PERFORMANCE, BONE MINERALIZATION AND HAEMATOLOGICAL INDICES OF BROILERS FED PHYTOGENIC FEED ADDITIVES

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE
UNIVERSITY OF NIGERIA, NSUKKA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE UNIVERSITY OF NIGERIA, NSUKKA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (MSc) IN ANIMAL SCIENCE, (ANIMAL NUTRITION AND BIOCHEMSITRY) OF THE UNIVERSITY OF NIGERIA NSUKKA

BY

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CERTIFICATION

I, AKURU, EUNICE AMAKA, a postgraduate student in the Department of Animal Science, with registration number, PG/M.sc/13/65784, have satisfactorily completed the requirements for research work for the award of the degree of Master of Science in the Department of Animal Science (Animal Nutrition and Biochemistry). This work is original, and has not been submitted in part or full, for any other diploma or degree of this or any other University.

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DEDICATION

In memory of my late Father, Mr. Nonyelum Nathaniel Nwokoro, who inspired my desire and drive to acquire knowledge.

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ABSTRACT

A 35-day experiment was carried out in a completely randomized design to determine the efficacy of two phytogenic feed additives as veritable alternatives to antibiotics using 320 day-old unsexed commercial Ross 308 broiler chicks. The chicks were randomly divided into four groups of 80 birds each and assigned to four isocaloric (12.60, 13.2 and 13.4 MJ/Kg AMEn) and isonitrogenous (25.0, 22.5 and 20.07 % CP levels) diets designated as follows: diet 1- maize-soybean based diet without additives (negative control), diet 2- maize-soybean based diet containing 10% tylosin (positive control), diet 3-maize-soybean based diet containing phytonutrient (product 1) and diet 4-maize-soybean based diet containing phytonutrient (product 2). Diet 2 contained 200g/ton of antibiotics, while diets 3 and 4 contained 1L/ton each of phytogenic feed additives. Each treatment was replicated 8 times with 10 birds per replicate. Birds had *adlib* access to feed and water. At 35th day of the experiment, 8 birds per treatment, one from each replicate pens were randomly selected, electrically stunned at 70V and killed by cervical dislocation for meat quality, carcass and organ weights determinations. The birds had their left and right tibias removed for bone strength and mineral assessment. Eight birds per treatment were also randomly selected and euthanized according to standard ethical procedures and used for assessment of haematological parameters. Inclusion of phytogenic feed additives (products 1 and 2) resulted in significant reduction ($p<0.05$) in feed intake and significantly ($p<0.05$) enhanced performance of birds at week 7 of the trial. Birds fed diets containing phytogenic feed additives product 2 had significantly higher ($p<0.05$) thigh weights. The inclusion of phytogenic feed additives (products 1 and 2) in some of the diets reduced fat content of meat significantly ($p<0.05$) compared to the control groups. Treatments did not have significant ($p >0.05$) effect on bone parameters. Dietary inclusion of phytogenic feed additives (products 1 and 2) significantly improved broiler feed efficiency in week 1, reduced broiler meat fat content and improved the immune status of broiler birds. Treatments had no significant ($p >0.05$) effect on white blood cells (WBC), neutrophils, eosinophil, red blood cells (RBC), haemoglobin concentration (Hb), haematocrit value (HCT), mean corpuscular value (MCV), mean heamoglobin count (MCH), mean cell haemaglobin concentration (MCHC), red blood cell distribution width (RDW) and platelet count. Leucocyte differential values such as lymphocytes, monocytes and basophils were significantly ($p<0.05$) affected by treatments.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Rearing ruminant animals is exorbitant and time demanding. These have prompted a paradigm shift towards early maturing and fast growing poultry such as broiler chickens. Chickens are one of the major sources of meat and this has made broiler farming a profitable venture due to increased consumer demand for broiler meat. In other words, broilers, apart from fast growth are priced highly by humans as a result of the high protein quality of their meat. Hence, the aim of producing broilers on a commercial scale over the years has been to increase performance within the shortest possible time. This increase in performance is directly correlated with performance indices like daily gain, feed intake and feed conversion ratio (Longo *et al*., 2005).

Low animal protein intake has remained a major human nutritional problem in Nigeria, especially for the non-wage and low income earners (Amaefule *et al*., 2009). According to Schonfeldt and Hall (2012), dietary energy is an index of diet composition. Unlike the developed countries of the world whose two main sources of protein are animal products and cereals, low income countries still derive most of their dietary protein from cereal based staple food, with only 3% of the total dietary energy coming from meat and offal (FAO, 2008). Although, the production of livestock has increased in recent times in developing countries, nevertheless, low protein intake is continuously increasing. Hence, under nutrition, including insufficient consumption of protein remains a persistent problem in most developing countries of the world (Schonfeldt and Hall 2012). In the light of the aforementioned facts, the problem of inadequate protein supply in most developing countries like Nigeria can be solved to a large extent if more intensive commercial broiler production is encouraged and embarked upon.

1.2 PROBLEM STATEMENT

The poultry industry has for decades achieved the aim of improved performance and fast growth in broiler chickens with antibiotic growth promoters (AGPs) (Niewold, 2007; Hajati and Rezaie, 2010). These AGPs reputed for growth promoting and disease control activities have been used at low, sub-therapeutic doses to improve growth performance, feed efficiency and health condition of poultry species. However, long-term use poses serious threats to the health of these birds and may also have carcinogenic effects on human consumers. Pathogenic resistance to

antibiotics and imbalance in normal beneficial intestinal micro flora of poultry birds is also on the increase. To this end, the use of AGPs in poultry nutrition has aroused researchers, consumers and government regulators to advocate for alternative measures. In view of this, the search for unconventional botanical feed additives as alternatives to antibiotics is imperative (Niewold, 2007; Hajati and Rezaie, 2010; Sen *et al*., 2012). These botanical feed additives are also known as phytobiotic or phytogenic substances, and are regarded as natural growth promoters due to naturally occurring phyto-constituents that abound in them.

1.3 OBJECTIVES OF THE STUDY

The broad objective of this research was to ascertain the performance, bone mineralization and haematological indices of broilers fed phytogenic feed additives. Specific objectives were to:

1. Determine the performance of broiler birds to diets containing two phytogenic feed additives.
2. Determine the effect of phytogenic feed additives on meat quality, carcass characteristics and skeletal parameters of broiler chickens.
3. Determine the organ weights and gizzard erosion scores of broilers fed diets containing two phytogenic feed additives.
4. Determine the effect of phytogenic feed additives on haematological parameters ofbroiler chickens.

1.4 JUSTIFICATION OF THE STUDY

In view of the ban on antibiotics by the European Union in 2006, the use of phytogenic feed additives has received increased attention as veritable alternatives to antibiotics in poultry nutrition. It has been reported that these feed additives, commonly known as phytobiotics (ie plant antibiotics), botanicals or phytogenic feed additives are safe, affordable and contain important pharmacologically- active substances such as curcumin, allicin, papain and a host of others. Among the spectrum of phytogenic feed additives that have received increased attention as alternatives to antibiotic growth promoters, acidifiers, probiotics, prebiotics, exogenous enzymes, non- starch polysaccharides (NSP), essential oils, and various classes and blends of medicinal plants and resins seem to be the most researched (Hajati and Rezaei, 2010). Phytogenic feed additives (PFA) have been implicated in exerting beneficial effects in animal nutrition, particularly in appetite stimulation, improvement of endogenous digestive enzyme secretion, activation of immune responses and numerous other therapeutic and pharmacological potentials (Toghyani *et al*., 2010,

2011). Due to the biochemical constituents of these additives, they are able to play essential roles in improving growth performance of animals, particularly in livestock and poultry (Windisch *et al*., 2008; Jacela *et al*., 2010). These additives are made up of diverse groups of herbs, spices and products derived thereof, and are mainly essential oils.

According to (Huyghaebaert *et al*., 2011), antibiotic growth promoters function in such a way that stimulates antibacterial action and as a result lead to a reduction in the incidence and severity of subclinical infections. Subsequently, the microbial usage of nutrients decreases but their absorption from the intestine is improved as a result of thinning of the intestinal mucosal. This indirect impact of AGPs mode of action translates into higher growth rate. Hypothetically, AGPs do not exert growth promoting effect in germ-free animals. In view of these facts, for any phytogenic feed additive to effectively replace antibiotics in livestock nutrition it is expected that they have the same beneficial effects as AGPs when included in the diets. It is expected also that just like AGPs, these alternative compounds should have modulatory effects on the gut microbiota and immune system (Huyghaebaert *et al*., 2011). To this end, a number of these plant-based substances have been found to possess qualities comparable to antibiotics, thus making them potent alternatives. However, there are reports that the mode of action of most of the phytogenic feed additives is not very clear.

The place of phytogenic feed additives as alternative compounds cannot be over-emphasized particularly with reference to their role in poultry nutrition. Exploring these additives in feeding of poultry species is not only beneficial to the animals but also to the human consumers. Different phytogenic feed additives have been used in broiler nutrition with varying results. A study was conducted to ascertain the effect of varying dietary levels (0.0, 0.25, 0.5 and 0.75%) of turmeric rhizome powder (TRP), and 50mg/kg vitamin E on the thigh meat of broiler chickens after slaughter. It was reported that the content of Malondialdehyde in the thigh meat of both birds fed medium (0.5%) turmeric rhizome powder, and those fed 50mg/kg vitamin E were much lower when compared to the groups whose diets were free of TRP. From the research findings it was evident that dietary inclusion of 5mg/kg TRP can increase the thigh meat shelf-life storage and quality in broiler chickens after slaughter (Daneshyar, 2012). Daneshyar *et al*. (2011) also reported that dietary supplementation of turmeric rhizome powder has the potential to reduce the concentrations of saturated fatty acids and triglycerides in thigh meat and improve the meat quality of broiler meat. Dietary supplementation of garlic meal reduced the population of pathogenic coliform in the gut of broiler chickens as evident in the reports of Sarica *et al*. (2005). It is reported that garlic has the

ability to lower the cholesterol and triglyceride levels of poultry meat and egg products, and also prevent atherosclerosis. Garlic is also reported to possess anti-thrombotic, anti-platelet, anti-hypertensive, anti-lipidemia properties (Amagase, 2006; Rahman and Lowe, 2006; Rahman, 2007). Acamovic *et al*. (2007) assessed the effects of dietary inclusion of 5 different culinary herbs or their essential oils on the growth, digestibility and intestinal microflora status of 165 female broiler chicks from 0-28 days of age. The authors reported that use of 10g/kg of these plant extracts (thyme, rosemary herb, oregano, marjoram) and/or 1g/kg of their essential oils affected the performance, gut health and endogenous secretions of the broiler birds. However, it is important to ascertain the chemical composition of the extract before optimal benefits can be obtained.

Against these backdrops, this study was conducted to investigate the performance, bone mineralization and haematological indices of broilers fed phytogenic feed additives.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW ON PHYTOCHEMICALS

Phytogenic plant substances, or phytobiotics as often called, have been reported as veritable alternatives to antibiotics due to the key roles they play in digestion, nutrient metabolism and growth promoting activities in poultry species. Though, not regarded as essential nutrients, phytogenic plant substances contain important compounds or phytochemicals that enable them to ward off diseases and aid growth. Phytochemicals are chemical compounds with bioactive components that are naturally found in plants. They endow plants with protective ability to resist and withstand attacks from pollution, stress, drought, ultra-violent exposure and pathogens. Recently, phytochemicals are reported to also possess health promoting potentials, and perform essential roles other than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999; Gibson *et al*., 1998; Mathai, 2000). Phytochemicals with disease preventing abilities are majorly dietary fibres, antioxidants, anti-carcinogenic, detoxifying agents, immunity-potentiating agents and neuro-pharmacological agents. There are different criteria for classifying phytochemicals, but the most employed are the protective function, chemical properties and physical attributes. Hence, over 4000 of them have been catalogued with only 150 studied extensively (American Cancer Society, 2000; Meagher and Thomson, 1999).

Based on the role of phytochemicals in plant metabolism, two main groups have been identified; namely primary and secondary constituents. Primary constituents are the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls, etc. Secondary constituents include plant compounds like alkaloids, terpenes, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Hahn, 1998). Of the secondary constituents, the phenolics are the most numerous and structurally diverse plant components that are made up of mostly flavonoids, phenolic acids and polyphenols. The polyphenolic components of higher plants may act as either antioxidants or agents of other mechanisms contributing to anti-carcinogenic or cardio-protective action. According to Hertog *et al*. (1993), polyphenols are multi-factional in that they either act as reducing agents, hydrogen donating antioxidants, singlet oxygen quenchers, or even as metal chelates. On the other hand, flavonoids are polyphenolic compounds with cytoprotective properties found in dietary plants and vegetables. Flavonoids constitute a wide range of substances

that protects biological systems against the deleterious effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids and DNA (Atmani *et al*., 2009). Phenolic acids are however, phenols with one carboxylic acid functional group, which are notable for numerous biological activities. In view of this, phenolic acids and flavonoids are two powerful antioxidants that have potential to scavenge free radicals and reactive oxygen species (ROS), thus protecting the body from oxidative damage (Saxena *et al*., 2013). Table 2.1 summarizes the important phytochemicals found in phytogenic plant substances and their essential constituents and roles.

Table 2.1: Important Phytochemicals Found in Botanical Additives

Classification	Bioactive Components	Biological Relevance	References
NSP (Non-Starch Polysaccharide)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids	Santos Jr. *et al*., 2004b.
Antibacterial and Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, lower the risk of fungal infection	Molyneux *et al*., 1996; Morrissey and Osbourn, 1999; Traore *et al*., 2000
Antioxidants	Polyphenolic compound, flavonoids, carotenoids, tocopherol, ascorbic acid	Oxygen free radical quenching, inhibits lipid peroxidation	Cohen and Kennedy, 2010; Cheung *et al*., 2010; Atmani *et al*., 2009.
Anticarcinogens, mutagenic and chemopreventive agents	Carotenoids, polyphenols, curcumin, flavonoids, phenolic acids, isothiocyanates, tocopherols	Inhibitors of tumour. Inhibits the development of lung cancer and possess anti-metastatic property. Chemopreventive roles. Induces apoptosis and modulate cell-cycle progression of highly proliferative carcinogenic cells	Fimognari *et al*., 2004; Saxena *et al*., 2013
Detoxifying agents	Reductive acids, tocopherols, phenolics, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen, activation, inducers of drug binding carcinogens, inhibit tumourogenesis.	Serrano *et al*., 2009; Meagher *et al*., 1999.
Immunomodulatory agents	Flavonoids, terpenoid, curcumin, organosulfuriccompouds	Improvement of immune responses through reduction of oxidative stress	Wenk, 2000.
Anti-inflammatory	Flavonoids, phenolic acids, tannins, tetrahydroxylcurcumin, organosulfuric compounds	Effective on a number of modulators of anti-inflammatory pathways influencing specific cytokines and DNA expression	DôBrant, 2012; Silva *et al*., 2007; Saxena *et al*., 2013
Anti-microbial agents	Phenolic acids, flavonoids, terpenoids, saponins, curcumin, organosulfur compounds, essential oils	Inhibitors of mould growth, Inhibits proliferation of some strains of Staph. Aureus. Improvement of quality and shelf life of perishable food products	Lacaille-Dubois and Wagner, 2000
Hepatoprotective Agents	Anthocyanins, alkaloids, resveratrol, stilbenoids, phenolic acids, terpenes, monoterpenes, tannins.	Stimulation of hepative function. Aid in regenerating hepatic cells	Madrigal-Santillan *et al*., 2012; Neto, 2007; Sandova *et al*., 2008; Dani *et al*., 2008
Others	Alkaloids, terpenoids, volatile flavour compounds, biogenic amines	Neuropharmacological agents, antioxidants, chemoprevention	Saxena *et al*., 2013.

Modified from: Saxena *et al*. (2013): Phytochemistry of medicinal plants. Journal of Pharmacology and Pharmacognosy, 1: 6, 168-181.

2.2 IMPLICATION OF THE USE OF ANTIBIOTIC GROWTH PROMOTERS IN LIVESTOCK NUTRITION

Antibiotic growth promoters (AGPs) refer to any medicine that destroys or inhibits bacteria and is administered at a low, sub-therapeutic dose (Butaye *et al*., 2003). The use of antibiotics in livestock production became increasingly necessary on account of intensified livestock production, to such an extent that animal protein supply may run below supply for the teeming human population. According to Huyghebaert *et al*. (2011) antibiotics have been used both for prophylactic and growth promoting purposes in animal production. As prophylactics, antibiotics are known to improve the health and well-being of animals, whereas as growth promoters, antibiotics have been used to improve production parameters such as growth rate and feed conversion efficiency. Antibiotics have been included in livestock diets since the mid-20th century (Dibner and Richards, 2005; Moore *et al*., 1946; Jukes *et al*., 1950). Although the mechanism of action of antibiotics is not fully understood, a number of researchers have given insight on the possible ways in which these growth promoters play beneficial roles in the metabolism, digestion, absorption and assimilation of nutrients in food producing animals.

According to the hypothesis of Niewold (2007), AGPs act as growth promoters by reducing the number of microbes present in the gut. This implies that they may promote growth by hindering macrophages from producing and excreting cytokines following accumulation of AGPs by these immune cells. Cytokine on release causes an acute phase response with consequent loss of appetite and breakdown of muscle tissue. Although inflammation of cells leads to decrease in performance (Humphrey and Klasing, 2003), AGPs have however been noted for their ability to equally shift the composition of the normal gut microflora toward one that is less capable of causing inflammation. Thus, AGPS act by reducing the total intestinal microbial load resulting in lowered inflammation rates and less energy expended by animals. Regardless of the mechanism of action of antimicrobial growth promoters, the main thing is that an alternative substance or compound must be able to improve performance as effectively as an antimicrobial growth promoter would do.

2.3 THE NEED FOR AND CHARACTERISTICS OF GOOD ALTERNATIVES TO AGPS

In view of the numerous public health implications associated with the use of antimicrobial growth promoters in poultry nutrition, the need and search for alternative growth promoting substances is imperative (Niewold, 2007; Hajati and Hazaei, 2010; Saleha *et al*., 2009). According to Huyghebaert *et al*. (2011), any alternative compound should possess beneficial properties comparable to those of AGPS. Although, it has not been completely elucidated how AGPs exert their beneficial roles, the most potent explanation for their mode of action is that AGPs possess antibacterial properties which enable them to enhance performance and growth in several ways which includes amongst others: (1) reduction of the incidence and severity of subclinical infection, (Brennan *et al*., 2003), (2) reduction of the microbial use of nutrients (Sydney and Wostmann, 1987), (3) improving the absorption of nutrients, due to the thinning of intestinal wall and (4) reducing the amount of growth-depressing metabolites produced by Gram-positive bacteria (Knarre-borg *et al*., 2004. It implies from the mode of action of AGPs that they do not exert growth promoting effects in germ- free animals (Coates *et al*., 1953).

For any compound to sufficiently replace antimicrobial growth promoters in poultry nutrition, it should be able to play growth promoting roles and bring about enhanced performance and improvement in productivity just like AGPs. A number of compounds with microbiota modulating and immune-modulatory compounds are considered as potential alternatives to APGs. However, it is important to note, that finding a total replacement for AGPs is difficult in the meanwhile (Huyghebaert *et al*., 2011). Nevertheless, it is believed that a combination of different phytogenic compounds working synergistically can help bridge the gap created by a ban on AGPs.

2.4 PHYTOGENIC PLANT SUBTANCES AS ALTERNATIVES TO ANTIMICROBIAL GROWTH PROMOTERS

2.4.1 PROBIOTICS AND PREBIOTICS

2.4.1.1 PROBIOTICS

Probiotics are non-pathogenic live intestinal organisms whose use in livestock nutrition, particularly when in substantial amounts, have been reported to enhance growth performance, nutrient retention, gut health and intestinal microflora of the host (Reid *et al*., 2003; Shim *et al*., 2010, Mountzouris *et al*., 2010; Awad *et al*., 2010). The line of action of probiotics is mostly localized in the gastrointestinal tract of animals. Probiotics function through various mechanisms, including

protection of host organism by improving water quality and modulating immune response, production of specific metabolic by-products such as short chain fatty acids and $H_20_{2,}$ prevention of invading bacteria from colonizing hostôs gut system thereby improving intestinal balance and interaction with receptor sites. Probiotics also function by altering metabolism through increased digestive enzyme activity, production of intermediary metabolites with antibacterial property, competing with toxin-producing bacteria for adhesion receptors in the gut epithelium for nutrients and space, amongst other beneficial roles (Jin *et al*., 2000; Sherman *et al*., 2009; Cutting, 2010; Lee, 2010a).

Two main groups of probiotics have been identified; the colonizing species which include lactobacilli, bifido-bacteria and Enterococcus spp, and the free-flowing non-colonizing species such as the spore forming Bacillus spp and *Saccharomyces cerevisae*. The effectiveness of probiotics in functioning as an alternative to antibiotics often depends on the probiotic strain used, and usually, its mechanism of action is not clearly understood. This is due to the fact that varied bacteria species found in the normal microbiota of the broiler gut happens to attain an equilibrium state after about one week post-hatch depending on location in the gastro-intestinal tract, integrity of mucosa and transit time of chymus. In addition, these normal microbiota exhibit specific multifactorial barrier 'impact' which enables them to induce anatomical and physiological changes in the intestinal cell wall structure, bring about immunological modifications in the gut and also boost the immunity of the birds to resist enteropathogenic bacteria like *C. perfringens*.

Among the various strains of bacterial used as probiotics, the Bacillus species happen to be the most preferred and the most-well known. This is because the effectiveness of probiotics depends in addition to the particular strain used, to a number of other factors such as the level and method of administration, ability of selected strain to survive certain environmental temperature and long term storage and still remain viable. Bacillus are spore forming bacterial species that have been reported to be heat stable, have extended shelf-life at room temperature, survive low gastric pH and also with a high possibility that nearly all ingested bacteria reaches the small intestinal tract (Hong *et al*., 2005; Lee, 2010a; Cutting, 2010). Lactic acid bacteria have been extensively used for human and animal health. Report show that lactic acid produced in vitro by lactic acid bacteria is used by the strictly anaerobic butyrate producing bacteria of Clostridial clusters IV and XIVa for the production of large concentrations of butyric acid (Duncan *et al*., 2004). This mechanism is referred to as cross-

feeding and is one of the reasons why lactic acid bacteria administration can beneficially affect performance.

2.4.1.2 USE OF PROBIOTICS IN NUTRITIONAL TRIALS

In livestock nutritional studies, probiotics has been reported to increase feed efficiency and growth rates, prevent possible intestinal disorders and facilitate the pre-digestion of anti-nutritional factors that can be present in feed. Dietary inclusion of probiotics in broiler nutrition has also proven that probiotics possess growth promoting and performance enhancement potentials. This is evident in their ability to enhance intestinal microflora, inhibit pathogenic growth and bring about intestinal histological changes. The ability to perform immunomodulatory functions, coupled with improvement in the sensory characteristics, and microbiological quality of dressed broiler meat, also lends credence to the fact that probiotics are growth enhancers (Kabir, 2009). In a study involving dietary inclusion of five bacteria species probiotics in broiler diets, Mountzouris *et al*. (2010) reported that probiotics had significant effect on broiler growth responses, apparent digestibility coefficients and caecal microbial composition of broiler chickens. There are also reports that probiotics supplementation in broiler nutrition has positive effects on carcass traits and haemato-biochemical parameters of broiler chickens (Cavazzoni *et al*., 1998; Kabir, 2009).

In a study conducted by Kioumarsi *et al*. (2012) to investigate the effect of probiotics on the carcass and internal organs of broilers, three probiotics (protexin, primalac and calciparine) were used. Results showed that there were significant ($p < 0.05$) differences among the probiotics treated groups in carcass weight and internal organs like carcass, empty carcass, head, neck, brain, and ileum weights. The use of probiotics had no negative effects on the performance of the broiler birds. However, the effects of probiotics on other parameters measured such as gizzard, crop, lung, testicles, colon, rectum duodenum and bursa of fabricus were non-significant ($p > 0.05$). Table 2.2 shows the effect of probiotics on the carcass and internal organs of the chickens used in the experiment.

Table 2.2: Effects of probiotics on carcass and internal organs of broilers

Traits	Control group	Protexin	Primalac	Calciparine
Full carcass weight (g)	2276.56±198.93[b]	2658.25±465.54[a]	2641.25±274.81[a]	2580.25±256.58[a]
Empty carcass weight (g)	1870.20±180.20[b]	2250.22±450.21[a]	2270.30±200.60[a]	2195.12±210.10[a]
Head weight (g)	63.03.±4.10[b]	61.50±8.22[a]	62.02±9.40[a]	55.40±3.51[a]
Gizzard (g)	58.22±5.84	61.27±16.50	55.19±10.55	56.45±9.28
Crop (g)	7.68±1.16	8.07±1.85	7.65±0.60	7.61±1.93
Lung (g)	11.01±0.53	11.31±2.09	10.77±1.99	8.40±3.37
Bursa of fabricius(g)	1.94±0.45	4.87±1.81[a]	3.53±1.06[ab]	2.78±1.20[b]
Brain weight (g)	3.19±0.23[a]	2.84±0.08[ab]	3.13±0.32[ab]	2.73±0.36[b]
Testicles weight (g)	0.800±0.313	0.855±0.318	0.770±0.289	0.635±0.094
Pancreas weight (g)	6.50±0.57	6.39±1.01	6.26±0.75	6.94±0.73
Duodenum weight (g)	13.83±0.76	18.52±6.02	15.59±3.02[a]	14.09±1.60
Ileum weight (g)	9.20±2.40[b]	17.62±4.85[a]	14.22±3.44[ab]	12.50±1.73[ab]
Colon weight (g)	1.96±0.55	1.86±0.48	1.55±0.42	1.63±0.25
Rectum weight (g)	1.82±0.20	1.98±0.36	2.13±0.58	2.30±0.24

[a, b, c] Means within rows for different groups with different superscripts differ ($p < 0.05$).
(Kiourmarsi*et al*., 2012).

From the study, the beneficial effects of the probiotics on the parameters measured was attributed to the fact that probiotics once established in the gut, have the potential to produce substances with bactericidal or bacteriostatic properties like lactoferrin, lysozyme, hydrogen peroxide and other organic acids . These substances help to lower gut pH, thereby promoting a better flora balance and exerting negative effects on the harmful bacteria. Probiotics also compete for nutrients and energy with toxin- producing bacteria, thereby attempting to suppress the activities of pathogenic species, and also ensure increased protection for host through improved intestinal mucosa immunity and subsequently improve hostôs health and growth performance. It has been observed that there have been inconsistent reports in published studies that evaluated the effect of probiotics on performance of birds. The differing opinions could be attributed to differences in dose and method of administration, probiotic strain used and their relative intestinal concentration, stability of strain during feed manufacturing and also in the GIT, physiological state of bird and actual microbiota balance in the gut of the animal. High quality probiotics are regarded as ideal when they possess attributes like being rapidly proliferating and resistant to effects of feed processing, acidity, bile

salts and digestive enzyme. Hence, there is need to screen bacteria intended for use as probiotics for antibiotic resistance to ensure that no undesirable antibiotic resistance is transferred into the intestinal environment.

2.4.2 PREBIOTICS

Prebiotics is proposed as one of the strategies for confronting the challenges associated with the use of in-feed antibiotics in poultry production. Prebiotics refers to non-digestible feed ingredients whose activities in the gastro-intestinal tract benefits host through selective stimulation of the growth and activity of one or a limited number of colonic bacteria (Gibson and Roberfroid, 1985). Due to its potential to reduce enteric diseases and subsequent contamination of poultry products along with probiotics, the use of prebiotics is regarded as a promising approach for enhancing the role of endogenous beneficial organisms in the gut (Bezkorovainy, 2001). Prebiotics provide the substrate for beneficial GIT microbes, with large amount of bacteria present in the small intestine of monogastrics being capable of utilizing the non-digestible carbohydrates for energy (Hilman, 2001).

Oligosaccharides are regarded as main components of prebiotics consisting of any of the hexose monosaccharide like glucose, fructose, galactose or mannose (Durst, 1996) with a polymerization degree of 2- 20 monosaccharide. They are a group of carbohydrates made up of 2-10 sugar units, each with varied chemical structure and are also naturally occurring constituents of plants and vegetables such as onions, banana, chicory roots, Jerusalem artichoke, etc. While the most common natural sources of oligosaccharides are grain legumes (which exist as raffinose, stachyose and verbascose), synthetic forms of oligosaccharides are produced either from direct polymerization of disaccharides, or fractionation of both vegetable and microbial cells. It is noteworthy that the physio-chemical properties of oligosaccharides depend on their chemical structure and composition.

Among the various types of oligosaccharides, the fructo-oligosaccharides (FOS) have been extensively researched as the main components of prebiotics. These short chain lengths of chicory inulin consisting 20 fructose units are the only products that meet the criteria allowing classification as prebiotics. Prebiotics enhance hostôs health through various mechanisms which include increasing the osmotic value in the intestinal lumen, lowering gut pH through lactic acid production, inhibiting or preventing gut colonization by pathogens such as Salmonella enteriditis and E. coli, modifying metabolic activity of normal intestinal flora and stimulation of the immune system (Chio *et al*., 1994; Gibson and Wang, 1994; Bengmark, 2001; Demigne *et al*., 1986). Prebiotics have also

been shown to prevent colon cancer and reduce cholesterol and odour compounds (Cummings and Macfarlane, 2002).

2.4.2.1 IMPLICATIONS OF PREBIOTIC USE IN BROILER NUTRITION

Although, prebiotics are reputed as growth promoters and are also capable of exerting other beneficial effects in poultry nutrition, there are however conflicting reports on the effects of prebiotics in enhancing performance characteristics in broilers. While some authors reported that prebiotics had significant positive effects on performance parameters like feed intake, weight gain and feed conversion ratio, a number of other authors reported that prebiotics cannot be considered as an alternative to antimicrobial growth promoters in broiler nutrition. This is evident in the reports of Li *et al*. (2008) and Yang *et al*. (2009) whose study revealed that dietary inclusion of fructo-oligosaccharides resulted in 5-8% improvement in weight gain and 2-6% improvement in feed conversion ratio in broilers. Zikic *et al*. (2008) reported that significant positive effect on performance and intestinal villus height of small intestines occurred on account of including prebiotics in broilers diets. Reports of Kannan *et al*. (2005) showed that dietary supplementation of 0.5g/kg and 1g/kg of yeast extract of prebiotics caused significant reduction in the abdominal fat pad of broiler birds. However, Biggs *et al*. (2007) reported a 2% decrease in gain in broiler birds fed fructo-oligosaccharide.

Prebiotics like Mannan-Oligosaccharide (MOS) also led to a 6% improvement of weight gain and feed conversion ratio in fattening chickens as reported by Roch (1998) and Newman, (1999). According to Maczulak *et al*. (1993), the lack of any beneficial effect in the use of prebiotics might be related to the non-specificity of the process of hindgut fermentation. This is because ingestion of prebiotics by livestock species results in stimulation in the growth and/or metabolic activity of different bacteria species including species that are both potentially harmful and beneficial.

In an experiment conducted by Kamran *et al*. (2013), MOS and 3 different AGPs (zinc bacitracin, Furazolidone and enramycin), were used at varying dietary levels which included 1kg/ton, 0.5kg/ton, 0.1kg/ton and 0.12kg/ton of feed, respectively. The aim was to ascertain the efficacy of MOS as alternatives to antibiotics in broiler production. The results of the 22 days experiment showed that feeding either MOS or any of the AGPs did not significantly affect the feed intake of birds. Although, the weight gain and feed conversion ratio of broilers fed MOS diet were superior ($p<0.05$) to those of control group, it was however inferior ($p <0.05$) to that of all of the AGPs fed groups. It was also observed that weight gain and feed conversion were not significantly different

among birds fed diets containing various AGPs. Table 2.3 shows the performance parameters evaluated in the study.

Table 2.3: Weight gain, feed intake and feed conversion ratio of broilers birds fed dietary levels of MOS and AGPS from 1-22 days of age1(Kamran et al., 2013)

Diets[2]	Weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio (g:g)
Control	789[c]	1227	1.56[a]
MOS	821[b]	1246	1.52[b]
Zinc bacitracin	851[a]	1263	1.49[c]
Furazolidone	862[a]	1271	1.48[c]
Emramycin	863[a]	1280	1.48[c]
Pooled SEM	8.9	12.9	0.06
ANOVA		Probability	
Diets	<0.0001	0.075	<0.001

[a-c] Means within a column with different superscripts differ significantly P<0.05>
[1] Means of 4 replicates with 10 birds each. [2] Control (no supplementation); MOS (1kg/ton of feed); Zinc bacitracin 10% (0.5kg/ton of feed); Furazolidone (0.1kg/ton of feed) and enramycin (0.12kg/ton of feed).

When compared to the control groups, the improved weight gain and FCR observed in the MOS and AGPs fed groups, showed that MOS can positively affect growth performance of birds. Nevertheless, the growth performance of broilers on the MOS diet was found to be lower than those of AGPs fed groups as the AGPs fed groups had better FCR, compared to control and MOS groups. This was attributed to better improvements in weight gain that resulted in better FCR than those of MOS and control groups. It can be said that broilers fed MOS had better performance than those groups fed the control diet. However, when these performance indices were judged against the overall performance of the AGPs fed groups, it was concluded that MOS cannot possibly be a potent substitute for the commonly used AGPs, especially during broiler starter phase in which this study was done. Hence there is a possibility that the shortfall may have been caused by the low inclusion level of the MOS, hence, higher doses may be able to make the difference in terms of better results.

2.4.3 EXOGENOUS ENZYMES

NSPs are polymeric carbohydrates with varied compositions, structures, physical activity, made up of a spectrum of polysaccharide molecules. These structural polysaccharides are commonly found in animal feedstuffs, especially (hemi) celluloses, oligosaccharides, pectins, mixed ɓ-glucans and arabinoxylans with either soluble or insoluble fractions (Parsippany, 2008). Poultry species are only able to digest the soluble NSPs up to a degradation level of 80-90%, but cannot digest the insoluble NSP. However, the digestion of soluble NSP by poultry also has some attendant challenges. This is because even with effective digestion, soluble NSPs are still capable of entrapping other nutrients in the gut, and decreasing nutrient digestibility, with subsequent reduction in the apparent metabolizable energy (AME) of the diet, and FCR values (Hetland *et al*., 2004; McNab and Boorman, 2002; Bedford and Schulze, 1998). Thus, depolymerisation of NSPs requires a particular kind of enzymes whose activities happen to be specific to the main and side chain structure of NSP (Dalibard and Geraert, 2004; Anderson *et al*., 2003; Bhat, 2000).

The application of feed enzyme in poultry diets for the enhancement of nutrient availability had been reported as far back as 1926 (Clicner and Follwell, 1926). Enzymes such as xylanase and ɓ-glucans have the ability to breakdown NSPs which are in the cell wall of cereal grains, thus making nutrients readily available to animals (Beford, 2000; Choct, 2006). Poultry diets have been supplemented with exogenous enzymes primarily for improvement of production efficiency and nutrient utilization (Choct, 2006; Acamovic, 2001). Evidence abounds to affirm that the use of exogenous enzyme in monogastric nutrition has important benefits (Acamovic, 2001). However, these beneficial effects depend on factors such as dietary composition, diet processing, enzyme type and a host of others. The most visible direct impact of NSPs in animal nutrition, particularly with regard to gut physiology and morphology, is the increase in gut viscosity which results from ingestion of NSPs compounds. In fact, to a large extent, the anti-nutritive effects of NSPs are directly linked to their solubility. When NSPs are ingested, they are often solubilized, leading to an increase in the viscosity of digesta with a concomitant decrease in digestion rate and performance. This is because the increased bulk and viscosity of the intestinal contents decreases the diffusion rate of substrates and digestive enzymes and hinder their effective interaction at the mucosal surface. There is also a resultant inducement of the mucous layer on the intestinal mucosa to thicken (Hedemann *et al*., 2009). An increase in gut viscosity also results in reduced throughput and feed intake, to the end, that the rate at which nutrients are assimilated becomes limited. It is important to note that the increased gut viscosity arising from ingestion of NSPs is usually regarded as a useful

indicator of the anti-nutritive effects of these polymeric carbohydrates rather than their mechanism of action (Williams *et al*., 1997).

The efficiency of nutrient utilization by gut microbiota in broiler chickens is also indirectly affected by the viscous nature of NSPs. This is evident in the manner in which these gut microbiota induce small intestinal volatile fatty acids production (VFA) by stimulating the fermentation of NSPs. Usually, when broilers are fed diets containing low amounts of NSPs, facultative anaerobes are more prominent in the small intestine, and in most cases the entire caecal microbiota may be found consisting of strict anaerobes. However, with a diet rich in non-starch polysaccharide compounds, a significant increase in the concentration of VFA results, particularly at the distal ileal lumen. This increase is attributed mainly to the large amount of fermentation taking place at this juncture, which is coupled with an increase in the number of fermentative microflora which slowly impacts the activity of hindgut microbiota (Bjerrum *et al*., 2006; Lu *et al*., 2003; Choct *et al*., 1996, 1999). Proliferation of fermentative bacteria increases their competition with the host for nutrients, especially nutrients like starch and proteins. Enzyme-free diets containing soluble- NSP rich cereals (wheat) have been shown to induce lymphocyte infiltration in the gut wall and induce apoptosis of epithelial cells much more than cereals such as maize that have low levels of soluble NSPs (Teirlynck *et al*., 2009). A number of these intestinal bacteria also produce bile acids degrading enzymes, thereby reducing the capabilities of host for lipid and protein digestion. This is because bile acids are known to stabilize pancreatic proteases in the lumen of the intestine, but when found lacking, protein digestion is compromised (Bedford, 1993). Non-starch polysaccharides (NSPs) also act as a physical barrier to endogenous enzymes. Since feed processing methods, such as pelleting and grinding does not entirely destroy the cell walls of the endosperm of grains, the likelihood for NSPs to entrap nutrients in the endosperm when grains are ingested is high, resulting in reduced digestibility of feed and utilization of starch and proteins (Classen, 1996).

Dietary inclusion of AGPs help to counter-balance the negative effects associated with feeding high NSP diets. Failure to do so often cause reduction in the concentration of ileal VFA and an increase in caecal VFA concentration since materials that are slowly fermented enters the caecum. This is because fermentation occurring in the caecum leads to the conversion of indigestible compounds into VFAs that are readily absorbed and utilized. Basically, dietary NSP-enzymes function by lowering the viscous nature of small intestinal digesta, thereby increasing digesta passage, and nutrient digestion rate, and providing less substrate and less time for the proliferation of

fermentative organisms. This may restore the normal and efficient endogenous enzymatic digestion of nutrients in the small intestine. Hence, exogenous enzymes help to partially counterbalance the adverse effect of soluble NSP on performance (Bedford and Classen, 1992). It is not possible to measure the relative contribution resulting from improvement in the utilization of nutrients, or the selective reduction in the proliferation of bacteria. Nevertheless, there are research findings that attest to the fact that the reduction in digestion rate brought about by the ingestion of NSPs, is more prominent in the presence of intestinal microbiota, due to the fact that there is not only a breakdown of digestive enzymes and bile salts, but also because there is microbial colonization at the absorptive surface. Hence, greater enzymatic responses will be recorded when AGPs are not included in the diets, especially in less digested diets. In addition, NSPs degrading enzymes will also reduce the proliferation of pathogenic bacteria such as *C. perfringens* (Jackson *et al*., 2003). To this end, all broiler diets are currently being formulated to contain enzymes such as xylanase and ɓ - glucanase which are capable of breaking down NSPs, thus making the feed readily available to animals.

The use of NSP-degrading enzymes in barley and wheat based broiler diets are well established and accepted (McNab and Boorman, 2002; Bedford, 2009). The main objective of using NSPs-degrading enzyme in wheat and barley based diet is to increase the apparent metabolizable energy of the wheat or barley, since the variability of the apparent metabolizable energy content of wheat is related to the anti-nutritional effect of NSPs present in the wheat and barley (Choct *et al*., 1999). Results of digestibility trials involving the use of wheat, rye and barley in broiler nutrition have shown that there are positive effects of these grains on production performance of broilers and improvement in nutrient digestibility of the feed (Choct, 2006; Bedford, 2000). NSPs-degrading enzymes (of which the most exploited are xylanase, ɓ-glucanase, pectinase, cellulase, mannanase and galactanase), have only recently been investigated in maize-soya diets (Choct, 1999; Meng and Slominski, 2005). The inclusion of these feed enzymes in maize-soya diets have been reported to improve nutrient digestibility, broiler production performance and reduce the variability in the nutrient content of different feed samples (Bedford, 2000; Choct, 2006). However, there are considerable variations in the type and amount of NSPs found in the same as well as different feed raw materials, owing to differences in varieties and geographical locations where the grains are produced. Whereas, maize contains almost insoluble NSPs, wheat and barley contain more of insoluble NSPs, (the ratio of soluble to insoluble is about 1/6). This ratio is about 3/4 in rye, making this cereal one with particularly high levels of soluble NSPs (Choct, 2002).

2.4.4 ORGANIC ACIDS

Organic acids are widely distributed normal components of either plant or animal tissues that results from the microbial fermentation of carbohydrates which are more prominent in the caeca of poultry birds. Organic acids are naturally made up of a spectrum of compounds differing in physical and chemical attributes. They are used as supplements in drinking water, feed additives (acidifiers), and a number of them exist as either sodium, potassium or calcium salts or as partially esterified compounds (Van Der Wielen *et al*., 2000). Usually, those existing in salt forms are preferred to the acids due to the ease of handling, particularly during feed manufacturing because of their solid and volatile form. They are also less corrosive, more soluble in water and generally odourless

Organic acids have been reported to possess growth promoting potentials. Acidifiers are increasingly been used as veritable alternatives to AGPs in recent years (Radcliffe, 2000). Acidifiers are essential in that they are not only capable of inhibiting the colonization of hostôs intestine by bacterial species which compete with them for available nutrients, but also have potential to reduce any toxic metabolic by-product that may possibly emanate from these bacterial organisms. In view of this, several authors have implied that the ability of acidifiers to function effectively as an alternative to AGPs may be attributed mainly to the potential which organic acids have to affect the concentration of bacteria in the caeca and small intestine (Vogt *et al*., 1982), and also effectively act as a bactericidal agent for salmonellae in the crop of host (Thompson and Hinton, 1997; Hinton and Linton, 1988).

Additional benefits also accrue from the use of organic acids in poultry nutrition, and this is evident in the ability of butyric acid to lower the incidence of sub-clinical necrotic enteritis caused by *C. perfringens*. These benefits are of high importance to and critical to the economics of production in the poultry industry (Timbermont, 2009). There are also reports that dietary supplementation of butyric acids enhanced growth of the gastro-intestinal tract mucosa. Butyric acids have been reported to have positive intestinal impact on rodents, humans and livestock. These fatty acids are also regarded as an essential source of energy for epithelial cells and are capable of stimulating epithelia cell proliferation and differentiation (Dalmasso *et al*., 2008). Their ability to strengthen gut mucosal barrier by increasing the production of antimicrobial peptides in mucous and expression of tight junction proteins makes them important anti-inflammatory compounds (Hodin, 2000; Mariadason *et al*., 1997; Schauber *et al*., 2003; Bordin *et al*., 2004; Peng *et al*., 2007). In view of

the antibacterial property and other essential roles of butyric acids and other medium chain fatty acids, organic acids are regarded as veritable alternatives to AGPs.

The mechanism of action of organic acid depicts their potentials as antibacterial agents and these includes decreasing the pH of drinking water, reducing the buffering capacity of the feed, and this also affects the physiology of the crop and proventriculus (Val Immerseel *et al*., 2006). Environmental pH affects the ability of organic acids to change from undissociated to the dissociated form, invariably enhancing their antimicrobial effect. The non-dissociated (non-ionized, more lipophilic) organic acids can penetrate the lipid membrane of the bacterial cell and decrease intracellular pH thereby disrupting the normal physiology and death of certain bacteria types (Dhawale, 2005).

Scientific evidences have shown that the use of organic acids in broiler diets not only improve digestibility of proteins and minerals such as Ca, Mg, P and Zn, but also serve as substrates in their intermediary metabolism, and also lower pathogenic colonization and production of toxic metabolites (Kirchgessner and Roth, 1998). Dietary supplementation of organic acids has also been reported to have positive effects on body weight and FCR of broiler birds (Skinner *et al*., 1991) and reduce damage to epithelial cells (Langhout, 2000). Organic acids are also capable of lowering chyme pH and the growth of bacteria that are intolerant to pH changes, thus, enhancing protein digestion (Pelicano *et al*., 2005; Pigoliev *et al*., 2008; Ao *et al*., 2009). Organic acids are also reputed for stimulating pancreatic secretion and providing better intestinal villus integrity, thus providing broiler birds with better intestinal health for maximum nutrient absorption (Dibner and Buttin, 2002).

However, conflicting reports abound on reports on the use of acidifiers in poultry nutrition. These could be attributed to the fact that the antibacterial activity of organic acids depends on a number of factors which include the chemical form of the acid (does the acid exist in esterified, acid, salt or coated forms or not?), pka value of the acid, (the value at which 50% of the acid is dissociated), chemical formula and molecular weight of the acid, buffering capacity of the feed, the nature of the bacterial species to be destroyed, the micro-organism related MIC-value of the acid animal species, and site of action of organic acids (Patten & Waldroup, 1988; Hernandez *et al*., 2006; Thompson and Hinton, 1997). It follows, therefore that the microbial activity of each acid is different depending on pH range, membrane structure and cell physiology of the microbiota species. In practice, mixtures of organic acids are often used, due to the array of pka values accruing from

different blends and the broader spectrum of activity that result from combining the good qualities of the different acids used

2.4.5 OTHER ALTERNATIVE PHYTOGENIC FEED ADDITIVES

2.4.5.1 RESINOUS SUBSTANCES: PROPOLIS

Propolis is a natural resinous substance produced as a result of biochemical alterations made possible by enzymatic glandular secretions from *Apis Mellifera Spp* (bees). Also referred to as bee glue, propolis is harvested from different plant seedlings and buds. These include the resins of poplars, conifers, birch, pine, alder, palm, willow, *Baccharis dracunculifolia* and *Dalbergia ecastaphyllum* (Mahmoud *et al*., 2014; Kosalec *et al*., 2004; Bankova *et al*., 2000; Burdock, 1998). This natural glue-like substance is often used by workers bees to seal cracks in the beehive, mummify dead bodies of invaders to prevent decomposition and disease spread and also position the honeycomb within the hive. The use of propolis during brood period helps to slow down evaporative water loss from cracks and crevices within the beehive. Propolis is gaining increased attention as an antioxidant capable of protecting biological systems from oxidative damage. The use of propolis supplementation in broiler diets has come a long way with spectacular research findings. Propolis is reported to exert positive effects on feed intake and body weights of broiler chickens (Seven *et al*., 2008). This is due to improvement in the taste of broiler diets owing to abundance of bioactive compounds particularly the phenols and terpenoids. As already noted, phenols are the most widely studied phyto-constituents due to a spectrum of biological potentials embedded in them. In addition to their numerous immense therapeutic potentials, phenols are also phytogenic substances with hypocholesterolemic effects (Ikeda *et al*., 1992).

Propolis is composed of a structure and consistency that is dependent on prevailing temperature. Its colour varies from light yellow to dark brown according to geographical area and plant source. It is hard and brittle at 0-15^{0}C and malleable at 30^{0}C, sticky between 30 and 60^{0}C and liquefies at a temperature higher than 70^{0}C. Regardless of origin and geographical location, propolis has similar microscopic structure (Stangaciu, 1999). The chemical composition of propolis depends on the geographical location, botanical origin (Salatino *et al*., 2011; Toreti *et al*., 2013; Bankova, 2005; Silici and Kutluca, 2005) and bee species (Silici and Kutluca, 2005). This is the main reason for the striking diversity in the chemical composition between tropically grown propolis and temperate region propolis.

The most biological active component of propolis are flavonoids, hydroxyl-flavonoids, dehydro-calcons, sugars, lactones, alpha and beta amylase, ketones, phenols, vitamins, minerals, trans-hydrogenase, nucleic acids, aldehydes, cinnamic acid and its derivatives, sterols, terpenoids, amino acids, insoluble parts and unidentified materials (Stangaciu, 1999). Propolis also contain about 5-10% short chained volatile oils and aromatic acids, 30-40% waxes, in addition to resins, balms and pollen grains all of which are rich sources of essential elements like magnesium, nickel, calcium, iron and zinc (Dobrowolski *et al*., 1991).

2.4.5.2 BIOLOGICAL RELEVANCE OF PROPOLIS

Propolis is an extremely complex mixture of phytochemicals that contain all the 22 nutrients required by the body for optimum performance and health. Propolis has been used as a natural antibiotic by ancient Greeks for the treatment of wounds, burns, sore throat, stomach ulcer etc. and also in the treatment of bacterial and viral originated diseases in poultry breeding (Hegazi and Abd El Hady, 1994; Hagazi *et al*., 1999). Propolis has become a subject of intense chemical and pharmacologic studies in the last 30 years, although it has been used in folk medicine for at least 300 years. Its biological properties are quite diverse and directly related to the quality of exudates collected by bees (Ghisalberti, 1979; Banskota *et al*., 2001; Bankova, 2005).

The biochemical constituents in propolis make it an important substance with antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, cytostatic and immunomodulatory properties. This has lent it a lot of attention from researchers the world over as a veritable replacement for antibiotics in poultry diets. Extensive studies have been done on the immunomoulatory effect of propolis on production of factors which lead to inflammation such as cytokines, prostaglandins, chemokines and a host of others. Hence, propolis has been shown to be capable of performing immunomodulatory roles in animals, particularly activation of macrophages, synthesis of antibodies and lymphoid organ weight (Lofty *et al*., 2006; Kosalec *et al*., 2005; Cetin *et al*., 2010; Fischer *et al*., 2010).

Propolis contains complex mixtures of substances including resins, waxes, essential oils, pollen and various organic compounds (Wagh, 2013). This makes it useful as a bio preservative in food processing and also storage of pharmaceutical products (Erkmen and Ozcan, 2008; Pavilonis *et al*., 2008). However, the percentage of its biological content depends largely on the plant source, location and time of collection (Markham *et al*., 1996). To this end, dietary supplementation of propolis in animal nutrition is seen as being capable of affecting the health and performance of

poultry, economics of feed mixtures for poultry and overall economics of production (Seven *et al*., 2008). Table 2.4 shows important compounds that are contained in propolis.

Table 2.4: Important compounds identified in Propolis resin

Chemical Groups	Compounds
Alcohol	Benzene methanol, cinnamyl alcohol, glycerol, Ŭ-glycerol phosphate, hydroquinone, isobutenol, phenethyl alcohol
Aldehydes	Benzaldehyde, caproic aldehyde, p-hydroxybenzaldehyde, isovanillin, vanillin
Aliphatic acids and aliphatic esters	esters Acetic acid, angelic acid, butyric acid, crotonic acid, fumaric acid, isobutyric acid, methylbutyric acid, isobutyl acetate, isopentyl acetate
Amino acids	Alanine, ɓ-alanine, Ŭ-aminobutyric acid, ɔ-aminobutyric acid, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Aromatic acids	Benzoic acid, caffeic acid, cinnamic, coumaric, acid, ferulic acid, gallic acid, gentisic acid, hydroxycinnamic acid, p-hydroxybenzoic acid, isoferulic acid, 4-methoxy cinnamic acid, salicylic acid, vanillic acid
Aromatic esters	Benzyl acetate, benzyl benzoate, benzyl caffeate, benzyl coumarate, benzyl ferulate, benzyl isoferulate, benzyl salicylate, butenylcaffeate, butyl caffeate, cinnamyl benzoate, cinnamylcaffeate, ethyl benzoate, coniferyl benzoate, coniferylferulate.
Chalcones	Alpinetinchalcone, naringeninchalcone, pinobanksinchalcone, pinocembrinchalcone, sakuranetinchalcone
Flavonones	FlavanonesNaringenin, pinobanksin, pinobanksin-3-acetate, pinobanksin-3-butyrate, pinobanksin-3-hexanoate, pinobanksin-3-methyl ether, pinobanksin-3-pentanoate
Flavones and flavonols	Acacetin, apigenin, apigenin-7-methyl ether, galagin, galagin-3-methyl ether, izalpinin, isorhamnetin, kaempferol, quercetin, ramnetin, ramnocitrin, aglycone
Waxy acids	Arachid acid, behenic acid, cerotic acid, lauric acid, linoleic acid, lignoceric acid, montanic acid, myristic acid, oleic acid, palmitic acid, stearic acid
Ketones	Acetophenone, dihydroxy-acetophenone, methylacetophenone
Terpenoid and other compounds	Ŭ-acetoxybetulenol, ɓ-bisabolol, 1,8-cineole, Ŭ-copaene, cymene, limonene, styrene, xanthorreol, naphthalene, sesquiterpene alcohol, sesquiterpenediol
Steroids	Calinasterol acetate, b-dihydrofucosterol acetate, ucosterol acetate, stigmasterol acetate
Sugars	Fructofuranose, Ŭ-D-glucopyranose, ɓ-D-glucopyranose, galactitol, gluconic acid, 2-*0*-glycerylgalactose
Vitamins and minerals	Vitamins A, B, C, E. Minerals like Ca, Mg, Na, Al, B, Ba, Cr, Fe, Mn, Ni, Sr, Zn, Co, Rb, Sb, Sm, and toxic elements such as As, Cd, Hg and Pb.

Source: Madrigal- Santillan *et al*. (2013)

2.4.5.3 NUTRITIONAL TRIALS INVOLVING PROPOLIS

Based on the rich amount of bioactive substances contained in propolis, it has been reported to possess antioxidant, antiviral, antibacterial, antifungal properties (Kumazawa *et al*., 2004; Tosi *et al*., 2007; Erkman and Ozcan, 2008; Talas and Gulhan, 2009; Daneshmand *et al*., 2012), in addition

to its antimicrobial, cytostatic, anti-mutagenic, anti-inflammatory, and immune-modulatory properties (Bankova *et al*., 2000; Prytzk *et al*., 2003; Wang *et al*., 2004). In addition, terpenoid in propolis endows it with anti-carcinogenic, anti-malarial, anti-ulcer, hepaticidal or diuretic properties (Langenheim, 1994; McCaskill, and Croteau, 1998; Dudareva *et al*., 2004).

Dietary inclusion of propolis in broiler diets has proven that propolis is a natural growth promoter with immense attributes. The study of Seven *et al*. (2008) revealed that dietary supplementation of ethanolic extract of propolis was more effective than vitamin C on performance and carcass characteristics of heat-stressed broiler birds. Shalmany and Shivazad (2006) reported that dietary inclusion of propolis in broiler chickens diets is capable of improving growth performance. Hassan and Abdulla (2011), showed that supplementation of broiler diets with propolis at 400mg/kg increased body weight and feed intake at 8th week of broilers age. Treatment also resulted in an improvement in feed conversion efficiency at the 2nd, 3rd and 4th week of the birdôs age.

Improved liver, heart, and thigh weights and also dressing percentage were observed among the propolis fed birds. However, Usama Mahmoud *et al*. (2013) reported that ethanolic extract of propolis had no beneficial effect on performance and carcass characteristics of broilers. Daneshmand *et al*. (2015) also reported that supplementing broiler diets with propolis extract resulted in decreased body weight, whereas, inclusion of propolis, probiotic and their combinations caused an immunomodulatory effect in broiler chickens.

Propolis supplementation in broiler diets has also been shown to be an inducer of immune responses in broiler chickens. According to Krocko *et al*. (2012), one alternative for improving broiler health and performance may be incorporation of propolis and bee pollen into broiler diets. Propolis was reported to have effect on the immune system by increasing macrophage activity, changing gut and intestinal microbial population, and stimulating lymphatic tissues. Also, the antioxidant and anti-inflammatory agents in propolis are capable of inhibiting prostaglandin synthesis, thus resulting in better humoral response (Namgoong *et al*., 1994).

Bone metabolism involves a complex balance between the deposition of matrix and mineralization and resorption. Scientific evidence abound to suggest that dietary components and herbal products can influence these processes, particularly by inhibiting bone resorption, thus having beneficial effects on the skeleton (Putnam *et al*., 2007). According to Ang *et al*. (2009), caffeic acid phenethyl ester in propolis is capable of supporting bone growth and development. This bioactive compound is regarded as potent treatment for bone lytic diseases and also a therapeutic agent for osteoporosis.

Propolis has also been found to significantly improve the availability and utilization of dietary calcium and phosphorous which are mineral compounds with beneficial effects on the skeleton (Haro *et al*., 2000)

Gut microflora is a nutritional ñburdenò in fast- growing broiler chickens, since an active microflora component may have an increased energy requirement for maintenance, and a reduced efficiency of nutrient utilization (Dibner and Richards, 2005; Lan *et al*., 2005). Hence, the focus of alternative strategies is to prevent proliferation of pathogenic bacteria and modulate indigenous bacteria leading to improvement in performance, health and immune status (Adil *et al*., 2011). The essential compounds found in either crude or ethanolic extract of propolis promote gut health, by controlling the growth of pathogenic micro-organisms in the gastrointestinal tract, with consequent improvement in digestive and absorptive functions (Eyng *et al*., 2014)..

2.4.5.4 ESSENTIAL OILS AND HERBAL PRODUCTS

Essential oils are products of herbal plants and consist of aromatic and volatile substances (Jang *et al*., 2004). Medicinal plants have also received attention as alternatives to antibiotics. A number of plant families are recognized as a result of important plant secondary metabolites which they contain. The *Lamiaceae* plant family has received the greatest attention in poultry nutrition with thyme, oregano and peppermint being the most popular representatives. These medicinal plants contain important bio-active components, for instance, peppermint leaves contain about 0.5-4% essential oils consisting of 25-77% menthol, 14-36% menthone, 1.5-10% isomenthone, 2.8-10% menthyl acetate, 3.5-14% cineol (Aziz *et al*., 2011; Bupesh *et al*., 2007). Thymol and carvacrol are the main bioactive constituents found in thyme and oregano essential oils (Huyghebaert *et al*., 2011). According to AL-Kassie (2009), birds fed essential oil derived from cinnamon had significant higher feed intake, body weight gain and FCR compared to control group.

Plants of the *Zingiberaceae* and *Alliceae* family to which turmeric and garlic belong are also considered important due to the essential phytochemicals found in them. Virtually all of these medicinal plants have been reported to possess antimicrobial, antibacterial, anti-inflammatory, immunomodulatory, anti-mutagenic, anti-carcinogenic, antioxidant and a host of other properties. Differences abound in the composition of essential oils and medicinal plants as a result of variations in plant species, geographical locations, planting and harvest conditions, manufacturing processes and storage conditions. Hence it is important to identify and quantify the several claims that have been linked to medicinal plants possessing beneficial properties.

The mechanism of action of medicinal plants is such that they are capable of stimulating digestive enzyme secretions, increasing feed intake, thereby adding to the nutrient requirement of animals, as well as increasing the nutrient utilization and absorption (Wenk, 2003) capabilities of these animals. They are also able to enhance the immune and endocrine system of animals. The primary site of action for medicinal plants is in the digestive tract of animals where they act to increase the population of beneficial micro-organisms and lower that of pathogenic micro-organisms (Wenk, 2000). According to Cowan (1999), this antimicrobial attribute of medicinal plants, coupled with their immune stimulating abilities are the two main mechanisms by which they bring about improved animal welfare and growth performance. There are reports that constituents of essential oils in herbal products work synergistically, and this makes it quite difficult to point out the actual components that exert the acclaimed beneficial effects in livestock production. Based on the antimicrobial activities of aforementioned medicinal plants, Adams (1999), ranked the antimicrobial activity of each of them into weak, medium and strong. The author stated that antimicrobial activity is rather weak for ginger and pepper, medium for cumin (p-cymene), coriander (lialol), oregano (carvacrol), rosemary (cineol), sage (cineol) and thyme (thymo), and strong for clove (eugenol), mustard (allysothiocyanate) cinnamon (cinnamaldehyde) and garlic (allicin).

Oleforuh-Okoleh *et al*. (2015) reported that dietary inclusion of ginger and garlic in broiler diets led to improved performance and general well-being, as well as enhanced immunity, hence both plant-based substances were recommended by the authors as important phytobiotics in broiler production. Findings from Fadlalla *et al*. (2010) showed that 0.3% garlic inclusion in broiler feed brought about positive improvements in the growth performance and carcass yield broiler of broiler chickens. Guo *et al*. (2004) carried out an in vivo trial aimed at studying the effects of polysaccharide extracts of two mushrooms, *Lentinus edodes* and *Tremella fuciformis*, and an herb *Astragalus membranaceus*, on growth performance, organ weights and GIT of broiler chickens. These extracts were included at the rate of 0.5, 1, 2, 3 and 4g/kg, and compared with an antibiotic treatment group fed 20mg/kg virginiamycin (VRG), as well as the non-supplemental groups. The result of the study showed that at the end of the 28 day trial, birds fed plant extracts performed better than those on the non-supplemented diets, but their performance was not significantly ($p>0.05$) different from those on the antibiotics-VRG diet.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION

The study was conducted at the Mariendahl Experimental Farm of Stellenbosch University located near Stellenbosch, Western Cape Province, South Africa. The experiment which was carried out during the summer months (October-November) lasted 35 days.

3.2 MANAGEMENT OF EXPERIMENTAL ANIMALS

A total of 320, vaccinated day-old unsexed Ross 308 broilers chicks, procured from a reputable hatchery were used for this study. The birds were housed in a temperature controlled bioassay automated unit equipped with wire cages (0.9x0.6m, 10birds/m^2), each containing a tube feeder and two nipple drinkers. The birds were housed in this unit until slaughter age of 35days. Prior to the arrival of the birds, the cages were covered with newspapers and the tube feeders filled with feed, whereas the nipple drinkers were adjusted according to the specifications of the breeder farm. Brooding lasted two weeks, during which time, the temperature and relative humidity of the house were maintained based on the specifications of the primary breeder. The birds had *ad lib* access to water and feed. Temperature and lighting in this house was according to Ross 308 standard. A minimum of six air changes per minute was provided for the birds. Record of mortality was kept. All experimental procedures and methods were practiced according to the ethical requirements that have been approved by Animal Ethics Committee of the Stellenbosch University, reference number: SU-ACUD15-00048.

3.3 EXPERIMENTAL DIETS

The birds were assigned to different isoenergetic (12.60, 13.2 and 13.4 MJ/Kg AMEn) and isonitrogenous (25.0, 22.5 and 20.07 % CP levels) diets designated as follows: diet 1- maize-soybean based diet without additives (negative control), diet 2- maize-soybean based diet containing 10% tylosin (positive control), diet 3-maize-soybean based diet containing phytonutrient (product 1) and diet 4-maize-soybean based diet containing phytonutrient (product 2). Diet 2 contained 200g/ton of antibiotics, while diets 3 and 4 contained 1L/ton each of phytogenic feed additives. The 35- day trial comprised the starter (0-18 days), grower (18-28 days) and finisher (28-35 days) phases, respectively. This methodology align with that of Pieterse *et al*. (2014), Nkukwana *et al*. (2014), and Ao *et al*. (2011) where birds were kept for 32, 35, and 35 days respectively and included the starter, grower and finisher phases. The birds were fed 1000g/bird, 1300g/bird and

1500g/bird at the starter, grower and finisher phases respectively. A description of the different diets is given in Table 3.1, whereas Table 3.2 describes the nutrient composition of experimental diets. All diets were given in mash forms and were mixed at the poultry section, Mariendahl experimental farm, Stellenbosch University, Matieland, South Africa.

Table 3.1: Description of the four dietary treatments comparing two different phytonutrient products with the negative and positive control diets

Treatment	Description
Negative control	Control diet without antibiotic (AGP)
Positive control	Control diet with antibiotic PROMOTE (10% tylosin), 200g/ton of feed= 20g tylosin/ton of feed
Product 1	Control + phytogenic feed additive, product 1 (1L/ton of feed)
Product 2	Control + phytogenic feed additive, product 2 (1L/ton of feed)

Table 3.2: Ingredient and calculated nutrient composition of experimental diets on as fed basis (g/kg)

	Starter	Grower	Finisher
Ingredients (g/kg)			
Maize	453.337	468.794	540.825
Soybean full fat	116.423	485.838	404.824
Soybean 46	277.129	-	-
Fish meal 65	53.108	-	-
L-lysine Hcl	1.793	1.393	1.450
DL methionine	3.973	3.748	3.254
L-threonine	1.137	0.986	0.902
[1]Vit+min premix	1.500	1.500	1.500
Limestone	14.506	14.584	14.224
Salt	1.241	2.796	2.754
Monocalcium phosphate	12.229	14.362	13.288
Sodium bicarbonate	1.631	1.156	1.279
Sunflower oil	60.961	3.843	14.699
[2]Product 1	1.000	1.000	1.000
[3]Product 2	1.000	1.000	1.000
[4]AGP (10% tylosin)	0.200	0.2000	0.200
Calculated nutrient composition (g/kg) [5]			
[6]AMEn (MJ/Kg)	12, 650	13, 200	13, 400
Crude protein (%)	25,000	22,509	20,073
Dry matter (%)	89,347	88,693	88,517
Lysine (%)	1,601	1,388	1,216
Methionine (%)	802	699	623
Cysteine (%)	389	380	352
Methionine+ cysteine	1,190	1,079	0,975
Threonine	1,074	1,079	975
Tryptophan (%)	292	261	227
Arginine (%)	1, 662	1,528	1,334
Isoleucine (%)	1,134	1,010	886
Leucine (%)	2,086	1,924	1,773
Histidine (%)	666	613	549
Phenylalanine (%)	1,107	1,043	926
Tyrosine (%)	922	792	705
Phenyl+ tyrosine (%)	2,029	1,835	1,631
Valine (%)	1,254	1,105	988
Ash (%)	5,034	4,421	4,067
Crude fibre (%)	3,023	3,703	3,416
Crude fat (%)	10,859	11,150	11,000
Calcium (%)	1,050	900	850
Phosphorous (%)	813	772	718
Available phosphorous (%)	500	450	420
Sodium (%)	160	160	160
Chloride (%)	230	230	230
Potassium (%)	936	941	837
Linoleic acid (%)	3,562	5, 823	5,419
Choline	1.547681	1.680149	1.493108

[1]Vitamin+ mineral premix provided (per kg of feed): 8160 IU vit A, 1700 IU vitamin D_3, 30.6 IU vitamin E, 2.7mg vitamin K_3 205mg vitamin B_1, 2.03mg vitamin B_2, 27.2mg niacin, 10.2mg calcium

pentothenate, 2.02mg vitamin B12, 4.1mg vitamin B6, 1.7mg folic acid, 0.068mg biotin, 120mg ronozyme P500, 350mg choline, 0.08mg I, 0.34 mg Co, 0.2mg Se, 70mg Mn, 70mg Zn, 6mg C and 50mg Fe.

[2] Phytonutrient sample 1 provided by Beonics Feed Supplements (Pty) Ltd, Cape Town, South Africa

[3]Phytonutrient sample2 provided by Beonics Feed Supplements (Pty) Ltd, Cape Town, South Africa

[4]Antibiotic growth promoter (PROMOTE with 10% Tylosin at 200g/ton of feed)

[5]Formulated not analyzed nutrient levels

[6]AMEn = Apparent metabolizable energy.

3.4 EXPERIMENTAL DESIGN AND TRIAL PROCEDURE

The birds were randomly allocated to each of 32 cages consisting of 8 replicate groups of 10 birds. Birds were also randomly assigned to each of four dietary treatments (Table 3.1) in a completely randomized design with dietary treatment as main effect.

The experimental model is shown below:

$Y_{ij} = U + A_{ij} + E_{iZ}$

Where $Y_{ij=}$ individual observation

U= population mean

$A_{ij=}$ effect of phytogenic feed additives inclusion

$E_{iZ=}$ experimental or random error

3.5 DATA COLLECTION AND MEASUREMENTS

3.5.1 FEED INTAKE AND BODY WEIGHT MEASUREMENTS

Feed allocation was recorded at the start of the trial and thereafter, at weekly intervals, i.e. 7, 14, 21, 28 and 35d respectively. Cage average for feed intake (FI, g) was calculated every week as feed offered minus feed refused. Body weight of all birds in each treatment group was recorded at the beginning of the experiment and thereafter, on a weekly basis until slaughter. The methodology for both weekly feed intake and body weight determinations as done in this work, agree with the work of Pieterse *et al*. (2014) and Wati *et al*. (2015). Individual weights were then calculated as an average. Data obtained were used to calculate feed conversion ratio (FCR), average daily gain (ADG) and European production efficiency factor (EPEF) (Awad *et al*., 2009). The FCR and EPEF were calculated using the formulae given below:

□□□□ = □□□□□□□□□□ □%□∗ □□□□ □ □□□□□□□□/ □□□ □□□□□∗ □□□ ∗ 100.

Where:

EPER = European production efficiency factor

Liveability (%) = percentage of number of birds remaining at the end of the trial

FCR = feed conversion ratio (feed per gram gain).

3.5.1.1 AVERAGE DAILY GAIN (ADG)

ADG was calculated by means of a simple linear regression analysis, by fitting birdôs weight over time. An analysis of covariance was done to determine line differences, where the intercept from the individual ADG linear regression function was included as a covariable. The slope obtained from the regression function termed as ADG, was used to compare the performance of animals between treatments.

3.5.2 PROXIMATE ANALYSIS OF MEAT SAMPLES

3.5.2.1 SAMPLE PREPARATION

Proximate analysis of the meat samples (n=32) was done using the left breast muscle. After the deboned portions were thawed at 4^0C, homogenization followed. The samples were re-vacuumed packed and frozen at -18^0C until the proximate analysis commenced when the samples were thawed at 4^0C. All of the analyses were performed in duplicates.

3.5.2.2 PROXIMATE ANALYSIS METHODOLOGIES

The proximate analysis involved determination of moisture, ash, crude protein, and crude fat of chicken left breast muscles of chickens from each replicate pen.

3.5.2.3 MOISTURE CONTENT (%)

Determination of % moisture was done by oven-drying a 2.5g homogenized meat sample at 100^0C for 24 hours. Procedures were according to the Standard Techniques of the Association of Official Analytical Chemist's (AOAC) using method 934.01 (AOAC, 2002a).

3.5.2.4 ASH CONTENT (%)

The oven-dried meat samples, already in porcelain crucibles, were placed in a furnace for 6 hours at 500^0C. Ash determination was done according to AOAC method 942.5 (AOAC, 2000b).

3.5.2.5 CRUDE FAT/LIPID DETERMINATION (%)

The crude fat/lipid content of a 5g homogenized raw meat sample was analyzed using the chloroform: methanol method (1:2 v/v) extraction method of Lee *et al.* (1996).

3.5.2.6 CRUDE PROTEIN (%)

To determine the crude protein content (%), a 0.15g defatted, dried and finely ground meat sample was analyzed using a Leco Nitrogen/Protein Analyzer (FP-528, Leco Corportaion). The Leco was calibrated with EDTA calibration samples before each of the analysis sessions. The Dumas combustion method 992.15 (AOAC, 2002c), was used. The results were expressed in % nitrogen (N). The nitrogen (%) was multiplied with the conversion factor of 6.25, to determine the crude protein (%) present in the meat samples.

3.5.3 ANALYTICAL METHODOLOGIES OF FEED SAMPLES

Analytical methodologies were performed at the Department of Animal Science, Stellenbosch University, South Africa. At each stage of the introduction of the three phase diets used in the trial, a 500gram representative sample of each treatment diet was collected and frozen at -20 ^{0}C until needed for laboratory analysis. Proximate analysis was done on the starter, grower and finisher diets, and all of the analysis was done in duplicates.

3.5.3.1 DRY MATTER DETERMINATION

Dry matter of the feed samples were determined according to the Association of Official Analytical Chemists (AOAC, 2002), Official Method 934.01. Representative feed samples were taken from each feed bag and the feed size reduced with a knife-tech machine into relatively fine samples to make for a more homogenized sample. Sample feed was weighed and placed in a crucible and was oven-dried at 1000^{0}C for 24 hours. Thereafter, the dry samples were weighed, and the DM calculated as follows:

$$\% \text{ Moisture} = \frac{(A + B) - C}{B} \times 100$$

% Dry Matter =100-% Moisture

Where:

A= Weight of empty and dry crucible

B=Weight of air- dried test sample

C=Weight of crucible and moisture free test sample

3.5.3.2 ASH DETERMINATION

The sample used for moisture determination with original known crucible mass (A), was then placed in a furnace at 500^0C for 6 hours, according to the methods of AOAC (2002). Ash was calculated as follows:

$$\% \text{ Ash} = \frac{D - A}{B} \times 100$$

Where:

D= Mass of crucible and ash, in g

A= Mass of empty, clean, dry crucible

B= Mass of sample, in g

3.5.3.3 FAT DETERMINATION

A representative feed sample (2g) was weighed using a PS 4500/C/1 scale (RADWAG wagi Elektroniczne, Poland). The crude fat content of the feed samples was determined by acid hydrolysis with Hcl method, followed by extraction of hydrolyzed lipid materials with mixed ethers. Thereafter, the ethers were evaporated and the residue heated to constant weight and expressed as % crude fat as follows:

$$\text{Fat \%} = \frac{\text{(Mass of fat cup plus fat) - (mass of fat cup)}}{\text{Mass of sample}} \times 100$$

All procedures were done according to the methods described by AOAC (2000).

3.5.3.4 CRUDE FIBRE DETERMINATION

Crude fibre was determined using the gravimetric method. This method involves chemical digestion and solubilization of other components present in the feed (i.e. protein, starch and other digestible carbohydrates) with diluted sulphuric acid and sodium hydroxide. First, about 1g of the feed samples is boiled in dilute acid and then in dilute alkali. The acid hydrolysis removes free sugars and starch. The alkaline hydrolysis removes protein and some carbohydrates. The fibre mass is then corrected for ash after ignition. The whole determination is done in a fibretech system according to the methods of AOAC (2002). The fibre content of the feed is calculated as follows:

$$\% \text{ Crude fibre} = \frac{A - B}{\text{Sample mass, in g}} \times \frac{100}{1}$$

A = Mass of residue in crucible after drying, in g

B = Mass of residue in crucible after ashing, in g

3.5.3.5 CRUDE PROTEIN DETERMINATION

A feed sample (0.10g), representative of the whole feed, was weighed into a foil cup using a sensitive scale. Thereafter, the weighed out samples were analyzed using a Leco Nitrogen/Protein Analyzer (FP-528, Leco Corportaion). The Leco was calibrated with Alfalfa calibration samples before each of the analysis sessions. The Dumas combustion method, 992.15 (AOAC, 2002) was used, and the results were expressed in % nitrogen (N). The nitrogen (%) was multiplied with the conversion factor of 6.25 to determine the % crude protein present in the feed sample.

3.5.4 BONE PARAMETERS

3.5.4.1 BONE BREAKING STRENGTH

At day 35, one bird per replicate (eight birds per treatment) was selected randomly from around the mean weight of chickens in each replicate. The birds were slaughtered according to standard commercial practices, including electrical stunning (50-70 volts, 3-5 seconds), followed by exsanguination within 10 seconds of stunning. Both tibias were removed and frozen at -20C for further analysis. The left tibias were left to thaw, cleaned of adherent tissues and weighed. The tibias were kept cool until breaking strength was determined. The three point destructive bending test prescribed by Fleming *et al*. (1998) was used to determine the breaking strength. This test uses an Instron TTDM compression test machine (Model-INSTRON TTDM) fitted with a 3-point bend ring, with a load cell capacity of 500N and crosshead speed of 30 mm/min. Using a Vernier caliper of 0.1mm accuracy, the length of the tibia and mid diaphyseal diameter were measured. The centre point was marked on the diaphysis with a marker and placed between two 14 mm retaining bars, set 38 mm apart. The 18 mm diameter crosshead probe approached the anterior side of the tibia at 30 mm/min until the bone was broken. The breaking strength (N) was recorded as the point of maximum load before failure occurred. Calculation of the bone breaking strength (Pa) was done using the formulae:

Bone Breaking strength (Pa) = Force (N)* tibia length (mm)/pi*radius^3

The right tibia bones were allowed to thaw, broken into halves and cleaned of adherent tissues and cartilages. Thereafter, they were placed in petroleum ether for 48 hours (Rama and

Reddy, 2001). The defatted tibias were placed in dry porcelain crucibles and oven-dried (100 ^{0}C for 24 hours) to determine fat free dry bone weight. Determination of fat-free bone ash percentage was done by placing the oven-dried tibias in a furnace at 600^{0}C for 24 hours (Zhang and Coon, 1997). All weight measurements of the bone were done using a PS 4500/C/1 scale (RADWAG wagi Elektroniczne, Poland) with accuracy of 0.0001g.

3.5.4.2 BONE MINERAL ANALYSIS

The ash bone samples were ground to powder and used for mineral analysis. Mineral analysis of the tibia was performed at the Institute of Animal Production, Western Cape Department of Agriculture. Mineral composition was determined according to the combustion method (method no.6.1.1.) in ALASA (1996). The tibia ash samples had 5 ml of 6M hydrochloric acid added to each sample individually. The samples were placed in an oven for 30 minutes at 50^{0}C, after which 35 ml distilled water was added and the solution filtered into a bottle and made up to a final volume of 50 ml with distilled water. Elements were measured on an iCAP 6000 series inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Element concentrations were calculated using iTEVA Analyst software.

3.5.5 CARCASS AND MEAT QUALITY CHARACTERISTICS

At 35 days of age, one bird per replicate (eight birds per replicate) was selected with a body weight close to the mean body weight of broilers in each replicate pen. Standard commercial practice, including electrical stunning, followed by exsanguination was used to slaughter the birds. The broilers were scalded; de-feathered and eviscerated (this included the removal of all internal organs, feet and neck). Live weight and hot carcass weight were recorded. Initial muscle pH (pH_i) of the breast and thigh was determined 15 minutes post mortem using a calibrated portable Crison pH25 meter (Allela, Barcerlona) by means of a small incision on the centre of both thigh and breast muscle. Ultimate muscle pH (pH_u) was determined 24 hours post mortem in the same manner and position as described for pH_i. Following the initial pH measurement, the carcasses were hung in cold storage at 4^{0}C for 24 hours. At the completion of the pH measurements, chilled carcass weight, 24 hours post mortem were recorded. Dressing percentage was calculated as the percentage difference between the live weight of the chicken and the weight of the hot carcass.

Commercial portion yields were determined by first cutting the cold carcasses in half, using a portion cutter. Subsequently, the thigh and drumstick were removed by cutting above the thigh towards the acetabulum and behind the pubic bone. The drumstick and thigh were separated by cutting perpendicular towards the joint connecting these two cuts. The wings were removed from the carcass by cutting through the joint between the scapula and the coracoid. The separate portions were weighed using a DS-673 Digital weighing scale (Teraoka Seiko, Japan). Percentage component yields were then calculated by expressing these weights as a percentage relative to chilled carcass weight. Subsequently, the breast was dissected into muscle, skin a d subcutaneous fat combined and bone. These fractions were weighed and expressed as a percentage relative to the total breast weight.

3.5.5.1 MEAT COLOUR MEASUREMENTS

The dissected breast muscle was placed on a flat surface and allowed to bloom for 45 minutes (Warris, 2000) at 8^0C. Meat colour (L*, a*, b* measurements) were measured using a CIE-Lab colour meter (BYK-Gardner GmbH, Gerestried, Germany). Lightness in the meat is represented by L*, while a represent the red-green range and b* represents blue-yellow range (Nollet *et al*., 2007). Negative a* values would represent blueness in the meat colour, whereas, positive values would represent yellow colour in the meat. Measurements were taken in triplicate over the total area of the muscle and the average calculated and used for statistical analysis. According to Warris (2000), a blooming period between 15 to 60 minutes is adequate.

3.5.6 ORGAN WEIGHTS DETERMINATION

The heart, liver, spleen and bursa of Fabricius were excised from the hot carcass and weighed using a DS-673 Digital weighing scale (Teraoka Seiko, Japan). The organs were expressed as percentage of live weight. The gizzard was removed and cut open longitudinally and rinsed under running water. Once rinsed, it was scored for gizzard erosion on an ordinary scale of 1-5, as described in table 3.1, below:

Table 3.3: Gizzard Erosion Scoring Description

Score	Description
1	No erosion
2	light erosion (roughness of the epithelia)
3	modest erosion (roughness and gaps)
4	Severe erosion (roughness, gaps and ulcers on the stomach wall showing haemorrhaging)
5	Extreme erosion (roughness, gaps and haemorrhagic ulcers on stomach wall and separation of epithelia from stomach wall)

3.5.7 DIGESTIVE TRACT PH

The pH of the duodenum, jejunum, ileum, proventriculus and caecum were measured using a calibrated portable Crison pH 25 meter (Allela, Barcelona) (with standard buffers pH of 4.0 and 7.0 at 25 ^{0}C). The pH readings were taken by inserting the pH electrode into the centre of the area of the digestive tract to be measured. The probe was thoroughly rinsed with distilled water between readings.

3.5.8 HAEMATOLOGY PARAMETERS

Blood samples (4 ml) were collected from one bird per replicate (8 birds per treatment) on the 35th day signaling the end of the trial. Blood collection was done by cervical cutting. The blood was immediately discharged into the EDTA collection tubes with anticoagulant. The blood tubes were put inside a cooler with crushed ice to prevent coagulation. Whole blood counts were performed using the Celldyne 3700 haematology analyzer, Abbot Diagnostics, USA with the Veterinary package (GMI, 2016).

3.6 STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance using the general linear model (GLM) and ANOVA procedures of SAS version 9.3 of the SAS system for windows with treatment as the main effect (SAS, 2009). Gizzard erosion scores were analyzed using the Chi-squared test of SAS version 9.3 of the SAS system for windows (SAS, 2009). Tests were done on the 95% confidence levels where a P-value less than 0.05 indicates that there is a difference between dietary treatments and P-value greater than 0.05 indicates that there are no differences between dietary treatments. Data were tested for homoscedasticity and normality. Means were separated with Bonferroni post hoc test (SAS, 2009) version 9.3.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 PERFORMANCE OF BROILERS FED EXPERIMENTAL DIETS

4.1.1 LIVE WEIGHT AND WEIGHT GAIN

Data on the mean live weights and mean cumulative live weight gains of broilers fed two phytogenic feed additives from day1-day 35 are shown in Tables 4.2 and 4.3. There were no significant differences ($p > 0.05$) among broilers fed the dietary treatments in average live weights and cumulative weight gains. Proximate composition of experimental diets is shown in Table 4.1.

Table 4.1: Proximate Composition of Experimental Diets on Dry Matter Basis

Treatments	Starter Phase					
	Dry matter %	CP %	Crude fat %	Crude fibre %	Ash	NFE
Negative Control	91.39	25.38	11.62	2.63	7.44	52.28
Positive Control	91.28	21.97	11.42	3.31	6.56	56.74
Product 1	92.02	21.75	11.1	3.2	6.69	49.28
Product 2	91.27	24.78	9.84	3.46	7.04	54.88
			Grower Phase			
	92.22	21.88	12 .88	4.49	6.17	55.40
Negative Control	92.53	21.81	12.80	3.53	6.27	55.59
Positive Control	92.22	20.53	12.17	3.34	6.22	57.74
Product1	92.32	20.34	12.87	3.53	6.27	57.00
Product 2						
			Finisher Phase			
Negative Control	92.19	20.00	13.30	3.73	5.85	57.12
Positive Control	92.07	19.27	12.53	3.63	6.37	58.00
Product 1	91.52	17.72	12.18	4.33	5.38	60.39
Product 2	91.96	19.06	12.62	3.55	5.76	59.01

Table 4.2: Mean (±standard deviation) live weights (g) of broilers fed two different phytonutrient compounds, antibiotics and no antibiotic diets

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
NEG CON	49.38±0.00	155.15±2.91	451.88±15.10	871.88±27.64	1448± 56.57	2148.75±98.04
POS CON	49.43±0.00	149.49±6.67	441.63±25.01	871.88±36.15	1472.5± 39.73	2197.5±78.69
PRODUCT 1	47.16±0.00	150±7.29	441.25±19.23	882.50±24.79	1473.38±47.04	2166.25±95.31
PRODUCT 2	47.73±0.00	146.03±7.84	435.63±24.56	875.63±29.09	1486.25±39.62	2240±141.22

a,b Means within columns with different superscripts differ significantly ($P<0.05$)

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton) NS- Non-Significant.

Table 4.3: Mean (±standard deviation) cumulative weight gains (g) of broilers fed two different phytonutrient compounds, antibiotics and no antibiotics diets

Treatments	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
NEG CON	105.79± 4.79	402.49±15.07	822.49±25.36	1398.67±54.93	2097.85±97.27
POS CON	100.06±6.00	392.23±25.84	822.51±361.2	1423.14±39.61	2145.22±79.14
PRODUCT 1	102.84±8.74	394.09±20.93	835.34±26.47	1426.22±48.99	2116.57±96.94
PRODUCT 2	100±8.06	387.89±23.95	827.89±28.11	1438.51±38.62	2155.39±79.79

a,b Means within columns with different superscripts differ significantly (P<0.05)

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton) NS- Non-Significant

4.1.2 FEED INTAKE

Data for cumulative weekly feed intake of broilers fed two different phytonutrient compounds in comparison to the negative and positive control groups are presented in Tables 4.4. There were significant ($p < 0.05$) differences between the treatment means in cumulative feed intake only at week 1 (day 0-7). However, for experimental periods- 0-14; 0-21; 0-28 and 0-35 days respectively, feed intake was not significantly influenced ($p > 0.05$) by dietary treatments. At week 1, the feed intake of birds in sample 1 group (treatment 3) was markedly reduced, and this was statistically ($p < 0.05$) lower than the feed intake of birds in the negative control group. Birds on treatments 2 and 4 had similar feed intakes with those on treatment 1, but this was not statistically different from the feed intake of birds on treatment 3.

Table 4. 4: Mean (±standard deviation) feed intake of broilers fed phytogenic feed additives

Treatments	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
NEG CON	130.51±6.15^{a}	498.8±24.49	1168.55±27.59	2072.02±58.42	3260.88±93.38
POS CON	128.81± 7.08^{ab}	503.45±24.10	1181.29±98.55	2085.25±130.89	3316.29±143.11
PRODUCT 1	121.59± 2.11^{b}	494.62±18.57	1175.25±22.19	2042.71±33.91	3236.11±82.23
PRODUCT 2	125.57 ±8.03^{ab}	493.00±26.31	1165.50±30.93	2078±38.34	3298±60.87
P-value	0.041	0.814	0.939	0.698	0.385

a,b Means within columns with different superscripts differ significantly (P<0.05)

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.1.3 FEED CONVERSION RATIO

Data on cumulative feed conversion ratio (FCR) are shown in Table 4.5. There were no observed significant ($p > 0.05$) differences among broilers fed the treatment diets in FCR, for the period of the experiment, except at day 7. Birds on treatment 3 (product 1 group) had better feed

conversion ratio at day 7, when compared to birds on treatment 2 (positive control). This was similar to those of treatments 1 and 4 (negative control) and (product 2 groups).

Table 4.5: Mean (±standard deviation) cumulative feed conversion ratio of broilers fed two different phytonutrient compounds, antibiotics and no antibiotic diets

Treatments	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
NEG CON	1.23±0.04[ab]	1.24±0.07	1.42±0.07	1.48±0.07	1.55±0.07
POS CON	1.29±0.04[a]	1.28±0.07	1.44±0.12	1.46±0.09	1.54±0.07
PRODUCT 1	1.18±0.09[b]	1.25±0.07	1.41±0.04	1.43±0.04	1.53±0.06
PRODUCT 2	1.26±0.06[ab]	1.27±0.06	1.41±0.05	1.45±0.03	1.53±0.04
P-value	0.03	0.58	0.85	0.41	0.81

a,b Means within columns with different superscripts differ significantly (P<0.05)

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.1.4 European Production Efficiency Factor (EPEF) and Liveability

Data on the production parameters such as European production efficiency factor (EPEF), liveability (%) and average daily gain (ADG) are presented in Table 4.6. There were no significant (p> 0.05) differences between treatments in EPEF, liveability and ADG.

Table 4.6: Mean (±standard deviation) EPEF, liveability (%) and ADG of broilers fed two different phytonutrient compounds, antibiotics and or no antibiotics

Treatments	EPEF	Liveability (%)	ADG
NEG CON	389.09±43.76	98.75	62.98±0.45
POS CON	397.57±42.42	97.50	62.45±0.43
PRODUCT1	399.95±31.27	98.75	62.76±0.43
PRODUCT 2	418.57±33.66	100	62.14±0.46
P-value	0.48		0.001

Means within a column with the same superscript do not differ significantly (P>0.05)

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.2 CARCASS YIELDS OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES, COMPARED TO ANTIBIOTICS AND NON-ANTIBIOTICS DIETS

Data on carcass characteristics of broilers are shown in Table 4.7. Treatments did not significantly (p>0.05) affect the various portions of broiler carcasses, with the exception of the thigh which was significantly (p <0.05) affected by treatments. Treatment also did not affect (p >0.05) the dressing percentages of the broiler carcasses. Data on the diffe8rent portions of the breast tissue, (fat

and skin, bone and muscle) are shown in Table 4.8. No significant differences (p>0.05) were observed in the percentage fat and skin, and also bone and muscle.

Table 4.7: Mean (±standard deviation) of the breast, thigh, leg, and wing of chilled carcasses obtained from broilers fed the experimental diets

Treatments	% Breast	% Thigh	% Leg	% Wing	Dressing Percentage
NEG CON	20.64±2.11	13.79±0.99^{b}	6.57±0.75	12.44±1.96	71.43±2.73
POS CON	21.19±1.45	14.84±0.63^{ab}	6.35±0.67	12.61±1.57	71.42±2.66
PRODUCT 1	22.62±2.05	14.75±1.18^{ab}	6.89±0.79	12.43±1.50	72.16±2.45
PRODUCT 2	22.02±1.83	15.2±0.72^{a}	6.80±0.90	13.00±1.79	69.99±1.82
P-value	0.19	0.03	0.55	0.90	0.36

a,b Means within columns with different superscripts differ significantly (p<0.05)

Treatment -Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

Table 4.8: Mean percentage (±standard deviation) of skin, fat and skin and bone of the breast obtained from broilers fed the experimental diets

Treatments	% Muscle	% Skin/fat	% bone
NEG CON	62.23±3.96	6.89±1.90	30.88±3.12^{NS}
POS CON	60.36±5.19	6.49±1.68	33.14±4.19 ^{NS}
PRODUCT 1	63.71±1.65	6.95±1.14	29.27±1.86 ^{NS}
PRODUCT 2	61.21±4.66	6.56±1.47	32.23±4.24 ^{NS}
P-value	0.41	0.92	0.16

a, b Means within columns with different superscripts differ significantly (p <0.05), NS- Non-Significant

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.3 MEAT QUALITY ATTRIBUTES OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES COMPARED ANTIBIOTICS AND NON-ANTIBIOTICS DIETS

4.3.1 PH OF MEAT

The pH for the breast and thigh muscles is shown in Table 4.9. There were no significant (p>0.05) differences among treatment means in the initial (pH_i) and ultimate (pH_u) pH of the breast and thigh muscles, respectively.

4.3.2 MEAT COLOUR

Data on the colour readings of the breast muscle are shown in Table 4.9. No significant (p >0.05) differences were observed among the different dietary treatments in the L*, a* and b* colour readings taken.Van Laack *et al*. (2000) also stated that normal broiler meat will have an L*, a* and b* colour reading values of 55.1, 2.2 and 9.6, respectively. The values obtained for a* and b* colour values were found to be slightly higher than the normal range. Birds in the antibiotic group had the highest a* and b* values whereas the lowest b* value was recorded in the negative control group.

According to Muthukumar *et al*. (2011), birds with slaughter weights higher than 2000g had higher a* and b* and lower L* values than birds with lower slaughter weights.

4.3.3 PROXIMATE COMPOSITION OF MEAT

Data on proximate composition of the left breast muscle of experimental chickens are shown in Table 4.9. There were no significant ($p > 0.05$) differences among treatments in moisture, ash and dry matter values. However, significant ($p < 0.05$) differences existed among the treatments in fat, nitrogen, protein (óAs isô) and DM minus fat percentage. Broilers fed treatments 4 and 3 had significantly ($p < 0.05$) lower fat content of 2.25 and 2.44%, and this was statistically different from the fat content of birds fed the negative and positive control groups, which were 3.5 and 3.39. Birds fed treatments 4 and 3 also had significantly ($p > 0.05$) higher N (óAs isô) value of 3.67 and 3.63, and this was statistically different ($p < 0.05$) from the nitrogen content of breast muscle of broilers in other treatment groups. The least nitrogen content was recorded for broilers in the negative control group. The same trend was also observed for protein content of breast muscle with treatment 4 birds having significantly higher protein percentage ($p > 0.05$) compared to birds fed the other treatment diets, whereas, broilers fed treatment 1 diet had significantly ($p > 0.05$) lower protein value than birds fed treatments 2, 3, and 4 diets respectively.

Table 4.9: Carcass and Biochemical Indices of broilers fed Experimental Diets

Carcass and Biochemical indices	Diets				P-value
	NEG CON	POS CON	PRODUCT 1	PRODUCT 2	
Chilled carcass weight (g)	1520±0.15	1530±0.05	1530±0.08	1550±0.04	0.94
Initial (pHi) and ultimate (pHu) pH of broiler breast muscle					
pH_i breast	6.30±0.21	6.19.±0.17	6.32±0.19	6.14±0.18	0.19
pH_u breast	5.90±0.12	5.92±0.0.10	5.95±0.11	5.86±0.14	0.57
pH_i thigh	6.04±0.24	6.17±0.11	6.09±0.35	5.96±0.0.14	0.31
pH_u thigh	6.17±0.78	6.07±0.09	6.03±0.13	6.01±0.09	0.90
Meat colour characteristics					
L*	51.63±2.03	51.99±1.94	50.75±0.69	51.17±2.30	0.57
a*	4.44±1.38	4.44±1.44	4.09±1.54	4.42±1.25	0.96
b*	12.99±1.73	14.09±2.15	13.51±1.47	13.51±0.91	0.854
Meat proximate composition					
DM %	25.04±0.89	25.36±0.45	25.18±0.99	25.33±0.56	0.83
N óAs isô	3.39±0.13^{b}	3.54±0.17^{ab}	3.63±0.21^{ab}	3.67±0.15^{a}	0.02
Protein óAs isô	21.19±0.83^{b}	22.11±1.09^{ab}	22.71±1.32^{a}	22.91±0.94^{a}	0.01
Fat	3.50±0.67^{a}	3.39±0.67^{a}	2.44±0.74^{b}	2.25±0.50^{b}	0.00
Ash	1.12±0.14	1.01±0.13	1.09±0.09	1.07±0.11	0.29
DM %- Fat %	21.54±0.90^{b}	21.97±0.54^{ab}	22.74±0.83^{a}	233.08±0.98^{a}	0.00

a,b Means within columns with different superscripts differ significantly ($p < 0.05$), Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton). óAs isô= as the meat is.

4.4 BONE PARAMETERS OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES, COMPARED TO ANTIBIOTICS AND NON-ANTIBIOTIC GROUPS

4.4.1 BONE BREAKING STRENGTH

Data on bone breaking strength are shown in Table 4.10 and expressed as the total force in Newton (N) required in breaking the tibia, or the strength of breakage of the bone expressed in standard unit (MPa). There were no significant ($p > 0.05$) differences among treatments in the force of breakage and breaking strength for all the tibia bones.

Table 4.10: Mean (± standard deviation) tibia breaking strength of broilers fed two different phytonutrient compounds, antibiotics and no antibiotics

Treatments	Bone Breaking Force, N	Breaking strength (MPa)
NEG CON	344.23±36.83	181.25± 32.62
POS CON	396.31±52.85	189.44±31.54
PRODUCT 1	382.68±83.19	217.42±46.93
PRODUCT 2	379.11±41.81	205±29.84
P-value	0.37	0.23

a,b Means within columns with different superscripts differ significantly ($p < 0.05$), NS- Non-Significant

Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.4.2 BONE ASH AND MINERAL CONTENT

Data on fat-free dry bone weight, bone ash contents and the amount of different macro and micro minerals present in the tibia bone ash are shown in Table 4.11. No significant ($p > 0.05$) differences were observed in all the treatments in fat-free dry bone weight, bone ash content and mineral composition of the tibia of birds.

Table 4.11: Mean (±standard deviation) bone ash, tibia breaking strength and mineral contents of broilers fed the experimental diets

	TREATMENTS				P-value
	Negative Control	**Positive Control**	**product 1**	**product 2**	
Fat -free dry- bone weight (g)	4.37±0.43	4.03±0.83	4.62±0.48	4.72±0.49	0.13
Fat -free bone ash (%)	49.82±4.08	52.71±2.56	50.12±2.19	50.30±1.62	0.21
Ca: P ratio	2.05	2.02±0.09	1.99±0.06	2.03±0.09	
Minerals as % of bone ash					
Calcium (Ca)	86.16±8.52	80.16±3.32	84.62±8.81	88.38±8.10	0.29
Phosphorus (P)	42.11±5.03	39.99±2.71	42.41±4.64	43.51±3.64	0.47
Potassium (K)	0.92±0.21	0.93±0.09	0.98±0.20	1.0±0.14	0.68
Magnesium (Mg)	1.94±0.21	1.83±0.13	1.92±0.06	1.91±0.09	0.33
Sodium (Na)	2.35±0.26	2.22±0.11	2.38±0.29	2.46±0.25	0.33
Iron (Fe)	0.04±0.01	0.03±0.00	0.04±0.00	0.03±0.00	0.60
Copper (Cu)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.29
Zinc (Zn)	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.50
Manganese (Mn)	0.002±0.00	0.002±0.00	0.002±0.00	0.002±0.00	0.35
Boron (B)	0.02±0.01	0.02±0.01	0.04±0.02	0.02±0.01	0.09
Aluminum (Al)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.47

4.5 ORGAN AND LYMPHOID CHARACTERISTICS OF BROILERS FED EXPERIMENTAL DIETS

4.5.1 Relative Organ Weights

The weights of the organs expressed as percentages of body weight are shown in Table 4.12. Treatments had no significant ($p > 0.05$) effect on gizzard, heart, liver, and spleen weights.

4.5.2 Relative lymphoid organ weight percentage

Data on relative lymphoid organ weights are shown in Table 4.12. Treatments had no significant ($p > 0.05$) effect on the weights of spleen, bursa or spleen: bursa ratio

Table 4.12: Mean (± standard deviation) Carcass characteristics of broilers fed phytogenic feed additives as alternatives to antibiotics

Organs	Diets				P-value
	NEG CON	POS CON	PRODUCT 1	PRODUCT 2	
	Relative and lymphoid weights (% hot carcass weight)				
Gizzard	1.41±0.51	1.52±0.32	1.57±0.25	1.40±0.17	0.55
Liver	2.06±0.33	1.99±0.28	1.99±0.31	1.93±0.11	0.83
Spleen	0.10±0.03	0.09±0.02	0.09±0.03	0.09±0.02	0.74
Bursa	0.22±0.04	0.21±0.04	0.23±0.04	0.28±0.08	0.08
Heart	0.64±0.09	0.63±0.06	0.63±0.09	0.65±0.13	0.84
	Spleen to bursa ratio				
	0.48±0.12	0.43±0.13	0.43±0.15	0.37±0.11	0.04

a,b Means within columns with different superscripts differ significantly (P<0.05) Diets 1: NEG CON- Negative control (no additive); POS CON- positive control (antibiotic growth promoter); sample 1 diet (phytonutrient sample 1-1L/ton); sample 2 diet (phytonutrient sample 2-1L/ton).

4.6 GIZZARD EROSION SCORES OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES

Data on gizzard erosion scores are shown in Table 4.13. There were no significant (p >0.05) differences between treatment means in gizzard erosion scores.

Table 4.13: Mean (±standard deviation) of gizzard erosion scores of broilers fed two different phytonutrient compounds, antibiotics and no antibiotics

	Gizzard Erosion Scores				
Treatments	1	2	3	4	5
NEG CON	4	3	0	1	0
POS CON	2	2	2	1	0
PRODUCT 1	3	2	3	0	0
PRODUCT 2	3	2	1	2	0
Chi-Square P value			0.46		

a,b Means within columns with different superscripts differ significantly (p <0.05), NS- Non-Significant

1Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.7 DIGESTIVE ORGANS pH OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES

Data on pH of the digestive organs are shown in Table 4.14. Treatments had no significant (p >0.05) effect on the pH of the proventriculus, duodenum, jejunum, ileum and caecum.

Table 4.14: Mean (±standard deviation) pH of various areas of the digestive tract of broilers fed two different phytonutrient compounds, antibiotics and no antibiotics

Treatments	Proventriculus	Duodenum	Jejunum	Ileum	Caecum
NEG CON	2.93±0.78	6.13±0.23	6.16±0.34	6.57±0.22	6.94±0.21
POS CON	2.93±0.89	6.09±0.23	6.06±0.22	6.56±0.42	6.74±0.34
PRODUCT 1	2.52±0.78	5.77±0.36	5.98±0.18	6.57±0.58	6.99±0.25
PRODUCT 2	2.65±0.86	6.2±0.21	6.03±0.39	6.54±0.27	6.56±0.31
P-value	0.70	0.02	0.65	0.99	0.04

a,b Means within columns with different superscripts differ significantly (p <0.05), NS- Non-Significant

1Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.8 HAEMATOLOGICAL PARAMETERS OF ROSS 308 BROILERS FED PHYTOGENIC FEED ADDITIVES

Data on haematological parameters of the birds are shown in Table 4.15. Treatments had no significant ($p > 0.05$) effect on white blood cells (WBC), neutrophils, eosinophil, red blood cells (RBC), haemoglobin concentration (Hb), haematocrit value (HCT), mean corpuscular value (MCV), mean heamoglobin count (MCH), mean cell haemaglobin concentration (MCHC), red blood cell distribution width (RDW) and platelet. There were significant differences ($p < 0.05$) among treatment means in lymphocytes, monocytes and basophils. Birds fed treatments 2 and 3 had similar lymphocyte values with birds fed treatment 1, and these were significantly $p < 0.05$) higher than the lymphocyte value of birds fed treatment 4. Birds fed treatments 1 and 4 also had similar lymphocyte values. Birds fed treatments 1, 2 and 4 had similar monocyte values and these were significantly higher than the monocyte values of birds on treatment 3. Birds fed treatments 1, 2 and 3 also had similar monocyte values. Birds fed treatments 1, 2 and 4 had similar basophil values and these were significantly higher than the basophil values of birds fed treatment 3. Birds fed treatments 2, 3 and 4 also had similar basophil values.

Table 4.15: Mean (±standard deviation) haematological parameters of broilers fed phytonutrient compounds, antibiotics and no antibiotic diets

Treatment	Negative Control	Positive Control	Product 1	Product 2	P-value
WBC 10^9/L	24.19±23.00	30.45±15.91	45.95±18.27	23.64±20.55	0.13
Neutrophils %	36.91±6.47	32.28±11.76	29.34±8.97	43.34±20.34	0.11
Lymphocytes %	47.41±22.59^{ab}	54.21±10.31^{a}	65.14±9.78^{a}	26.18±25.68^{b}	0.00
Monocytes %	14.41±4.01^{ab}	7.84±4.15^{ab}	5.00±3.05^{b}	17.29±14.16^{a}	0.02
Eosinophil %	9.02±7.03	4.65±3.51	4.85±3.17	11.22±6.60	0.06
Basophil %	9.23±3.08^{a}	5.94±1.99^{ab}	3.11±1.28^{b}	6.90±3.64^{ab}	0.00
RBC 10^{e12}/L	2.33±0.23	2.15±0.26	2.25±0.30	2.38±0.24	0.36
HGB g/DL	13.17±1.56	11.79±1.72	12.51±1.64	13.13±1.32	0.30
HCT %	17.93±1.73	16.80±2.24	17.37±2.12	18.53±1.61	0.35
MCV Fl	77.35±2.42	77.83±2.73	77.34±2.51	77.79±2.53	0.98
MCH pg	55.39±5.12	54.54±2.10	55.66±1.68	55.35±1.97	0.89
MCHC g/DL	73.35±5.04	70.09±3.02	71.96±0.82	70.84±0.87	0.29
RDW %	11.93±0.66	12.05±0.72	11.66±0.74	12.55±0.41	0.07
Platelet 10^{e9}/L	17.22±19.84	18.34±17.02	13.96±13.83	37.34±25.73	0.11

a,b Means within columns with different superscripts differ significantly ($p < 0.05$), NS- Non-Significant

1Treatment 1-Negative control (no additive); 2 positive control (antibiotic growth promoter); 3 sample 1 diet (phytonutrient sample 1-1L/ton); 4 sample 2 diet (phytonutrient sample 2-1L/ton)

4.9 DISCUSION

4.9.1 PERFORMANCE OF BROILERS FED PHYTOGENIC FEED ADDITIVES

4.9.1.1 AVERAGE LIVE WEIGHTS

The result obtained for live weights in the present study compares with the findings of Toghyani *et al*. (2011) which showed that dietary supplementation of 2g/kg cinnamon markedly enhanced the body weights of birds. Ao *et al*. (2011) also reported that there were no significant ($p > 0.05$) differences in BWG, feed intake or FCR for birds fed fermented garlic powder, and those on the control diet throughout the 5 weeks experimental period. Aporn and Adcharatt (2008), recorded no significant differences in feed intake, average gain and FCR across treatments in broilers fed diets containing garlic powder. The result of this study on live weights however contrast the findings of Kirkpinar *et al*. (2011) which showed that oregano essential oil supplementation significantly decreased body weights at 42 days. A possible explanation for the lack of significant differences among treatment means in live weights for broilers fed diets containing phytogenic feed additives could be seen in the reports of Bedford (2000), who affirmed that growth promoters that act on the gut may not be able to elicit any effect in the absence of challenge with enteric pathogens. This is evident in a study that lasted for 39 days where the authors Wati *et al*. (2015) investigated the efficacy of a commercially available phytogenic feed additive (PFA) and an antibiotic growth promoter, bacitracin methylene disalicylate (BMD) on performance, nutrient retention, caecal colonization of bacteria and humoral immune responses against Newcastle disease in broiler chickens challenged orally with *Salmonella enteriditis* and *Escherichia coli*. The results of this study showed that supplementation of PFA improved body weight and FCR of broilers inoculated with enteric pathogens.

According to Mountzouris *et al*. (2011) and Jamroz *et al*. (2005) plant extracts and essential oils are capable of improving broiler performance and this was attributed to the fact that plant extracts like PFA, unlike antibiotics, may require some time to be able to bring about any shift in gut microbiota and may not also exert significant growth effect on birds in the absence of enteric pathogens. The PFA does this by decreasing the population of growth depressing microbial metabolites such as ammonia and biogenic amines (Jamroz *et al*., 2003; Windisch *et al*., 2008), selection of healthier microbial groups and increasing nutrient availability to the host (Anderson *et al*., 1999; Castillo *et al*., 2006). Hence, in the study of Wati, *et al*. (2015) it was reported that inoculating the birds with enteric pathogens pushed them to more of a ódiseasedô state and allowed

both BMD and the PFA to elicit their effects since both of them are gut modulators. A probable explanation for the inability of the phytogenic feed additives used in this study to exert significant growth promoting effects on birds could be inferred from the aforementioned facts that PFAs requires time, and can only function effectively as gut modulators in the presence of enteric pathogenic challenge on birds.

4.9.1.2 FEED INTAKE

It has been reported that the digestive tract of birds are usually not fully developed at the first week of growth (Kleyn, 2013). This may be the probable reason for the differences in feed intake only at week 1. With age, the birds were able to absorb nutrients effectively and compete equally. Kirkpinar *et al*. (2011) stated that phytogenic feed additives, such as essential oils play important role in stimulating appetite and this role is often dependent on dietary levels and birdôs age. The data obtained for feed intake at day 14-35, in which treatment did not affect feed intake, are in consonance with the findings of Onibi *et al*. (2009) and Fadlalla *et al*. (2010) which showed that there were no significant differences between control group and broilers fed garlic in both feed intake and body weights gain. Doley *et al*. (2009) also recorded that dietary supplementation of 0.25% turmeric and garlic powder had no significant effect ($p > 0.05$) in broiler live weight gain and feed intake when compared to the control.

4.9.1.3 FEED CONVERSION RATIO

The significant differences ($p < 0.05$) observed between treatments in feed conversion ratio at day 7 in this study agree with the reports of Najafi and Torki (2010) that broilers fed thyme-based diets had significantly higher feed conversion ratio compared to the control groups. Generally, the feed conversion ratios of the Ross 308 broilers used in this study were within the industry standards for 35 days old birds (Ross, 2014). Although, there were no significant ($p>0.05$) differences in feed conversion ratios between treatments at day 14 to day 35, the results obtained at day 7 in this study are similar to the findings of Toghyani *et al*. (2011). The authors reported that birds that received supplemented diets (cinnamon and garlic) at day 28 period had a significantly lower feed conversion ratio compared to the control group ($p < 0.05$). In the present study, the positive effects of the phytogenic feed additives on the digestive system and nutrient absorption of the broiler chickens was more pronounced at the younger age of the birds, particularly, at day 7.

There were no significant differences ($p>0.05$) between treatments in European production efficiency factor (EPEF), liveability and average daily gain (ADG) as presented in Table 4.6. EPEF

takes into account the age, liveability (%), live weight and FCR of broilers. The higher the EPEF value, the better the technical performance of the birds (Ross, 2007). According to Ross (2007), higher EPEF values are indicative of better technical performance in broiler birds. All the EPEF values obtained in the present study were much higher than 300 which are considered an excellent production efficiency value for a broiler flock (Basson, 2011). Liveability which is a representation of the percentage of chickens surviving till slaughter is considered appropriate at approximately 97%. Interestingly, in the present study, liveability was higher than 97% in all the treatment groups, with the treatment 3 birds having a liveability of 100%. Result on liveability or mortality in the present trial are in consonance with the findings of Safa *et al*. (2014) which showed that experimental treatments had no significant ($p > 0.05$) effect on mortality rate. In this study, the death of one bird each in both treatments 1 and 3, and 2 birds from treatment 2 could not be attributed in any way to the experimental treatments.

The flock mortality rate in this trial (liveability data in Table 4.1.5) was < 2%, indicating that the birds were exposed to minimal pathogenic challenge, and this could possibly be the reason for the non-significant differences for EPEF and ADG between dietary treatments. This result align with the reports of Banerjee *et al*. (2013) whose findings showed that the use of phytogenic growth promoters enhanced the productive performance of broiler birds in terms of body weight gain with minimal alteration of gut morphology, thereby significantly ($p < 0.05$) decreasing the possibility of pathogenic invasion. The decrease in pathogenic invasion subsequently results in reduced flock mortality rates.

No significant differences ($p > 0.05$) were observed among treatments in ADG for the overall growth period (Table 4.6). The result obtained in this study for ADG contrasts the findings of Acamovic *et al.* (2007) which showed that thyme oil and rosemary herb had the largest overall average gain over the study period, although only the ADG of birds on thyme oil was significantly ($p < 0.01$) greater than that of the control treatment.

4.9.2 CARCASS YIELD OF BROILERS FED PHYTOGENIC FEED ADDITIVES

As shown in Table 4.7, there were no significant differences ($p > 0.05$) among treatment means in the breast and leg portion sizes of the broiler carcasses. Only the weights of the thigh differed significantly ($p < 0.05$) among the various treatments. Earlier report, (Safa *et al*., 2014) had shown that the percentages of breast, drumstick and thigh in broiler meat were significantly ($p < 0.05$) affected by the different levels of Piper nigrum L. (black pepper). Birds fed the highest level

of black pepper of 1% produced significantly the highest percentages of commercial cuts while the lowest percentages of these cuts were produced by the control group. In a 42-day experiment, Marcincak *et al*. (2011), observed higher weights of breast and thigh muscles in groups fed basal diet enriched with 1% clove buds powder, in combination with 0.2% agrimony extract in drinking water, and in the group fed basal diet enriched with 1% clove buds powder combined with 0.2% lemon balm extract diluted in drinking water. Nevertheless, the results of this study partly align with the findings of Kirkpinar *et al*. (2011) which showed that carcass yields of 42-day old broilers were unaffected by dietary treatments. The reports of Onibi *et al*. (2009) also revealed that garlic supplementation had no significant effects on major carcass components and organ characteristics of broiler birds. Ghaedi *et al*. (2013) also stated in their study that chicks fed basal diet and served Piper nigrum L. extract, a phytonutrient, at 2mg/ml of drinking water had significantly ($p < 0.05$) higher carcass yield percentage as compared to the control group. Shahverdi *et al*. (2013) stated that dietary supplementation of 0.02% black pepper in broiler diets increased not only the drumstick but also the breast percentages of the broiler portions.

The importance of carcass and muscle yield of broiler birds cannot be over-emphasized. Usually, carcass weight, meat yield and lean meat quality are important criteria that affect the value apportioned to any given chicken carcass. However, demand for lean and healthier meat signifies demand and selection for meat with less abdominal fat. There is high demand for chicken meat sold as portions, and the modern consumers tend to prefer these chicken portions basically because of convenience and versatility (Kennedy *et al*., 2004). Nowadays, deboned and skinless chicken portions are also being sold. These demands for specific chicken portions have brought about an increase in selection for breast meat yield. This, coupled with selection for lower abdominal fat, has invariably increased the procurement cost of chicken meat, but not without offering the consumer a more desirable product (Young *et al*., 2001; Le Bihan-Duval *et al*., 1998; Guerrero-Legarreta, 2010).

Table 4.8, showed that no significant differences ($P>0.05$) were observed in the percentage fat and skin, and also bone and muscle among the different dietary treatments. To meet up with modern consumer needs, as well as with the demands of the slaughter industry, broilers are required to have a high slaughter yield and desirable carcass conformation (Bogosavljevic-Boskovic *et al*., 2010). Although the major objective of broiler breeding companies is to increase the lean muscle yield of modern chicken lines, however, attention should also be given to the importance of skin

yield. This is because skin and skin fat, including subcutaneous fat can represent as much as 11-15% of total carcass weight (Ferrini *et al*., 2008). This is pertinent to the poultry industry, particularly due to reduced fat content of poultry meat, due to significant subcutaneous deposition of fat (Fanatico *et al*., 2007). It is important to know that for the commercial producer, the total muscle yield of whole chicken is considered relevant. Thus, contribution of muscle, skin and bone to relative to chilled carcass weight becomes relevant when considering the total muscle yield.

Dressing percentage is calculated from the weight of the hot carcass expressed as a percentage of live weight. A higher dressing percentage is indicative of a higher market value for broiler carcasses. It has been observed that dressing percentage of carcass is affected by muscle and visceral growth. Usually, visceral weight or abdominal fat is regarded as a waste in broiler production. In view of this, an increase in abdominal fat results in a concomitant decrease in the dressing percentage. In the present study, treatments did not affect ($p > 0.05$) the dressing percentages of the various broiler carcasses (Table 4.7). Although quite high, the dressing percentages observed in the present study showed that broilers in the various treatment groups had reduced abdominal fat composition. This is comparable to the work of Mohammed *et al*. (2008) which recorded a dressing percentage of over 70%, for birds fed diets supplemented with mannan-oligosaccharide, although the different dietary supplementation did not significantly ($p>0.05$) affect the dressing percentage of birds. Zhang *et al*. (2005) also observed that birds fed essential oil exerted no significant ($p>0.05$) differences in dressing percentages. Nevertheless, Al-kassie *et al*. (2012) stated that dietary inclusion of 0.75 and 1% of a mixture of hot red pepper and black pepper improved significantly ($p <0.05$) the dressing percentage of broilers.

4.9.3 PH AND CIE-LAB MEASUREMENTS OF BROILERS FED PHYTOGENIC FEED ADDITIVES

4.9.3.1 PH OF MEAT

As shown in Table 4.9, treatments did not affect ($p > 0.05$) the initial and ultimate pH values of both breast and thigh muscles of broilers fed the experimental diets. Initial pH readings in the current study were recorded 15 minutes postmortem. Several conflicting results abound on the acceptable initial and ultimate pH values for normal, pale soft and exudative (PSE) and dark, firm and dry (DFD) meats. However, the initial pH values of breast meat as observed in this study falls within the normal range reported by Niewiarowicz and Pikol (1979), in which they stated that pH of normal breast meat 15 minutes post mortem (pH_{15}) ranged from 5.8-6.3, whereas a value of 5.77 signified PSE meat. Debut *et al*. (2003) and Battula *et al*. (2008) also reported that on average, pH

of meat after 15 minutes (pH_{15}) was between 6.3 and 6.6 for broiler breast meat that was considered as normal meat. Ristic *et al*. (2004) reported an initial pH of >6.0 as value for normal meat, and a value of 5.7 as meat with incidence of PSE. However, Zhang and Barbut (2005) reported that normal meat has a pH value of 5.91 after 15 minutes of slaughter, while 5.54 and 6.23 were indicative of PSE and DFD, meats, respectively.

According to Fernandez *et al*. (1994) pH is an indicator of meat quality. Hence, a low pH of less than 5.7 at 24 h postmortem is indicative of poor meat quality. None of the breast muscles of broilers in all treatments as observed in the present trial had a pH value less than 5.8 and as such can be regarded as normal meat. Van Laack *et al*. (2000) reported pHu values of 5.96 for normal and 5.70 for pale coloured breast meat. The result on initial pH (pHi) and ultimate pH (pHu) of breast muscle are similar to the findings of Corzo *et al*. (2009), in which none of the breast muscle had a pH of below 5.7, indicating that there were no quality problems with the breast meat from each treatment group. According to Soares *et al*. (2002), normal broiler meat has pH of >5.80 24 hours postmortem, whereas pH values <5.80 after 24 hours is indicative of PSE condition in the meat. All the ultimate pH values of thigh muscles recorded in this study did not exceed 6.1. Hence, the pH readings of the breast and thigh muscles of broilers in the present study shows that the meat were normal meat and had no quality related issues.

4.9.3.2 MEAT COLOUR

The results on meat colour readings in the present study (Table 4.9) is in consonance with the findings of Hong *et al*. (2012), Aao *et al*. (2011), Aporn and Adcharatt (2008) and Saenkhunthow (2004) which stated that the means of L*, a * and b* of chicken breast and thigh meats did not differ among treatments. The results also align with the findings of Young *et al*. (2003), which showed that supplementation of broiler diets with 30mg/kg oregano increased meat a* and b* values. Nevertheless, Simitzis *et al*. (2008) found that dietary supplementation with oregano decreased both a* and b* values of and meat. This may be due to a decrease in haemoglobin oxidation and activation of mechanism modified pigment distribution in animal tissues. In another instance, dietary inclusion of feed additives such as garlic, turmeric, oregano in the diets of lambs and pork were found to lower not only the L*, a* and b* reading values of meat, but also increase pH and water holding capacity of these meats (Chen *et al*., 2008; Smitzis *et al*., 2008). Uniform meat colour within a package is crucial, particularly when there is need to sell individual chicken parts.

Broiler breast meat quality is often evaluated using key attributes such as colour, pH, and water holding capacity, tenderness and sensory acceptability. This is due to the fact that consumers would normally go for a meat that is tender and juicy rather than hardd and pale meat (Van Laack *et al*., 2000; Fletcher and Smith, 2006). Colour is the major attribute of meat that determines its acceptance or rejection by a particular consumer (Fanatico *et al*., 2007). This is of particular significance considering the modern consumerôs demand for chicken meat portions such as chicken pieces and de-boned fillets. According to Fletcher *et al*. (2000) consumers prefer meat with pale tan-pink colour when fresh, and meat with tan-grey colour when cooked. Hence, meat quality defects determines to a large extent, whether or not a chicken part/processed product will be sold or bought, and not necessarily the quantity of meat that is to be procured. This focus towards quality as against quantity is what empowers the consumer to reject meat products when the colour falls short of expected standards (Fanatico *et al*., 2007; Qiao *et al*., 2001).

Following slaughter, oxygen and nutrient supply to the animal by the circulatory system ceases, and a conversion of glycogen to lactic acid occurs under anaerobic conditions. The latter dissociates into H^+ and lactate in the meat, leading to a drop in pH from neutral value of 7.0 to ultimate pH (pH_u) values of between 5.4-5.8, roughly 24 hours after slaughter (Heinz and Hautzinger, 2007). Ultimate pH influences the structure of myofibrils, and also the colour of meat. Compared to other meat products, post-mortem pH decline is more common and rapid in chicken meat, and this predisposes it to a more frequent occurrence of pale, soft and exudative (PSE), defect, a condition in meat caused by accelerated post-mortem muscle metabolism (Castellini *et al*., 2008). It is important to note that the rate at which this pH decline occurs, influences to a large extent, not only colour, but also other meat quality attributes such as tenderness, cooking loss, juiciness, shelf-life and water holding capacity (WHC) (Honikel, 2004). In other words, post-mortem pH changes are critical to the control of functional meat qualities.

A number of authors have reported a negative correlation between colour and pH of chicken breast meat (Barbut, 1993; Fletcher, 1995; Allen *et al*., 1998). According to Allen *et al*., 1998, meat colour is associated with pH in a way that lighter muscles (L*>50) have higher pH values than darker (L*<45) ones. Pale meats also have lower ultimate pH (pH_u), and a lower a* reading value, whereas darker meats have a higher pH_u and a lower L* value. Chicken meat with lower ultimate pH values has lower water holding capacity which influences cooking loss and drip loss. Drip loss is an undesirable meat quality as far as consumers are concerned. Increased drip loss in meat

decreases its tenderness due to moisture loss (Van Laack *et al*., 2000; Huff-Lonergan & Lonergan, 2005). Meat with higher ultimate pH is often characterized by increased tenderness, moisture absorption properties, improved shelf-life and better meat flavor (Froning *et al*., 1978; Barbut, 1993; Allen *et al*., 1997; Lawrie and Ledward, 2006).

4.9.3.3 PROXIMATE COMPOSITION OF BREAST MUSCLE

As shown in Table 4.9, no significant differences ($p > 0.05$) were recorded for moisture, ash and dry matter values of broiler breast meat. Treatments however significantly ($p < 0.05$) affected the percentages of fat, nitrogen and protein as well as DM-fat % across the various treatments. It is important to note that the proximate composition of moisture, protein, fat and ash contents of broiler meats recorded in the present study were all within the normal ranges reported by Keeton and Eddy (2004). Chicken meat also known as white meat is preferred above red meat due to its low fat and cholesterol contents (Charlton *et al*., 2008). Meat primarily consist mainly of five chemical attributes namely moisture, proteins, lipids (fats), carbohydrates and inorganic matter (ash or minerals). According to Keeton and Eddy (2004), lean meat comprises approximately 72-75% water. The moisture content of meat influences attributes such as the juiciness, tenderness and flavor of meat and these affect the palatability and overall acceptability of meat (Lawrie and Ledward, 2006; Warris, 2010). Moisture content of meat is inversely proportional to the fat content of the muscle (Pearson and Young, 1989). A meat sample with low fat content will definitely have high moisture content.

Lean chicken meat contains approximately 16-20% protein which is rich in essential amino acids. It is known that as fat content increases, the protein in meat decreases (Keaton and Eddy, 2004. Animal muscle tissue is comprised of approximately 2-5% fat, containing phospholipids, neutral lipids (triglycerides) and cholesterol (Keeton and Eddy, 2004). Poultry meat is reputed for its low fat content, with the breast portion which is white meat containing fat as low as 3% when compared to the red meat portions such as thigh and drumstick with 7.3% fat (Fanatico e*t al.*, 2007a; Mckee, 2003). According to Lawrie and Ledward (2006), red muscles have higher intramuscular contents than white muscles due to their ability to store intramuscular fat (IMF) within the muscle fibres, in the form of fat droplets. However, as stated by Fanatico *et al*. (2007a), poultry unlike other meat animals have specialized mechanism for storing fat subcutaneously rather than in the meat itself resulting in reduced lipid and higher moisture, protein and ash contents than other meat animals. Ash which has been found essential for human nutrition is known as the

mineral constituents of meat and it contains iron, potassium, phosphorous, in addition to oxides, silicates, sulphates and chlorides. About 1-2% of ash is found in poultry meat (Keeton and Eddy, 2004; Lawrie and Ledward, 2006).

The result on proximate composition of broiler breast muscle in this trial contradicts the findings of Hong *et al*., (2012) which showed that breast and thigh muscle dry matter and fat content were unaffected by any of the dietary treatments.

According to the study of Marcincak *et al*. (2011), addition of 1% clove buds powder, and 0.2 % agrimony (AC), in combination with 1% clove buds powder, combined with 0.2% lemon balm (LC) had no significant ($p > 0.05$) effect on the proximate composition of breast muscle. Experimental groups were comparable with control in protein, crude fat and dry matter. However, for the thigh muscle, the group AC had a significantly higher proportion of protein compared to other groups. Lower proportion of fat was also observed in the groups fed plant extract compared to the control group. DM was also significantly lower ($P<0.05$) in the experimental groups compared to the control.

4.9.4 BONE PARAMETERS OF BROILERS FED PHYTOGENIC FEED ADDITIVES AS ALTERNATIVES TO ANTIBIOTICS

4.9.4.1 BONE BREAKING STRENGTH

Table 4.10, showed that treatments did not affect ($p>0.05$) the bone breaking force (N) and bone breaking strength (MPa) of tibia bones across the various treatment groups. According to Stofanikova *et al*. (2011), there was significant reduction in tibia strength and elasticity, and also as high incidence of various leg deformities in about 10% of broilers receiving diets with no zinc additives, compared to the group on zinc supplementation. Hence, it was concluded that one key indicator of locomotory disorders in broilers is determining the weight bearing capacity of tibia bones, through assessment of its mechanical properties. Thus, the authors (Stofanikova *et al*., 2011), noted that zinc has significant effect in reducing locomotory disorders in broilers.

4.9.4.2 BONE ASH AND MINERAL CONTENT

As shown in Table 4.11, treatments did not have any significant ($p>0.05$) effect on fat-free dry bone weight, bone ash content and the mineral composition of the tibia bones between the various treatment groups. According to Shim *et al*. (2012), the percentage of ash in the bone is a key indicator of the level of mineralization that occurred in the bone. It also depicts the susceptibility level of birds to bone disorders, particularly, as chickens with bone disorders usually have a lower

bone ash percentage than that of healthy chickens. It has been observed that broilers with fast growth rate generally have a lower bone mineral content in comparison to the slow growing broiler lines (Venalainen *et al*., 2006). Nevertheless, it is pertinent to note that broiler diets have not been adjusted or improved upon to keep up with this increased demand on mineral requirements needed for increased growth rate (Thorp and Waddington, 1997).

Bone is a complex tissue that contains both organic and inorganic substances. The former provides elasticity to the bone, whereas the latter such as Ca and P are responsible for bone hardness and strength (Mutus *et al*., 2006). The state of the bone indicates the adequacy of minerals in poultry diets (Rath *et al*., 1999). Bone metabolism involves a complex balance between the deposition of matrix, mineralization and resorption. Scientific evidence abound to suggest that dietary components and herbal products can influence these processes, particularly by inhibiting bone resorption, thus having beneficial effects on the skeleton (Putnam *et al*., 2007).

Over the past few decades, commercial chicken meat hybrids have been selected mainly for fast growth, improved feed efficiency and meatiness. With time, high incidences of skeletal deformities became prominent. A large percentage of these skeletal abnormalities are linked with tibia-related problems, mostly osteoporosis (in laying birds) and tibia dyschondroplasia in meat-type chickens (Capps *et al*., 1995). Bone breakage and infections account for huge economic losses in the poultry industry, evident in the high mortality rates, low productivity, and increased carcass condemnation that occur in the broiler house. The degree of bone mineralization affects bone strength (Reichmann and Connor, 1977). Poor mineralization predisposes birds to higher fracture risks, deformity of the metatarsi and downgrading of meat. Weak legs also often results in reduced feed intake and low weight gain (Orban *et al*., 1999; Molnar, 2010). A number of invasive (bone ash, breaking strength, weight and bone volume), and non-invasive methods (ultrasounds) had been used to determine bone mineralization in poultry (Onyango *et al*., 2003).

The reduced mortality rates across treatments, coupled with results obtained on bone breaking strength, bone ash and mineral composition of tibia bones as observed in the present study shows that the bones had a high degree of mineralization. The feed intake and weight gains of the birds were up to the recommended standards set by the breeders, hence it can be inferred that the incidence of skeletal abnormalities were highly reduced among birds in the different treatment groups. Although the birds had fast growth rate, only two (>1%) birds died from incidence of tibia

dyschondroplasia and also had a high concentration of minerals (particularly P and Ca) as against what has been reported in literature.

4.9.5 RELATIVE AND LYMPHOID ORGAN WEIGHTS PERCENTAGE

In all the parameters measured based on organ weights, relative organ weight and relative lymphoid organ weight percentage, only the absolute weights of bursa was significantly different ($P<0.05$) between treatments (Table 4.12). Hernandez *et al*. (2004) reported that there were no differences ($p >0.05$) in gizzard, liver and pancreas weights of broilers which were fed diets containing wheat-soybean meal based diets, supplemented with two extracts (an essential oil extract from oregano, cinnamon and pepper, and a labiatae extract from sage, thyme and rosemary). Earlier report (Amad *et al*., 2011) showed that relative weights of the pancreas, spleen, liver and heart were not affected by the phytogenic feed additive used in the study. In a similar study, Dieumou *et al*. (2009), in a similar manner also reported that virtually all the organ weights of broilers used in the study were not affected by dietary treatments, except for a decrease in relative liver weight observed in the birds fed diets containing garlic oil.

According to Kirkpinar *et al*. (2011), treatments had no effect on the relative weights of the proventriculus, gizzards, duodenum, jejunum, ileum, colon, cecum, liver, pancreas, spleen, heart and bursa of broilers at the end of a 42-day trial. Sarica *et al*. (2005) also reported that dietary supplementation of 1g/kg thyme and garlic powder in wheat based diets did no significantly affect performance and relative weights of some internal organs of broilers. In the same vein, the above reports are consistent with those of Cabuk *et al*. (2005) and Demir *et al*. (2008) which showed that herbal powders and essential oils did not affect the relative weights of internal organs.

Nutrient deficiencies can lower an animalôs ability to withstand pathogenic invasion, for instance, when arginine levels are low in the diet, poor development of organs, results, including lymphoid organs (Kwak *et al*., 1999). The bursa of Fabricius, thymus and spleen are three major lymphoid organs that form part of the avian immune system (Yegani and Korver, 2008). Hence, their proper development is critical to the optimal functioning of the immune system. It has been shown that dietary factors such as Se and vitamin E, as well as aflatoxins, and antibiotics influences the lymphoid organ weights of poultry species leading to an adjustment of the immune responses to the desired level (Hamilton *et al*., 1972; Zyla *et al*., 2000; Huff *et al*., 2004). The well-known trace metals that play a role in immunity function are Zn, Se, Mn, and Cu (Kidd, 2004). With an increase in stress, heat stress, stocking densities or other forms of general stress, the chicken's lymphoid

organs tend to decrease in weight. Measuring the lymphoid organ weights is an accurate method of assessing the immunity of the chicken (Pope, 1991) with the weight of the bursa being the most accurate representation (Heckert *et al*., 2002). Decrease in bursa weight can be due to viral infections or stress (Pope, 1991). Nevertheless, an excessive or inappropriate immune response will unnecessarily depress performance (Collet *et al*., 2005). In the present study, neither treatments nor nutrient composition had any effect on the weights of the spleen, bursa or spleen: bursa ratio (Table 4.12).

4.9.6 GIZZARD EROSION SCORES

As shown in Table 4.13, no significant ($p > 0.05$) differences existed among treatments in gizzard erosion scores. Gizzard erosion involves the presence of lesions or extensive sloughing of the koilin lining of the gizzard, as well as thickening and loosening (Itakura *et al*., 1982; Fossum *et al*., 1988). This results in decreased feed intake and also reduced growth rate. Mortality can also be increased due to gizzard erosion (Tisljar *et al*., 2002). According to the International Association of Fish Meal Manufacturers (1987), occasional parcels of fish meals (gizzerosine) have been shown to cause erosion of the gizzard lining in broiler chickens, especially when fed at high levels in the broiler diet (7% or greater). However, research has shown that gizzard erosion is not uniquely associated with fish meal feeding, and can be caused by other components of the animal feed such as vitamin K, mould growth, etc. It can also be caused by other factors such as mycotoxins (Hoerr *et al*., 1982; Dorner *et al*., 1983), dietary copper sulphate levels (Fihser *et al*., 1973), stress (Dzaja *et al*, 1996) histamine and histamine antagonists such as gizzerosine. Gizzerosine is formed by the reaction of histamine or histidine during overheating of fish meal (Tao *et al*., 2012). Incidences of severe gizzard erosion were low in the present study, indicating that none of the diets contained mycotoxins or histamine antagonist which might have affected production negatively.

4.9.7 DIGESTIVE ORGAN PH

As shown in Table 4.14, treatment had no effect ($p > 0.05$) on the pH of the various digestive organs. In the same vein, Hong *et al*. (2012) reported that pH of different intestinal parts (duodenum, jejunum and ileum) did not differ significantly among treatments. The health of the chicken, the kind of nutrients in the digesta and the gut microbial population has significant impact on intestinal pH. The pH in specific areas of the GIT affects nutrient digestion and absorption (Rahmani *et al*., 2005). Dietary inclusion of phytogenic feed additive in broiler diets has been reputed for lowering sub-clinical immune stress experienced by animals. This is achieved through

an improved balance of gut microflora. Thus, the animal is afforded the opportunity to attain its full genetic potential by expending useful energy on growth related activities rather than on disease resistance (Wenk, 2000; Hashemi and Davoodi, 2011). A reduced microbial population also enhances nutrient availability, absorption and assimilation in the gastrointestinal tract, and invariably reduces the production of growth depressing toxins by disease causing microbes ((Windisch *et al*., 2008; Hahsemi and Davoodi, 2010).

The values of intestinal pH observed in the different treatments in the present trial were mostly acidic, with only those of the ileum and caecum falling within the generally accepted pH of the small intestine ranging from 6.5-7.7 as stated by Simon and Igbasan (2002). A reduction in intestinal pH is desirable and may improve nutrient absorption (Engberg *et al*., 2002; Rahmani *et al*., 2005). It has been reported that phytogenic additives are veritable alternatives to AGPs not only because they promote higher nutrient digestibility, and increase enzyme activity and gastric and pancreatic juice secretion, but also because they protect the intestinal microvilla and improve bird performance by antimicrobial activity (Hernandez *et al*., 2004).

The intestinal mucosa is a unique environment providing an interface where the often geno-toxic contents of the intestinal lumen come in contact with an epithelial population, characterized by a rapid turnover rate. Intestinal immunity is crucial to the maintenance of gut function (Churchill *et al*., 2000). The digestive, absorptive and immunological function of the intestine makes it an essential organ in maintaining animal health. The gut microflora plays a major protective function in maintaining the integrity of the intestinal mucosal. Impairing this integrity i.e. intestinal barrier dysfunction, can lead to progressive increase of mucosal permeability which facilitates pathogens infection (Lambert, 2009). As a bird gets older, its gut microflora changes, and the gut mucosal system becomes more susceptible to loss of integrity (Burel and Valat, 2009). Losing integrity encourages pathogenic bacteria at the expense of beneficial bacteria.

4.9.8 HAEMATOLOGY PARAMETERS

Treatments only affected ($p < 0.05$) the leucocytes differentials examined, namely the lymphocytes, monocytes and basophils among the various treatment means. There were however no significant ($p > 0.05$) differences between treatments in the values obtained for other blood parameters such as WBC, PCV, MCV, MCH, MCHC, RDW and platelet count (Table 4.15).

The lymphocytes values across the treatments were within the normal range of 45-75% reported by Glystorff (1983), and the 62% reported by Ghergariu *et al*. (2000). The normal values

for monocytes in broilers are usually 4%, as reported by Ghergariu *et al*. (2000). However, the values obtained in the present trial were all higher than it. The values obtained for monocytes were also higher than the normal values of 1.10-6.5% reported by Uray (1992), except for those of treatment 3 birds that were within this range. The basophils were all within the range of 1-17% reported by Hoffman (1961), but were higher than the 2% stated by Ghergariu *et al*. (2000) except for birds fed treatment 3, whose basophils were within this range. The differences in differential leucocyte counts (lymphocytes, monocytes and basophils) among the different treatment groups show that the birds were challenged with pathogenic agents which they had to contend with (particularly birds fed treatment 3). More so, the birds were not vaccinated during the experimental period, and that could have contributed to the exposure. Usually, to vaccinate the birds would require obtaining an ethical clearance, and a qualified veterinarian, both of which were not possible to obtain before the commencement of the trial.

The results of this study contradict the findings of Kung-chi *et al*. (2006) which indicated that intake of high dosage of garlic oil significantly increased white blood cells, and reduced red blood cell counts, haemoglobin, haematocrit, and mean corpuscular haemoglobin in rats. It also differ from the reports of Li *et al*. (2002) which indicated a higher count of red blood cell and haemoglobin concentration in chicks fed garlic-supplemented diets. The authors attributed the higher values obtained to imply that garlic may possess constituents that trigger the erythropoietic system to produce red blood cells. These constituents would play a role in the immune system stimulation and in the function of organs related to red blood cell formation such as thymus, spleen and bone marrow.

According to Ammen *et al*. (2007), Immune status is a function of leucocytes, neutrophils and lymphocytes. Lymphocytes are known to play key roles in immune defense system of both man and animals. When WBC neutrophils and lymphocytes fall within the normal range, it indicates the feeding patterns did not affect the immune system (Minka and Ayo, 2007). The use of blood analysis (Olabanji *et al*., 2009) and organ weights (Ewuola, 2009) is regarded as a readily available and fast means of assessing clinical and nutritional status of animals on a feeding trial. According to Toghyani *et al*. (2011), changes in the physiological state often reflect alteration of haematological values. Therefore blood indices are a fundamental tool used to monitor the effect of therapeutic, nutritional, and environmental management in human and veterinary medicine.

Olafedehan *et al*. (2010) posited that blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions. Haematological components such as RBC, leucocytes, MCV, MCH, MCHC are valuable in monitoring feed toxicity especially with feed constituents that affect blood as well as the health status of farm animals. According to Adamu *et al*. (2006) there is significant effect of nutrition on haematological parameters such as PCV, Hb and RBC. Togun *et al*. (2007) reported that when the haematological values are within the normal range expected for a particular animal, it means that diets did not exert any adverse effect on haematological parameters during the experimental period, but when the values fall below the normal range, it is indicative of anaemia. Low levels for haematological parameters as reported by Bawala *et al*. (2007) could be due to harmful effects of high dietary contents. The non-significant differences recorded in this trial for the PCV, MCV, Hb, RBC, MCH and MCH, across the various treatments can be interpreted to mean that treatments had no adverse effect on the haematological indices of the broiler birds. However, the PCV of the birds though not significant, was below the normal range reported for poultry.

CHAPTER FIVE

5.0 SUMMARY AND CONCLUSION

5.1 SUMMARY

Results showed that inclusion of phytogenic feed additives (products 1 and 2) resulted in significant reduction ($p<0.05$) in feed intake and significantly ($p<0.05$) enhanced performance of birds at week 1 of the trial. Birds fed diets containing phytogenic feed additives product 2 had significantly higher ($p<0.05$) thigh weights. The inclusion of phytogenic feed additives (products 1 and 2) also reduced fat content of meat significantly ($p<0.05$) compared to the control groups. Treatments had no significant ($p >0.05$) effect on white blood cells (WBC), neutrophils, eosinophil, red blood cells (RBC), haemoglobin concentration (Hb), haematocrit value (HCT), mean corpuscular value (MCV), mean heamoglobin count (MCH), mean cell haemaglobin concentration (MCHC), red blood cell distribution width (RDW) and blood platelets. Leucocyte differential values such as lymphocytes, monocytes and basophils were significantly ($p<0.05$) affected by treatments.

CONCLUSION

It is evident from the results obtained in the study that the inclusion of phytogenic feed additives (products 1 and 2) in the diets of broilers improved feed efficiency in week 1 and reduced the fat content of broiler meat.

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