Abdallah *et al*. (2013) conducted proximate analysis on ginger powder. The ash content, crude protein and nitrogen free extract of the air dried ginger are close to the values reported by Abdallah *et al*. (2013). The result on the metabolizable energy (ME) value of the air dried ginger in this study is in agreement with that of Abdallah *et al*. (2013). The minor variation in values as noted in this study may be due to the differences in variety, environmental condition or processing method employed.

Table 4.1: Proximate composition of ginger

Parameters (%)	Ginger meal	Fresh ginger
Moisture Content	20.21	83.45
Ash	7.08	0.94
Crude Protein	13.19	1.52
Crude Fibre	5.95	1.90
Ether Extract	0.39	0.08
Nitrogen Free Extract	53.18	12.12
[a]ME(MJ/kg)	2.60	1.74

[a]ME = Metabolizable Energy, calculated according to Lodhi *et al*. (1970).

4.2 Broiler Performance

The results on the performance of broiler birds fed different levels of ginger meal based on daily weight gain, daily feed intake and feed conversion ratio are shown in the Table 4.2. The daily weight gain improved as the level of ginger meal increased from 0 – 0.6 % in the diets, but revealed no significant difference ($P > 0.05$). Similar results were noted on daily feed intake and feed conversion ratio ($P > 0.05$) as revealed in this study. The result obtained in this study contradicts the findings of Mohammed *et al*. (2012) who reported significant differences ($P < 0.05$) in body weight, weight gain, feed conversion ratio and feed intake when broiler birds were fed diets containing 0.1% and 0.2% ginger. The non-significant effect of ginger observed in this study is in agreement with previous reports that indicated herbs, plant extracts, essential oil and/or the main components of the essential oil did not affect body weight gain, feed intake or feed efficiency in broilers (Demir *et al*., 2003; Cross *et al*., 2002; Botsoglou *et al*., 2004; Hernandez *et al.*, 2004; Amooz & Dastar, 2009; Freitas *et al.*, 2001 & Bampidis *et al*., 2005). Specifically, results from this study correspond with the findings of Dieumou *et al*., (2009) who fed ginger essential oils to broilers and found that, there were no differences between the birds on the ginger oil diets and the control in terms of feed intake, body weight gain and feed conversion ratio.

Although growth performance was not significantly affected ($P > 0.05$) when ginger meal was included in the diets, there was a tendency of broilers consuming ginger meal-supplemented diets to grow faster compared to broilers fed the control diet. El-Deek *et al.* (2002) observed that diet containing 1 g/kg of ginger did not affect the growth performance, whereas Farinu *et al*. (2004) reported that supplementation of ginger meal at levels of 5, 10, or 15 g/kg slightly improved growth performance of broilers. In contrast, Al-Homidan (2005)

Table 4.2: Effect of dietary inclusion of ginger meal on broiler performance

	Ginger meal levels (%)				
Parameters (g)	**0**	**0.2**	**0.4**	**0.6**	**SEM**
Initial liveweight (Day old)	42.00	43.00	42.00	43.00	0.00
Final liveweight (8 weeks)	2371.69	2429.18	2401.71	2532.24	61.78
Daily weight gain	41.60	42.61	42.14	44.45	7.72
Daily feed intake	106.24	106.55	105.91	107.06	12.86
Feed conversion ratio	2.55	2.50	2.52	2.41	0.05
[a]Feed cost/kg weight gain	259.19	261.13	270.29	265.94	5.66
[b]Feed cost/kg weight gain	228.55	231.13	240.09	237.02	5.01

Differences were not statistically significant ($P > 0.05$); [a]Feed cost is in Naira for starter phase; [b] for finisher phase.

observed reduced growth rate of starter broilers (1 – 4 weeks) when ginger was fed at the rates of 20 and 60 g/kg. Onu (2010) reported that the addition of ginger (0.25%) in the basal diet of broiler chicks resulted in improved feed conversion ratio and higher body weight, although feed intake was not affected. The non-significant difference in weight gain could be linked to the non-significant effect on feed intake.

There were no significant differences ($P > 0.05$) in the cost of feed per kg weight gain between the treatment groups. This contradicts Minh *et al*. (2010) who reported that supplementation of dried ginger to broiler diets led to improved performance and reduced feed cost. Ginger, like other spices (e.g. pepper) loses its pungency the longer it is stored post milling. Although the air dried and milled ginger used in this study was stored in air tight containers, it is likely that a significant amount of its potency were lost during storage. Thus, investigating the effect of storage period post milling on broiler performance is necessary.

4.3 Lipid Profile

The results on the lipid profile of broiler birds fed diets containing varying levels of ginger meal are shown in Table 4.3. The results showed no significant differences ($P > 0.05$) in cholesterol, triglyceride and LDL levels, but a significant difference ($P < 0.05$) was observed in HDL level. The birds fed 0.4% ginger meal diets were significantly higher in HDL level than those reared on 0.2% ginger meal diet. The results obtained in this study are not in agreement with Saeid *et al*., (2010), who reported significant decreases in total cholesterol, LDL, Very low density lipoprotein (VLDL) and an increase in HDL cholesterol concentrations in broiler chicken fed diets containing 0.4 % and 0.6 % aqueous ginger extract. Also, Najafi & Taherpour (2014) reported a significant difference ($P < 0.05$) in triglycerides, total cholesterol and LDL cholesterol concentrations, but no significant

Table 4.3: Effect of dietary inclusion of ginger meal on serum lipid profile and meat ether extract of broilers

	Ginger meal levels (%)				
Parameters (mg/dL)	**0**	**0.2**	**0.4**	**0.6**	**SEM**
Cholesterol	123.30	116.90	140.10	118.76	7.82
Triglycerides	83.14	39.54	61.74	59.46	15.04
HDL	42.96[ab]	25.38[a]	56.72[b]	42.72[ab]	10.12
LDL	63.70	78.22	75.70	64.16	12.97
Ether Extract (%)	12.32	10.65	15.81	16.38	1.98

[a,b] Means on the same row bearing different superscript are significantly different ($P < 0.05$)

HDL = High Density Lipoprotein

LDL = Low Density Lipoprotein

Cholesterol; 125 – 200 of serum (mg/100ml)

difference (P > 0.05) in HDL cholesterol concentration when ginger meal was fed to broiler birds at 0.8 % inclusion. Work done by Ademola *et al*. (2009) reported a significant difference (P < 0.05) in triglycerides, total cholesterol, LDL and HDL in broilers fed mixture of ginger and garlic at levels 1.5 % and 2.0 %. The result of this study indicated that, birds placed on 0.4 % ginger meal inclusion will have a better health outcome than those on the 0.2 % ginger meal inclusion. This could be explained by work of Gordon *et. al*. (1989) who reported that having large numbers of large HDL particles correlates with better health outcomes.

Evidence from results in this study and of previous reports strongly suggests hypolipidaemic and hypocholesterolaemic effects of ginger meal and its extracts. The hypotriglyceridemia effects in chickens fed with herbs are probably due to active ingredients in herbs leading to a decrease in the activity of lipogenic enzymes; thereby reducing re-synthesis (*de novo*) of fatty acids in liver and subsequently reducing blood LDL cholesterol level (Ciftci *et al*., 2010). The use of ginger extract was reported to reduce cholesterol levels in animals (Galib *et al*., 2010). According to some studies, active ingredients of medicinal herbs inhibit activity of hydroxymethyl glutaryl Coenzyme A reductase (HMG – CoA) in the liver (Crowell, 1999; Elson *et al*., 1989). This enzyme is a key regulatory enzyme in cholesterol synthesis. Although no significant differences (P > 0.05) were observed in ether extract from meat samples, it is likely that at higher levels of inclusion of ginger meal, significant reduction would manifest.

4.4 Haematology

Results on haematological parameters of broiler birds fed varying levels of ginger meal are shown in Table 4.4. Haemoglobin concentration, packed cell volume (PVC) percentage, mean cell haemoglobin (MCH) level and total white blood cell (TWBC), lymphocyte and

Table 4.4: Effect of dietary inclusion of ginger meal on haematology and bacterial load

	Ginger meal levels (%)				
Parameters	**0**	**0.2**	**0.4**	**0.6**	**SEM**
Haemoglobin (g/dl)	10.16[a]	11.96[b]	10.08[a]	10.60[a]	0.29
PCV (%)	32.22[a]	36.68[b]	32.78[a]	33.66[a]	0.89
RBC ($x10^6$/ul)	2.74	2.79	2.62	2.56	0.12
MCV (fl)	121.48	132.6	126.64	133.52	4.79
MCH (Pg)	34.26[a]	40.34[b]	37.60[ab]	40.02[b]	1.89
MCHC (g/dl)	29.36	30.38	29.86	29.74	0.69
Platelet ($x10^3$/ul)	24.00	27.10	24.80	25.60	1.17
TWBC (%)	65.04[a]	69.32[ab]	89.62[c]	76.78[b]	2.49
Lymphocyte (%)	86.80[b]	85.60[ab]	86.00[ab]	81.60[a]	1.56
Neutrophil (%)	13.20[a]	14.40[a]	14.00[a]	19.40[b]	1.42
Monocyte (%)	-	-	-	-	-
Eosinophil (%)	-	-	-	-	-
Basophil (%)	-	-	-	-	-
Bacterial Load	+ + +	+ + +	+ + +	+ + +	-

[a,b,c] Means on the same row bearing different superscripts are significantly different ($P < 0.05$)

+++ Heavy bacteria growth of Salmonella spp.

neutrophil percentages showed significant differences ($P < 0.05$), while red blood cell (RBC) counts, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and platelet counts revealed no significant differences ($P > 0.05$) across the treatment groups. There was no trace of monocyte, eosinophil and basophil percentages in all the groups studied. The values for haemoglobin concentrations were 10.16, 11.96, 10.08 and 10.60 g/dl for 0, 0.2, 0.4 and 0.6 % ginger meal inclusion, respectively. The haemoglobin concentration of 0.2 % ginger meal dietary group was significantly higher ($P < 0.05$) than the other dietary groups, whereas values for 0, 0.4 and 0.6 % ginger meal dietary groups were similar ($P > 0.05$). Similarly, the PCV of the 0.2 % ginger meal treatment group was significantly higher ($P < 0.05$) than values from the other treatment groups while the PCV of 0, 0.4 and 0.6 % treatment groups were similar ($P > 0.05$). Chicks fed the control diet were lowest in TWBC. There was a significant difference in TWBC between the 0.4 % and 0.6 % ginger meal dietary groups ($P < 0.05$).

Results on the effects of dietary inclusion of ginger on haematological profile have been largely inconsistent. Ademola *et al*., (2009) reported no significant difference ($P > 0.01$) in PCV, Haemoglobin and RBC but a significant difference ($P < 0.01$) in TWBC when broiler chicks were fed diets containing ginger, garlic and their mixture at rate 1.0, 1.5 and 2.0 % inclusions. This is in agreement with results from the present study. However, Kehinde *et al*., (2011) reported no significant difference ($P > 0.05$) in PCV, haemoglobin, RBC, TWBC and lymphocyte when gingerized diet was fed to white cockerel chicks at 2 weeks. Najafi & Taherpour (2014) reported a significant difference ($P < 0.05$) in RBC, haemoglobin and lymphocyte but no significant difference ($P > 0.05$) in TWBC and heterophil when ginger meal was supplemented in broiler diet at rates of 0.4 % and 0.8 %. Nonetheless, Mitruka *et al*., (1977) stated that the number of RBC in chicken is influenced by the condition of the animal, which may be a contributory source of variation.

Herbal oils such as ginger oil increase immunoglobins levels in the blood as well as the ability to destroy microbial cells by leukocytes due to terpinen-4-ol (Koenen *et al*., 2004). Increasing lymphocytes count in the blood following increase in white blood cells count can play an important role in stimulating the immune system of animals (Al-Kassi, 2009). This underpins the importance of higher TBWC levels associated with ginger meal inclusion in the diet. In all likelihood, the increase in TWBC and neutrophil counts in 0.4 % and 0.6 % ginger meal groups, respectively, represents the stimulation of the host's immune system. This implies that, anti-viral, anti-bacterial and anti-fungal properties of some of these herbs may be in relation to improvement of immune function and thus make the environment unfavourable for invading foreign agents. Ginger roots have also been reported to contain a number of compounds that exert other biological activities, including antioxidant action (Nakatani, 2000; Rababah *et al*., 2004), antimicrobial efficacy (Akoachere *et al*., 2002; Jagetia *et al*., 2003; Mahady *et al*., 2003) and numerous other pharmacological effects (Chrubasik *et al*., 2005; Ali *et al*., 2008).

4.5 Bacterial Load

The results on bacteria load on broiler meat from chicken fed diets containing varying levels of ginger meal are shown in Table 4.4. Result showed a heavy bacteria growth of Salmonella species. Sudrashan *et al*., (2010) reported that essential oil extracted from ginger resulted in a significant reduction in the bacterial counts of *Staphylococcus*, *Escherichia coli* and *Salmonella* when applied as a decontaminating agent in the ratio of 1:150, 1:250 and 1:500 to chicken meat. The different outcomes may be due to the form in which ginger was applied and the environmental condition in which this study was done. Chrubasik *et al*. (2005) suggested that, the processing method of ginger product affected its clinical efficacy.

There is dearth of data on effect of ginger incorporated in the diets on the shelf-life of broiler meat. Nonetheless, numerous authors have observed that plant extracts with anti-microbial properties can be used to increase refrigerated meat shelf-life (Skandamis *et al*., 2002; Djenane *et al*., 2003; Chouliara *et al*., 2007). Eugenol, clove, oregano and thyme extracts applied on meat were reported to be effective against *Lactobacillus monocytogenes*, *A. hydrophila*, and autochthonous spoilage flora at higher concentrations than those required *in vitro* assays (Hao *et al*., 1998a; 1998b; Skandamis & Nychas, 2001).

4.6 Carcass Characteristics and Organ Weights

There were significant differences ($P < 0.05$) observed in per cent neck, carcass weight and dressing percentage across the groups (Table 4.5). However, no significant differences ($P > 0.05$) were observed on head, heart, shanks, small intestine, gizzard full, gizzard empty and liver + gall bladder percentages. The percentage neck on 0 % ginger meal chicks was significantly lower ($P < 0.05$) than the 0.4 % ginger meal group, but was similar with those of 0.2 % and 0.6 % groups. Chicks fed the 0 % ginger meal diet (control) were lowest in carcass weight and dressing percentage, while those fed the 0.4 % ginger meal diet were the highest in dressing percentage ($P < 0.05$). Gradual and progressive increase in drumstick percentage was observed as the inclusion level of ginger meal increased, although the differences were not significant ($P > 0.05$).

Kamal & Abo Omar (2012) reported no significant differences ($P > 0.05$) in the carcass cuts and organ weights of broilers fed diets containing garlic powder (a herb like ginger) at the rates of 0.2 % and 0.4 %.

Table 4.5: Effect of dietary inclusion of ginger meal on carcass characteristics and organ proportions[1]

	Ginger meal levels (%)				
Parameters (%)	**0**	**0.2**	**0.4**	**0.6**	**SEM**
Head	2.10	2.12	2.22	2.39	0.24
Neck	3.25[a]	3.74[ab]	4.13[bc]	3.81[ab]	0.29
Heart	0.48	0.42	0.40	0.41	0.03
Shanks	3.06	3.27	3.60	3.74	0.29
Small Intestine	1.85	2.00	2.16	1.95	0.18
Gizzard Full	3.08	3.14	2.82	3.06	0.29
Gizzard Empty	2.26	2.35	2.23	2.32	0.24
Liver + Gall bladder	1.87	1.98	1.87	1.78	0.08
Drumstick	9.86	10.72	11.44	11.95	0.79
Dressing Percentage	68.19[a]	68.75[ab]	71.55[c]	70.87[bc]	0.80
Carcass Weight (g)	1876.80[a]	2221.40[b]	2123.8[b]	2257.40[b]	68.67

[a,b,c] Means in the same row bearing different superscripts are significantly different ($P < 0.05$)

[1] Percentage of live weight

In contrast, Ademola *et al*. (2009) reported significant differences ($P < 0.05$) in drumsticks, wings, shanks, liver, gizzard and GIT weights but no significant differences ($P > 0.05$) in head and neck weights of broilers fed diets containing mixtures of ginger and garlic at the rates 1.0, 1.5 and 2.0 %. The results on carcass weight and dressing percentage were in agreement with the result of Zhang *et al*. (2009) who observed that birds fed ginger as part of the diet produced higher carcass weights compared to untreated birds. Results obtained in this study showed that, inclusion of ginger meal up to the 0.6 % level had no effect on heart, liver + gall bladder and intestinal weights. This suggests that there were no adverse physiological or physico-chemical reactions. The slight improvements in percentage drumstick and significant increase in dressing percentage are recommendable positive effects. The implication is that, this inclusion of ginger meal in broiler diets may result in increased lean growth and overall improvement in carcass weight. Zhang *et al*. (2009) suggested that improved carcass quality of broiler may be associated with the antioxidant effect of ginger which enhances protein and fat metabolism.

4.7 Organoleptic Quality

The results showed no significant differences ($P > 0.05$) in juiciness, flavour and hedonic score, while a significant difference ($P < 0.05$) was observed in tenderness (Table 4.6). The meat from the 0.4 % and 0.6 % treatment groups were significantly ($P < 0.05$) more tender than the 0 % treatment group. Information on the organoleptic quality of broilers fed ginger meal supplemented diets is scarce in literatures. However, work done by Odoemelam *et al*. (2013) showed a significant difference ($P < 0.05$) in tenderness, flavour, juiciness and general acceptability of broilers fed diets containing varying levels of scent leaf meal, a spice like ginger. Many herbs, spices and their extracts have been added in a variety of foods to improve their sensory characteristics and extend shelf-life (Shahidi *et al*., 1992).

Table 4.6: Effect of dietary inclusion of ginger meal on meat colour[1], cooking loss and organoleptic rating[2]

	Ginger meal levels (%)				
Parameters	**0**	**0.2**	**0.4**	**0.6**	**SEM**
Meat Colour	2.80	2.80	2.20	2.60	0.21
Cooking Loss (%)	10.65	10.65	13.08	11.59	1.42
Tenderness	7.40[a]	8.00[ab]	8.20[b]	8.20[b]	0.24
Juiciness	6.80	7.20	7.20	7.00	0.32
Flavour	7.20	7.40	7.60	7.80	0.59
Hedonic Score	7.20	7.80	7.80	7.80	0.46

[a,b] Means in the same row bearing different superscript are significantly different ($P < 0.05$)

[1] The 3 points colour score used was: Normal colour = 3; slightly abnormal = 2; clearly abnormal = 1.

[2] The 9 points Category Rating Scale was used, according to AMSA (1978): Extremely tender/juicy/flavoured = 9; very tender/juicy/flavoured = 8; moderately tender/juicy/flavoured = 7; slightly tender/juicy/flavoured = 6; neither tender/juicy/flavoured, nor tough/dry/unflavoured = 5; slightly tough/dry/unflavoured = 4; moderately tough/dry/unflavoured =3; very tough/dry/unflavoured = 2; extremely tough/dry/unflavoured = 1.

Hedonic Scoring: Extremely liked = 9; very liked = 8; moderately liked = 7; slightly liked = 6; neither liked nor disliked = 5; slightly disliked = 4; moderately disliked = 3; very disliked = 2; extremely disliked = 1.

However, authors have reported that natural antioxidants have no effect on sensory characteristics of meat. Chaves *et al*., (2008) did not detect any effect of essential oil compounds (carvacrol and cinnamaldehyde) added to the diet of growing lambs, on the sensory characteristics of sirloins. The same was observed in pork where different essential oils were included in the diet (Janz *et al*., 2007). The result from the hedonic rating suggests that panellist seemed to like meat from all the groups equally.

4.8 Meat Colour

The results on the meat colour of broiler birds fed diets containing varying levels of ginger meal are shown in Table 4.6 above. Results of the analysis showed no significant difference ($P > 0.05$) in meat colour between the treatment groups. There is lack of information in literature on the efficacy of ginger meal in altering meat colour of broiler. However, the rhizome powder of turmeric (*Curcuma longa Linn*), a member of the Zingiberaceae family, has been extensively used for imparting colour and flavour to foods (Deshpande *et al*., 1997). Several authors have reported the effectiveness of rosemary and oregano extracts to reduce colour loss in some types of meats (Djenane *et al*., 2002). The result of this study shows that ginger meal inclusion to broiler diets did not affect the meat colour; this is due to its lack of pigmentation properties.

4.9 Percentage Cooking Loss

There were no significant differences ($P > 0.05$) in percentage cooking loss in the treatment groups (Table 4.6). The study is suggesting that, inclusion of ginger meal in broiler diets had no effect on post-mortem glycolysis or exudation of juice from the meat. Expectedly, during post-mortem aging, the antioxidant properties in ginger meal could have imparted its preservative effect which may have a moderating effect on cooking. In this study, the muscle samples were not aged as they were tested for cooking losses about 24 hours post-mortem.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The following conclusions were made:

i. There were changes in final live weight, daily weight gain, daily feed intake and feed conversion ratios of broilers, but were not significant ($P > 0.05$), when dietary ginger meal was included in the broiler diets..

ii. The cholesterol, triglycerides and low density lipoprotein (LDL) were not significantly affected ($P > 0.05$), but the 0.4 % ginger meal diet resulted in significantly higher ($P < 0.05$) serum high density lipoprotein (HDL) level.

iii. The ether extract of broiler meat was not significantly affected ($P > 0.05$) when the dietary ginger meal was made part of the chicks diets.

iv. The haemoglobin concentration, mean cell haemoglobin and packed cell volume, total white blood cell, lymphocyte and neutrophil percentages were significantly affected ($P < 0.05$), while the red blood cell counts, mean cell volume, mean cell haemoglobin concentration and platelet counts were not significantly affected ($P > 0.05$) by dietary inclusion of ginger meal

v. There was heavy bacteria growth of Salmonella species in the broiler meat of all the treatment groups after 10 hours of slaughter. The inclusion of ginger meal in the diet showed no anti-microbial impact on the meat.

vi. On organ proportion, significant difference was only seen in the neck proportion with the 0.4 % ginger meal groups recording the highest ($P < 0.05$). The internal organs were not affected even at 0.6 % level of inclusion.

vii. The dressing percentages were significantly different ($P < 0.05$) with the 0.4 % ginger meal groups diets recording the highest.

viii. The carcass weights of all the ginger meal treated groups were significantly higher ($P < 0.05$) than as noted in the control group.

ix. On organoleptic quality, there was a significant difference ($P < 0.05$) in tenderness with the 0.4 % and 0.6 % groups, which recorded the highest values, while juiciness, flavour and hedonic scores revealed no significant differences ($P > 0.05$).

x. The dietary inclusion of ginger meal did not have any significant effect on the cooking loss and meat colour of the broiler birds ($P > 0.05$).

5.2 Recommendations

In the light of the above conclusions, the following recommendations are made:

i. For better health outcome on birds and consumers, poultry producers should incorporate ginger meal at 0.4 % in broiler diets. It increased the HDL level of broilers, and having higher serum level of HDL particles correlates with better health.

ii. For an increased productivity and profitability, operators in the poultry industry should incorporate ginger meal in broiler diets; it increased values such as the carcass weight and dressing percentage. This will attract higher market prices for the broilers.

iii. In order to produce broilers with higher organoleptic quality, producers should incorporate ginger meal at 0.4 % or 0.6 % levels since with these levels, there was increased in the broiler meat tenderness.

iv. Further researches are necessary to investigate the efficacy of ginger meal inclusion in poultry diets for improved productivities and good quality meat.

5.3 Contribution to Knowledge

This research work show that the inclusion of ginger meal at 0.4% or 0.6% levels in broiler diets increases the serum high density lipoprotein level, dressing percentage, carcass weight and meat tenderness of broilers. Researchers, academics and broiler producers should find this contribution a significant addition to currently available body of knowledge.

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