# EVALUATION OF CHESTNUT (*Castenea sativa)* AND ZINC SULPHATE AS EUBIOTICS ON THE PERFORMANCE OF BROILER CHICKENS

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**MARCH, 2021**

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**B. Agric. (U.N.N) 2013**
**P16AGAN8016**

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

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## DECLARATION

I declare that the work in this dissertation entitled “Evaluation of Chestnut (*Castenea sativa*) and Zinc Sulphate as Eubiotics on the Performance of Broiler Chickens” was carried out by me in the Department of Animal Science, Faculty of Agriculture Ahmadu Bello University Zaria under the supervision of Prof. P.A Onimisi and Dr. (Mrs.) M. Afolayan. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another Degree or Diploma at this or any other Institution.

| | | |
|---|---|---|
| DIALOKE, Nnamdi Godswill | _____ | _____ |
| Name of student | Signature | Date |

## CERTIFICATION

This dissertation entitled "Evaluation of Chestnut (*Castenea sativa*) and Zinc Sulphate as Eubiotics on the Performance of Broiler Chickens" by Nnamdi Godswill DIALOKE meets the regulation governing the award of the degree of Masters of Science (Animal Science), of the Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

| | | |
|---|---|---|
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## DEDICATION

This dissertation is dedicated to God Almighty who has been seeing me through right from the day I was born till this stage and also to my parents, Mr. and Mrs. Innocent Dialoke for their continuous prayers, sacrifices, moral and financial supports.

## ACKNOWLEDGEMENT

My profound gratitude goes to God Almighty for the favour, courage, protection, peace of mind given to me during the course of this study even when friends adviced I should not come to the North.To my Head of Department Prof. M. Kabir, and all other lecturers in the Department of Animal Science, I want to say thank you for the immeasurable knowledge and wisdom you impacted on me. I will forever remain grateful to you all.

I express great appreciation to my supervisor, Prof. P. A. Onimisi, for his love, care, encouragement and fatherly advice despite all the stress and tight schedule he made out time to go through my work and never gave up on me and in his own way encouraged me to carryon to the end. My immense regards goes to my second supervisor, a virtuous woman, Dr. (Mrs) M. Afolayan, thank you for your love, care, financial support, encouragement and for creating out time to supervise the experiment, and to edit and develop this work. I pray that the Lord will richly reward both of you in double folds according to His riches in glory. Amen.

My sincere gratitude goes to my loving and ever caring parents, Mr. and Mrs. Innocent Dialoke, my relations Prof. S.A. Dialoke, Mr. John Dialoke, Engr. Nnabuchi Ogbobe, Mrs. Caroline Ogbobe, Mr. Basil Otti, who were there for me and above all their prayers and financial support which brought me thus far. I am sincerely indebted to you all. To my ever loving siblings Promise Dialoke, Favour Dialoke, Chidiebere Dialoke, Uche Dialoke, Chikamso Dialoke; I want to say thank you for your love and care and also believing in your brother. I wish to express my sincere gratitude to Dr. Emeka Okechukwu, and Dr. Chika Oyeagu, Miss. Edna Obianwuna and Mrs. Agbai Kalu Nnenna for their assistance, encouragement, understanding and support. To my course mates, Mr. KperunThaddeus, Mr. Kayode Oluwande, Miss. Eunice Moses and Davina Oluwa to mention but a few, thanks for your care and good company.

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## ABSTRACT

Two Experiments were conducted to evaluate the performance of broiler chickens fed diets supplemented with graded levels of Chestnut (*Castenea sativa*) Phytobiotics and Zinc Sulphate respectively, as eubiotics to replace antibiotic growth promoters (AGPs). In each feeding trial, 300-day-old cobb 500 broiler chicks were allotted in a completely randomized design to five dietary treatments each replicated thrice, with 20 chicks per replicate. In Experiment 1, Chestnut Phytobiotics was included at 0g, 100g, 125g and 150g/100 Kg diet while Oxytetracycline was included at 111g/100kg diet. In Experiment 2, Zinc Sulphate was included at 0mg, 10mg, 20mg and 30mg/100 Kg diet while Oxytetracycline was included at 111g/100kg diet. Data was collected on growth performance, haematological parameters, serum biochemical parameters, lipid profiles, nutrient digestibility, villi morphometry, intestinal microbial composition, carcass evaluation and economic indices. In Experiment 1, results for the starter phase showed that birds placed on 100g phytobiotics diet had significantly ($P<0.05$) high final weight and weight gain than other supplemented diets and the control. Weight gain was significantly ($P<0.05$) higher in the oxytetracycline based diet (1783g) for finisher phase however, there was an improvement in values for birds placed on phytobiotics. Haematological indices showed that values for white blood cell ($91.90x10^3/\mu L$) and Heterophils (20.63%) were significantly ($P<0.05$) higher in diets containing oxytetracycline than other treatment groups. Birds fed diets containing 100g phytobiotics were significantly ($P<0.05$) higher in values for red blood cell, monocytes, eosinophils and basophils. Chestnut phytobiotics reduced the total cholesterol values from 151.13 - 96.55mg/dL when compared to other treatment groups. Apparent nutrient digestibility showed higher significant ($P<0.05$) difference in crude protein for birds fed diets with 100g phytobiotics (76.15%) when compared to other treatment groups. Villi morphometry showed significant ($P<0.05$) differences in all the parameters measured except for crypt depth. There were significant ($P<0.05$) differences for values of *Lactobacillus spp* and *Bacillus spp*, whereas non-significant ($P>0.05$) differences were observed for *Escherichia coli, Clostridium spp and Salmonella spp*. Intestinal bacteria count revealed that *Lactobacillus spp,* a beneficial bacteria was significantly higher and best in diet containing 100g phytobiotics ($15.33x10^3$cfu/g) when compared to that of the control (5.00x103cfu/g) but similar to that fed diets with oxytetracycline ($10.67x10^3$cfu/g). The mean yield cost decreased as the levels of phytobiotics increased. In experiment 2, results for the starter phase showed significant ($P<0.05$) differences in all the

growth parameters measured except feed intake and mortality. Birds supplemented on diets with 10mg of zinc were significantly (P<0.05) higher in final weight (783.33g/bird) and weight gain (742.16g/bird). Birds which had access to 20mg ($93.33x10^3$/µL), 30mg zinc and oxytetracycline were significantly (P<0.05) higher in WBC when compared to the control group ($59.40x10^3$/µL) but statistically similar to the birds placed on 10mg ($72.97x10^3$/µL) zinc. The results for aspartate aminotransferase, alanine aninotransferase and alkaline phosphatase were significantly (P<0.05) influenced by the dietary treatment that resulted to the increase in the amount of these enzymes which were produced by the liver. Birds on the control diet had significantly (P<0.05) higher values of AST and ALT while those on diets with 30mg zinc had higher (P<0.05) value for ALP. Oxytetracycline inclusion resulted to a significant (P<0.05) increase in the total cholesterol (90.63mg/dL) whereas those on diets with 20mg zinc had the least (74.61mg/dL). Triglyceride was significantly (P<0.05) different across the treatment groups, with birds on diets with 30mg zinc (95.49g/dL) having a higher value while those on diets with 20mg zinc had the least (24.49g/dL). There were significant (P<0.05) differences in crude protein, digestibility diet with group fed diet 10mg (76.47%), 20mg (78.95%), 30mg (80.66%) and oxytetracycline (76.87%) level having higher values compared to that of the control (64.18%). Zinc and oxytetracycline inclusion greatly improved soluble carbohydrate digestibility and utilization.Villi area (29919µm$^2$), width (164.36µm), height/crypt depth ratio (5.03µm) were higher in birds fed control diet than other treatment groups except for oxytetracycline which was similar in villi height/crypt depth ratio (5.20µm). The value for villi width and height/crypt depth ratio decreased with an increase in the levels of zinc. *Lactobacilli spp* was significantly (P<0.05) higher and best in birds placed on control diet ($15.00x10^3$cfu/g), followed by 20mg zinc ($9.67x10^3$cfu/g) dietary level. *Salmonellaspp* was significantly (P<0.05) lower in birds which had access to 20mg zinc ($0.00x10^3$cfu/g) but statistically similar with those on the control diet ($1.67x10^3$cfu/g) and 30mg zinc ($2.67x10^3$cfu/g). Birds placed on diets with 10mg zinc and oxytetracycline were significantly higher in live weight, dressed weight and dressing percent. Feed cost/Kg (119.95₦/Kg) was the same across the treatment groups except for oxytetracycline (124.35₦/Kg) which had a higher value.It is concluded that chestnut phytobiotics though did not significantly improve feed intake above the antibiotic growth promoters for starters but however, improved the weight gain, feed conversion ratio and feed cost/Kg gain for both starter and

finisher chickens. Zinc did not significantly improve villi height/crypt depth ratio of broiler chickens, but significantly lowered mortality, feed conversion ratio and feed cost per kg gain.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Antibiotics

The use of antibiotics in poultry feed at sub-therapeutic level as an antimicrobial growth promoters (AGP) has been beneficial to growth performance and has reduced the populations in the gastrointestinal tract of potentially pathogenic organisms such as *Clostridium perfringens, Salmonella* and *Escherichia coli* and thus diseases associated with these pathogenic bacteria (Hume, 2011). Antibiotics are given in a small dosage for growth promoting and have positive effects especially in young growing animals. Using antibiotics as growth promoters causes weight increase, better feed conversion and low cost of therapy. Besides positive effects, it is possible for certain negative effects such as production of resistant strains of enterobacteria to occur. However, concerns of consumers over antibiotic-resistant bacteria and drug residues in poultry meat in recent years have generated controversial views concerning use of antibiotics.

The European Union has banned the use of antibiotics as growth promotants in animal feed since 2006. In the United States, the Food and Drug Administration initiated a voluntary plan to remove antibiotics in feed as growth promotantsat the end of 2013 (USDA, 2003). Now the poultry industry is looking for alternative products, such as prebiotics, probiotics, essential oils, organic acids, enzymes, minerals (Zn and Cu compounds), herbs and spices etc. with claim to affect the composition or activity of intestinal microbiota. The above-mentioned alternative substances are referred to as natural growth promoters (Panda *et al*., 2006) as most of them are of natural origin.

In recent years, some of those products have been described by the general term ‘eubiotics’, which is related to the Greek term ‘eubiosis’, referring to an optimal balance of microflora in the gastrointestinal tract. The main purpose of using such eubiotics is to maintain the intestinal eubiosis, which will result in an improved health status and performance in farm animals. The combination of eubiotics in one product has been shown to confer benefits beyond those of either on its own (Gallaher and Khil, 1999). Formulation of diets focused on specific effects on gut health is becoming a reality in the monogastric animal industries because the maintenance or enhancement of gut health is essential for the welfare and productivity of animals when antibiotics are not allowed in feed.

Among the slated alternatives, phytobiotics have drawn a lot of attention because of being natural, nontoxic and residue free. Phytogenic feed additives (phytobiotics or botanicals) are commonly defined as plant-derived compounds incorporated in to diets to improve the livestock productivity through amelioration of feed properties, improvement of nutrient digestibility, absorption and elimination of pathogens in the gut (Abdel-Azeem, 2005; Abou-Bakr, 2011). Phytogenic (phytobiotics) feed additives (PFA) include herbs, which are non-woody flowering plants known to have medicinal properties; spices, which are herbs with intensive scent or taste, commonly added to human food; essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits, and roots; and oleoresins, which are extracts derived by non-aqueous solvents from plant material (Windisch, *et al.*, 2008). However, the use of phytobiotics feed additives like plant extracts, hydrolysable tannin from Chestnut (*Castenea sativa)*and zinc compounds like zinc sulphate have potentials which can be harnessed in a study like this especially for gut health in broiler chickens.

Various plant extracts (essential oil) have been studied for their antimicrobial abilities. Due to their antibacterial activity they may be able to modify the composition of intestinal microbiota and to exert beneficial effects on performance of poultry (Mitsch *et al*., 2004; Hume, 2011; Broz and Paulus, 2015). Essential oil contains natural polyphenolic compounds or flavonoid as major active ingredients which have been identified as potential antimicrobial agents (Cruickshank 2001; Friedman *et al.* 2004). Thus, supplementation of broiler diets with essential oil can create a healthier gut microflora, aiding optimum digestion and improving bird performance (Cruickshank 2001).

Tannins can be classified into condensed and hydrolysable tannins (Scalbert, 1991; Haslam, 1996). Hydrolyzable tannins in particular are based on gallic acid, usually as multiple esters with D-glucose. Current scientific evidence suggests that there is significant potential in the use of tannins to enhance nutrition and animal health (Frutos *et al.*, 2004).

The protection of the gut environment is now known to play an important role in reducing disease in animals (Vidanarachchi *et al.*, 2005). Zinc's importance as an essential nutrient (trace mineral) has been recognized for many years, only recently have researchers understood the full impact of zinc as eubiotics on animal and human health. Zinc also appears to be directly involved in immune cellular functions and zinc deficiencies might also have indirect consequences on the immune system by failure to limit bacterial infections (Park *et al*., 2004). Zinc is critical and also required for the maintenance of immune functions, enzyme structure and function, and appetite regulation in all avian species (Tomaszewska *et al.*, 2016). Dietary zinc is relatively nontoxic to animals and humans; both exhibit considerable tolerance to high intakes of zinc (Fosmire, 1990). The role of additional supply of zinc has been studied and associated with various health parameters in animal studies, mainly related to immunity parameters (Park *et al*.,

2004). Inadequate intracellular concentration of zinc also causes damage to the lymphocyte function that is responsible for the ability of T- and B-cell proliferation (Vruwink *et al.,* 1993). The effectiveness of zinc inhibition of bacterial growth results from changing the active transport system and impeding the initial phase of bacterial mating (Sobocinski *et al.,* 1977). Immune parameters under this research was examined to confirm this hypothesis.

In this scenario raw plant extracts and derived tannins are showing promising results for food animal production (Huyghebaert *et al.,* 2011). Miadiasan® feed additive is a blend of two products comprising phytobiotics (plant extract, hydrolysable tannin) from Chestnut (*Castenea sativa)*which may help to maintain chickens intestine, health and improve immunity.

## 1.2 Justification

During the last 2 to 3 decades, a substantial growth in poultry industry has been observed, largely secluded to large and small scale organized poultry farming. This is mainly due to exploitation of various modern growth promoting strategies and appropriate disease preventive and control measures. Many antibiotics are used in poultry feeds as growth promoters for improving health of animals although this has led to proliferation of resistant bacteria in humans via the food chain. The use of feed additives like plant extracts, hydrolysable tannin, probiotics, prebiotics, synbiotics, organic acids, vitamins and minerals (Zn and Cu compounds), herbs show similar effects as antibiotics, but without residual effect. Plant extract, hydrolysable tannin and zinc compound like zinc sulphate are antimicrobial in nature which are needed to improve intestinal integrity which is a precondition for gut health, and provide the possibility to reduce the use of antibiotic treatments. Therefore, this study was designed to investigate the efficacy of chestnut extracts (*Castenea sativa*) and zinc sulphate as eubiotics and a replacement for antibiotics in broiler chickens.

## 1.3 Aims and objectives

The aim of the study was to evaluate the effect of Chestnut (*Castenea sativa)* phytobiotics and zinc sulphate as eubiotics in broiler chickens. The specific objectives are:

1. Evaluation of the optimum level of Chestnut *(Castenea sativa)* phytobiotics in broiler chickens diets and the effect on growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota and economic indices in Zaria Nigeria.

2. Evaluation of the optimum level of zinc sulphate in broiler chickens diets and the effect on growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota, carcass characteristics and economic indices in Zaria Nigeria.

## 1.4 Research Hypotheses

**Experiment 1**

**Null hypothesis ($H_0$):**There are no significant differences in the growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota and economic indices of broiler chickens fed diets containing graded levels of Chestnut (*Castenea sativa)* phytobiotics as eubiotics.

**Alternate hypothesis ($H_A$):**There are significant differences in the growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota and economic indices of broiler chickens fed diets containing graded levels of Chestnut (*Castenea sativa)* phytobiotics as eubiotics.

**Experiment 2**

**Null hypothesis ($H_0$):**There are no significant differences in the growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota, carcass characteristics and economic indices of broiler chickens fed diets containing graded levels of zinc sulphate as eubiotics.

**Alternate hypothesis ($H_A$):**There are significant differences in the growth performance, haematology, serum biochemical parameters, nutrient digestibility, villi morphometry, intestinal microbiota, carcass characteristics and economic indices of broiler chickens fed diets containing graded levels of zinc sulphate as eubiotics.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Phytobiotics in Poultry Production

Phytobiotics also known as phytogenics or botanicals are commonly defined as plant-derived compounds incorporated into diets to improve the livestock productivity through amelioration of feed properties, improvement of nutrient digestibility, absorption and elimination of pathogens in the gut (Abdel-Azeem, 2005; Abou-Bakr, 2011). They can be added to the diet of commercial animals to improve their productivity through enhancing feed properties, promoting animals' production performance, and improving the quality of products derived from these animals (Windisch *et al.* 2008). Phytobiotics are well known for their pharmacological effects and are thus widely used in human traditional and alternative medicine. In human nutrition, phytobiotics play an important role as flavours and food preservatives. The action of phytobiotics is caused by primary and secondary ingredients. A huge number of in vitro and in vivo studies have confirmed a wide range of activities of phytobiotics in animal nutrition like stimulation of feed intake, antimicrobial, coccidiostatic, anthelmintic and immunostimulating (Panda *et al.,* 2006).

In commercial poultry nutrition mainly whole seeds or extracts of black cumin (*Nigella sativa*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and chilli (*Capsicum annum*) are used singly or in combination as feed additives. In addition, Windisch *et al.* (2008) have recommended some other commonly used terms to classify different phytogenic compounds based on their origin and processing, including herbs (flowering, non-woody and non-persistent plants), spices (herbs with an intensive scent or taste commonly added to human food) like cinnamom, coriandor, pepper, chilli, rosemary,

oregano, anise, thyme and garlic. Essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation) and oleoresins (extracts derived by non-aqueous solvents). Within phytogenic feed additives, the content of active principles in products may vary widely, depending on the plant part used (e.g., seeds, leaf, root or bark), harvesting season and geographical origin.

## 2.2 Properties of Phytobiotics

Plant extracts have antimicrobial action, immune enhancement, anti-stress property (Chattopadhyay *et al*., 2005), antioxidant and gut microflora manipulation (Hashemi *et al*., 2009), nutrigenomics effects (Franco-Jimenez *et al*., 2007), digestibility enhancer (Cross *et al.*, 2007), stress lowering effect (Khaksar *et al*., 2012), cholesterol-lowering effect (Lee *et al*., 2003). Plant herbs have the underlisted kinds of properties to increase the animal's health and performance (Figure 2.1).

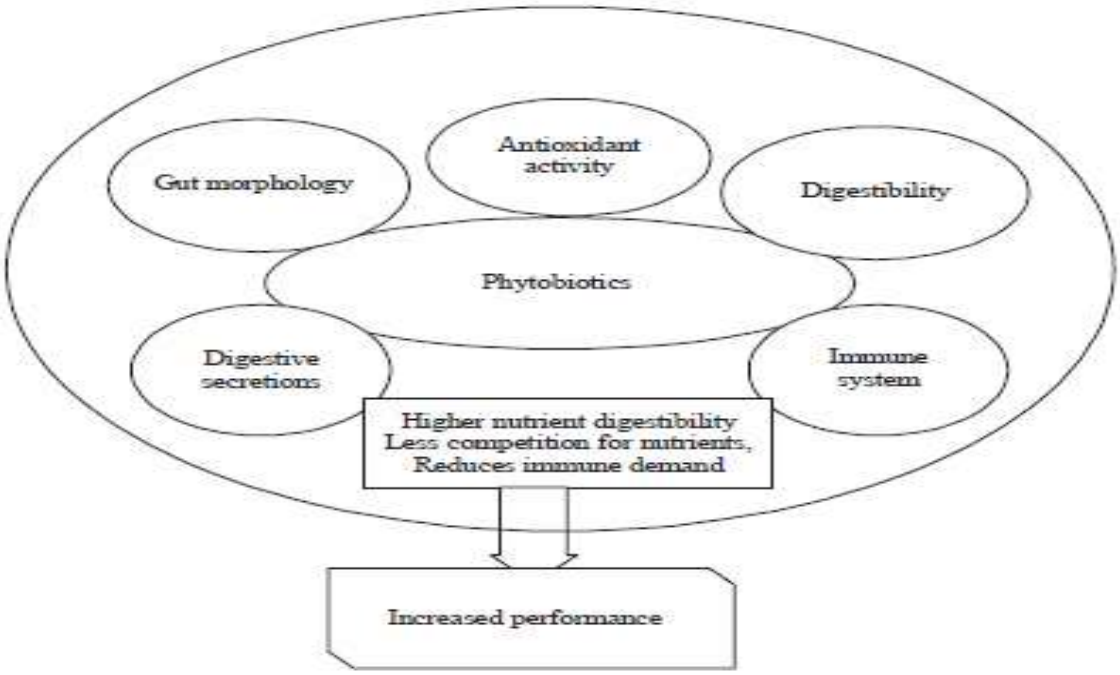


Figure 2.1: Activities of phytobiotics in poultry system (Govinthasamy*et al.*, 2016)

Phytogenic effects have been proven in poultry for feed palatability and quality (sensory aspects), growth promotion (improved weight gain and feed conversion ratio, reduced mortality), gut function and nutrient digestibility (improved growth), gut microflora (less diseases of the GIT, improved growth, reduced mortality), immune function (improved health), and carcass meat safety and quality (reduced microbial load, improved sensory)(Mountzouris *et al*., 2009).

## 2.3 Specifications of Phytobiotics

Leaves, roots, seeds, flowers and whole plants are used for production of phytobiotic products. Products may comprise the dried form of whole plants or their parts or extracts of some valuable ingredients. In general, phytobiotics are described by primary and secondary plant compounds (Figure 2.2). Primary compounds are main nutrients (e.g., content of protein, fat.), whereas, secondary compounds comprise essential (ethereal) and/or volatile oils, bitterns, hot stuffs, colourants and phenolic compounds (Wald, 2003). Therefore, secondary plant compounds are the main ingredients of interest.

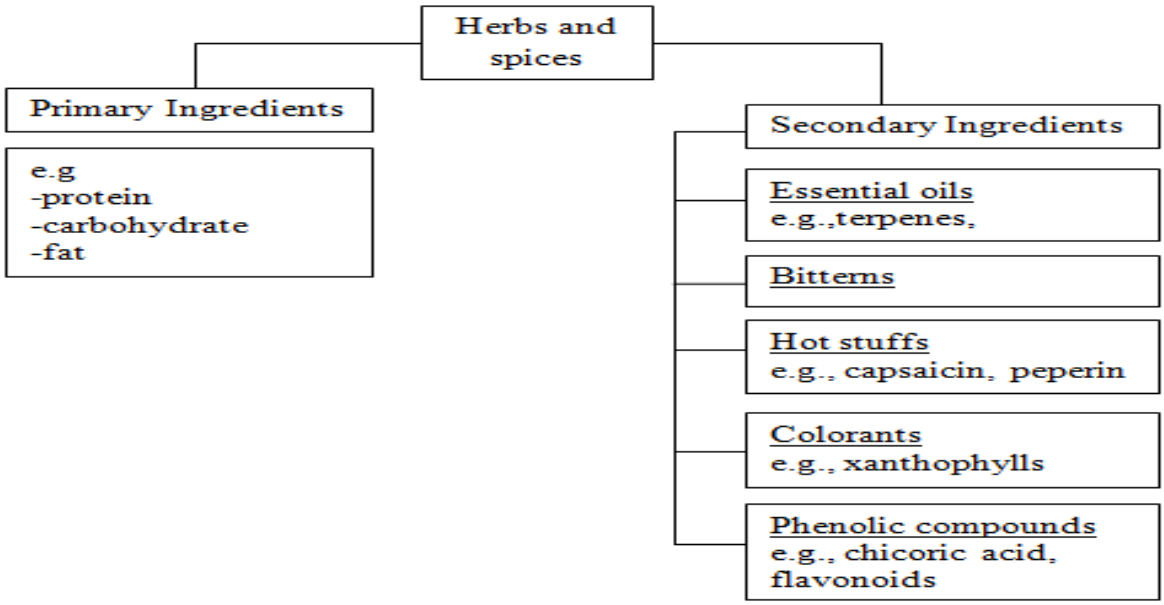


Figure 2.2: Composition and main ingredients of phytobiotics (Wald, 2003)

The main constituents of **essential oils** are lipophilic, liquid and volatile and belong to chemical groups of alcohols, aldehydes, esters, ethers, ketones, phenols and terpenes (Mathe, 2009). Essential oils as flavoursstimulate the digestive system and by this improve nutrient digestibility.

**Bitterns** are reported for thyme and sage (carnesol) and hot stuffs for chilli (capsaicin) (Heyland *et al*., 2006). It is well documented that poultry is in favour of bitter taste, whereas, high contents of hot stuffs result in feed rejection.

Natural **colourants** (xanthophylls) are included in most phytobiotics. The dominant xanthophylls are lutein, zeaxanthin, ß-carotene and lycopene. In chilli and bell pepper, capsanthin and capsorubin are responsible for the red colour. Xanthophylls are antioxidants and show partly pro-vitamin A activity. Antioxidative capacity of colourants is yet not very intensively investigated in poultry as the major antioxidants in diets are selenium and vitamin E. Pro-vitamin A activity is normally of minor interest as commercial diets are supplemented with sufficient amounts of vitamin A. Due to the normally low supplementation level of phytobiotics in broiler diets the colouring effect is less pronounced.

Various **phenolic compounds** are included in phytobiotics with flavonoids and chicoric acid being the dominating ones (Panda *et al*., 2006; Nasir and Grashorn, 2010). (Table 2.1 and 2.2)

## 2.4 Safety Issues of Phytobiotics

Phytobiotics contain many pharmacologically active components which playa major role in the defence system of the plant. From this point of view safetyconcerns cannot be excluded, although, phytobiotics are categorized as“generally recognized as safe” by the United States Food and Drug Administration (Wald, 2003; Mathe, 2009). In several experiments it was observed that bioactive ingredients or their metabolites may be transferred to tissues (Windisch

Table 2.1. Main volatile constituents of rosemary, sage, thyme, oregano and purple coneflower

| Plant | Source | Constituent | Amount, % |
|---|---|---|---|
| Rosemary | leaves | a-pinene | 2-25 |
| | | bornyl acetate | 0-17 |
| | | camfor | 2-14 |
| | | 1,8-cineole | 3-89 |
| Sage | leaves | camfor | 6-15 |
| | | a-pinene | 4-5 |
| | | ß-pinene | 2-10 |
| | | 1,8-cineole | 6-14 |
| | | a-tujone | 20-42 |
| Thyme | leaves | thymol | 10-64 |
| | | carvacrol | 2-11 |
| | | γ-terpentine | 2-31 |
| | | p-cymene | 10-56 |
| Oregano | leaves | carvacrol | <1-80 |
| | | thymol | <1-64 |
| | | γ-terpentine | 2-52 |
| | | p-cymene | <1-52 |
| Purple coneflower | leaves, roots | chicoric acid | 27 |

(Burt, 2004**,** Mountzouris *et al*., 2009; Heyland *et al*., 2006; Nasir and Grashorn, 2010)

Table 2.2. Most commonly used phytobiotics in traditional animal health care and livestock production

| Latin name | Common name | Parts/products used |
|---|---|---|
| Achillea millefolium | Yarrow | Infusion |
| Arnica montana | Arnica | Extract |
| Boswellia sacra | Frankincense | Resin |
| Carum carvi | Caraway | Seed, essential oil |
| Citrus sp. | Citrus oil | Essential oil |
| Curcuma longa | Curcuma | Rhizome |
| Foeniculum vulgare | Fennel | Seed |
| Matricaria recutita | Camomile | Infusion, essential oil |
| Mentha sp. | Mint | Infusion, essential oil |
| Pimpinella anisum | Aniseed | Seed, essential oil |
| Pinus sp. | Turpentine | Essential oil,(oleo) resin |
| Salvia officinalis | Sage | Infusion, essential oil |
| Syzygium aromaticum | Cloves | Buds, essential oil |
| Zingiber officinale | Ginger | Rhizome |

(Franz *et al.*, 2010).

*et al*., 2009). Some, concerns are reported for capsaicin (cancer causing), cyanide containing ingredients, carvacrol (in oregano) and glycosides. Little information is available on metabolites deposited as residues to tissues resulting in impaired sensory attributes (Kluth *et al.*, 2003). In

general, for probable negative effects the dose is important. High inclusion levels of oregano may affect the minimum inhibitory concentration (MIC) for antibiotics (Mountzouris *et al*., 2009). For rats, the LD50 value was reached for oregano oil for a dose 100 times the normal supplementation level in feed (Wald, 2003). The observed results indicate the necessity to check phytobiotics thoroughly for potential safety issues.

**2.5 Uses of Chestnut**

Chestnut trees are tannin-rich, with high levels of ellagitannins (hydrolysable tannins). This property has been exploited by using the wood for wine barrels and addition of wood chips directly to the wine; the leaching of these tannins from the wood provides the characteristic organoleptic properties in matured wines and brandies (Canas *et al.,* 1999; Simon *et al*., 2009). The bark and wood of chestnut trees are the prime sources of tannins for leather processing, transformation of hides and skins into leather and as retanning agents (Korel and Balaban, 2009).

The chestnut shell resulting from the fruit peeling process is used in some countries as fuel (Spain) and is also a rich source of phenolics and antioxidants (Hwang *et al*., 2001;Calliste *et al.*, 2005; Barreira *et al.,* 2008; Vazquez *et al.,* 2009; Zivkovic *et al.,* 2009). These antioxidants and other components present in shells, wood and leaves are used in animal feeds and could also be used as additives in human foods, allowing the recycling and decrease of vegetable waste together with a more environmentally friendly food production (Laufenberg *et al.,* 2003; Calliste *et al.,* 2005; Barreira *et al.,*2008; Vazquez *et al.,* 2009).

Previous studies showed that the addition of hydrolysable tannins extracted from *Castaneasativa* wood to chicken (0.20%) and rabbit (0.45%) diets may improve their health and even increase

their life spans. These tannins act by preventing or eliminating the gut colonization by parasites (Schiavone *et al.,* 2008; Zoccarato *et al.,* 2008).

### 2.5.1Chestnut Phytobioticsas Alternative to Antibiotics

Chestnut content of phenolics (gallic and ellagic acid), which have been linked to various positive effects on human health such as antioxidant activity, a decrease in the risk of cardiovascular diseases, anticancer mechanisms, and anti-inflammatory properties (De-Vasconcelos *et al*., 2010). Tannins also can be used in combination with other antibiotic growth promoter alternatives, as probiotics, showing a synergist effect in the promotion of gut health**.** Chestnut extracts has been reported to exhibite a surprising effect in improving the tolerance to gastric transit of *Lactobacilli*, while chestnut fibre mainly improved the tolerance to bile juice (Blaiotta *et al*., 2013).

Among the various plant extracts, tannins represent an important group often reported to have effects similar to antibiotics. Tannins belong to a group of secondary metabolites that contribute to the mechanism of plant defense against herbivores. The tannins can be divided into 4 groups based on their chemical structure, namely:

a. Condensing tannins, or proanthocyanidins from green tea, quebracho, grape, mimosa

b. Hydrolyzable tannins (ellagitannins) from chestnut

c. Phlorotannins from brown algae

d. Complex tannins associated with metals or proteins.

The tannins extracted from hard-woods, such as oak and chestnut, are particularly rich in hydrolyzable tannins. These tannins exhibit antiviral, antibacterial, and antioxidant activities in

vitro (Almeida *et al*., 2008; Dinis *et al*., 2012) and in gastrointestinal epithelium cells (Fernandes *et al*., 2009; Ma *et al*., 2011), in addition to antitumor activity (Arapitsas, 2012). Chemically hydrolyzable tannins have a low molecular weight, and primarily consist of gallic acid as the basic unit, which forms the gallotannin group. Gallic acid is associated with ellagic acid, which is the basic unit of ellagitannins (Mueller-Harvey, 2006; Arapitsas, 2012). In addition, castalin, vescalin, castalagin, and vescalagin (among others) are present in the tannin extracts of chestnut and oak wood (Vivas *et al*., 2004). Tannins have major potential as a feed additive for livestock. The use of plant extracts that have high antioxidant potential, along with selective antimicrobial and antiviral activities, is highly desirable among animal breeders and producers of healthful food. However, the lack of knowledge about the role and impact of individual tannin in animals hinders their use in modern livestock production.

### 2.5.2 Perspectives in the Use of Tannins as Alternative to Antibiotic

Tannin is a water-soluble polyphenolic compound and plant secondary metabolites that serve as a part of plant chemical defence system against invasion by pathogens and attack by insects. Tannins are widely distributed in plant kingdom, especially abundant in nutritionally important forages, shrubs, cereals and medicinal herbs (Salunkhe *et al*., 1982; Wang *et al*., 1999). They are found in many fruit species such as banana, blackberry, apple and grape as well as tea (Nonaka *et al*., 1984; Bravo *et al*., 1992; Mertz *et al*., 2007; Mercurio and Smith, 2008; Kheng, 2010).

Tannins have shown numerous biological activities and some of them, are very important to the modern feed animal production. The amounts of polyphenols in plant tissues can vary from 0-8 to 24 g/kg (Longstaff and McNab, 1991a, b; Helsper *et al*., 1996).

Chestnut (*Castanea sativa*) is particularly rich in esters of gallic acid. The molecular weight of different tannins can vary between 500 and 3000 Da. The content of vescalagin and castalagin in hydrolysable tannin may reach about 40 and 25%, these substances can be degraded in the digestive tract of animals to ellagic acid, then to castalin and vescalin and finally to the gallic acid and glucose or other monosaccharides (Hagerman *et al*., 1998; Karasov *et al*., 1992). In contrast, condensed tannins containing aromatic bonds, derivative of flavons, have no ester bonds and thus cannot be broken down in the intestine (Laurena *et al*., 1984). The large molecular size prevents direct absorption of tannins from digesta (Blytt *et al*., 1988; Bickley, 1991; Huang, 1992).

Hydrolysable tannins can interact with proteins of saliva and feeds, with sugars (Yan and Bennick, 1995; Naurato *et al.,* 1999), metals and other macromolecular substances. The bonds of hydrolysable tannins with proteins are less stable in low or high pH, 3-0<pH>8-5, in comparison to the condensed tannins. The binding of endogenous enzymes with tannins result in reduced digestibility of proteins (Asquith and Butler, 1986; Asquith *et al*., 1987; Karasov *et al.,* 1992; Charlton *et al*., 1996).

In the digestive tract, the tannins may form a thin film of insoluble, denatured proteins covering the surface of mucous membrane of intestinal walls (Sell *et al*., 1985; Ahmed *et al*., 1991). The thickness of the layer depends on the concentration of tannin in the diet (Zhao *et al*., 2001; Yoshimura *et al*., 2007). This phenomenon can explain both protective and inhibitory functions of tannins as a factor against colonisation of intestinal mucosa with pathogens and harmful bacteria. Controlled amounts of tannins can support the activity of antioxidant enzymes (Mitjavila *et al*., 1977) and can control the obstructive effect of microflora proliferation (Scalbert, 1991), due to their ability to create the layer of insoluble tannin-protein bounds on the

surface of bacterial cell membranes. In this way, the tannins may reduce microbial activity and toxins (Fuller *et al*., 1967; Huang, 1992; King *et al*., 2000; Kim and Miller, 2005).

Higher doses of tannins may prevent some digestive disorders and leakage of liquids from body tissues into intestine lumen and prevent diarrhoea (Chung *et al*., 1998). Tannins can negatively affect the protein digestibility in monogastric animals (Garcia *et al*., 2004), although the slow degradation of feed protein coupled with tannins had a beneficial effect in ruminants (Schragle and Muller, 1990; Bento *et al*., 2005).

In contrast to the beneficial effects of dietary tannins administered in controlled doses, the anti-nutritive effects of these substances present in different raw feedstuffs on feed consumption and utilisation, on growth of animals, activity of digestive enzymes, digestibility of carbohydrates, proteins, lipids, absorption of mineral substances have also been reported in numerous publications (Choct and Annison, 1992; Jansman *et al*., 1994; Mansoori and Acamovic, 1998, Garcia *et al*., 2004; Clauss *et al.,* 2007). Higher dietary tannin increases the secretion of salivary and intestinal muco-proteins as well as hepatic bile acids (Jamroz *et al*., 2009). There may be hypersecretion of endogenous enzymes, which can lead to endogenous losses of amino acids and minerals in monogastrics, especially in poultry. The condensed tannins may reduce amino acid digestibility, particularly the sulphur amino acids in poultry and proline and glycine in swine (Ortiz *et al*., 1993; Yu *et al*., 1996; Mansoori and Acamovic, 2007).

### 2.6 Use of Different Tannins in Monogastric Animals

Unlike for ruminants, tannins have traditionally been considered as ‘anti-nutritional’ factors in monogastric nutrition with negative effects on feed intake, nutrient digestibility and production performance (Butler, 1992; Redondo *et al.,* 2014). Therefore, it is almost a common practice in

feeding industry to minimize the use of tannin-containing feed in swine and poultry diets or to take measures to reduce their dietary concentrations if such feed are used. However, several recent reports showed that low concentrations of several tannin sources improved health status, nutrition and animal performance in monogastric farm animals (Schiavone *et al.,* 2008; Zotte and Cossu, 2009; Biagia *et al.,* 2010; Starcevic *et al.,* 2015). The mechanisms of growth promoting effects of tannins in monogastric animals are much less understood compared with those in ruminants. Although some reports indicated that low concentrations of tannins increased feed intake and thus increased performance of monogastric animals, given the astringent nature of tannins, it seems not justified that this is through improving the palatability of feed. Information available to date seems to suggest that the growth promoting action of tannins in monogastric animal relies on the balance between their negative effects on feed palatability and nutrient digestion through protein and enzyme complexation and positive effects on promoting the health status of intestinal ecosystem through their anti-microbial, anti-oxidant and anti-inflammatory activities. The final impact of tannins on animal performance depends on the type of animals and their physiological status, feed, type of tannins and their concentrations in the diets. Compared with other domestic animals, pigs seem to be relatively resistant to tannins in the diets, and they are able to consume relatively high quantities of tannin-rich feedstuffs without presenting any toxic symptoms (Pinna *et al.,* 2007). This is likely due to parotid gland hypertrophy and secretion in the saliva of proline-rich proteins that bind and neutralize the toxic effects of tannins (Cappai *et al.,*2014). Compared to the vast sources of tannins for ruminants, sources of tannins used for monogastric animals are rather limited and so far only few have been studied and showed potential as feed additive.

### 2.6.1 Chestnut Tannins (Hydrolysable Tannin-HT)

Hydrolyzable tannins from chestnut (*Castanea sativa*) have been assessed as feed additive for monogastric food producing animals. Schiavone *et al.* (2008) evaluated the effects of adding 0.15%, 0.20% and 0.25% of chestnut tannin product (77.80 % HT) on growth performances of broiler chicks. The results showed that inclusion of up to 0.20% of chestnut tannin increased daily feed intake and average daily gain. However, increasing its concentration to 0.25% seemed to lead to negative effects as all the measured parameters were the lowest. Jamroz *et al.* (2009) assessed the effects of dietary addition of 0.025%, 0.05% and 0.10 % of sweet chestnut tannins on the performance, intestinal microbial populations and histological characteristics of intestine wall in chickens. Their results showed that tannin supplementation had no effects on feed conversion and carcass quality, but tannin at 0.1% reduced final body weight. *Escherichia coli* and coliform bacteria in the small intestines of 28-d-old chickens were also reduced at the tannin levels of 0.05% to 0.10 %. In another study, Rezar and Salobir (2014) found that addition of 0.07% and 0.20 % of the same tannin product (0.05% and 0.10 % HT) did not affect broiler growth performance or the organic matter, crude protein, crude ash, calcium and phosphorus balance and utilization, but increased dry matter content of excreta. In a challenged study, Tosi *et al.* (2013) reported that chestnut HT at the dietary concentrations of 0.71% and 1.50 % reduced *Clostridium perfringens* (*Eimeria tenella, Eimeria acervulina, Eimeria maxima*) in the gut of broiler chicken orally challenged with these *coccidia*.

In vitro studies showed strong activities against parasites and pathogens residing in animal digestive tract (Chung *et al.,* 1998; Butter *et al.,* 2001), the in vivo assessments have yielded inconsistent results for animal performance. At the concentrations from 0.11% to 0.45% in swine diets, it was found that chestnut HT improved feed efficiency, tended to increase viable counts of

*Lactobacilli* in the jejunum and reduced caecal concentrations of ammonia, iso-butyric, and isovaleric acid, but had no effect on bacterial caecal counts, faecal excretion of Salmonella or colonization of the intestines (Biagia *et al.,* 2010; Parys *et al.,*2010). However, increasing concentration from 0.71% to 1.50 % reduced feed efficiency although feed intake, growth and carcass weight were not affected (Bee *et al.,* 2016). Stukelj *et al.* (2010) reported that chestnut HT at the level of 0.15% in combination with 0.15% of a mixture of acids had no effects on health status or growth performance of pigs whereas Brus *et al.* (2013) found that 0.19% of it in combination with 0.16% of a mixture of acids increased growth performance, increased lactic acid bacteria and reduced *Escherichiacoli* populations in the intestines.

Supplementations of chestnut HT at levels of 0.45% and 0.50% also have been shown to increase live weight gain and feed intake of rabbits (Maertens and Struklec, 2006; Zoccarato *et al.,* 2008). However, Liu *et al.* (2009) found that inclusion of chestnut HT at the concentrations of 0.50 % and 1.00 % had no effect on growth performance of rabbits. All the above informations suggest that depending on the type of animals and the type of diets, dietary addition of chestnut HT at the levels lower than 0.50 % for swine and rabbit and lower than 0.20 % for chicken may have positive effects on their growth performance and improve intestinal health. Higher chestnut HT concentration in the diets than the above mentioned will mostly lead to decreased animal growth performance by decreasing nutrient digestion and absorption (Iji *et al.,* 2004; Ebadi *et al.,* 2005).

### 2.6.2 Grape Tannins (Condensed Tannin-CT)

Extracts of grape (*Vitis vinifera*) seed and grape pomace contain significant amount of polyphenolic compounds including CT (Prieur *et al.,* 1994; Choy *et al.,* 2014), which have been assessed for their uses as natural feed additives to monogastric food producing animals. Wang *et al.* (2008) found that CT in grape seed extract at the dietary concentrations from 5 to 80 mg/kg

significantly decreased faecal shedding of *Eimeria tenella*, improved antioxidant status, reduced mortality and increased growth performance of *Eimeria tenella* infected broiler chicken and the most favourable results were observed with diets containing 10 to 20 mg CT/kg DM. Farahat *et al.* (2017) showed that grape seed extract possessed significant antioxidant and immunostimulant effects when fed to broiler chickens at the dietary concentrations of 0.125% to 2.00 % with 0.125% to 0.25% being the optimum dosages. Further increasing the concentration negatively affected birds' growth performance, protein and amino acid digestion (Chamorro *et al.*, 2013). Grape pomace, which includes skins, pulp and contain significant amount of CT and other simple phenolic compounds, is the byproduct of grape processing. Several studies evaluating the effects of grape pomace on swine and poultry performance indicated that addition of such tannin-rich product up to 10% of the diet had no effect on growth performance of broiler chicken, but enhanced anti-oxidant status and increased intestinal populations of beneficial bacteria (Brenes *et al.*, 2008; Viveros *et al.*, 2011; Chamorro *et al.*, 2015).

Choy *et al.* (2014) reported that adding 1.00% of grape seed extract to pig diets increased abundances of *Lachnospiraceae, Clostridales, Lactobacillus* and *Ruminococcaceae* in faecal microbiome. They found that oligomers (dimerepentamer) of grape tannin were only partially metabolized by the gut microbiota, producing phenolic metabolites that are known to be more readily absorbed. These phenolic compounds may have contributed to the altered bacterial populations thereby exerted the beneficial effects on the colon.

### 2.6.3 Tannic Acid

Tannic acid is a HT from varying plants including tara pods (*Caesalpinia spinosa*), gallnuts from *Rhus semialata, Quercus infectoria* or Sicilian Sumac leaves (*Rhus coriaria*). Tannic acid at the concentration of 0.50% increased growth performance and fat content in breast and thigh meat,

but reduced blood glucose concentration and cholesterol content in the liver of broiler chicken (Starcevic *et al.,* 2015). It is also reported that tannic acid at the dietary concentrations of 0.75% and 1.50% did not alter the numbers of *Salmonella typhimurium* in the cecal contents of broiler chickens (Kubena *et al.,* 2001). Increasing concentrations to 2.50% and 3.00% reduced weight gain and protein efficiency and impaired the immune function of growing chickens by decreasing weight of bursa of fabricius, thymus and spleen, reducing total immunoglobulin (Ig) M and IgG immunoglobulin levels and total white blood cells and absolute lymphocytes in a dose-dependent manner (Marzo *et al.,* 1990). Ebrahim *et al.* (2015) found that 1.00% of tannic acid decreased body weight gain and feed intake but improved the fatty acid profile of breast muscle of broilers under heat stress by decreasing monounsaturated fatty acids. From the above results, it seems that application rates of tannic acid in both poultry and swine are higher than those of other sources of tannins, but rarely result in positive effect on animal performance although increased antioxidant status were reported in several studies. High concentrations (e.g., ≥1%) appear to be toxic to animals in terms of decreasing production efficiency.

Lee *et al.* (2010) reported that addition of tannic acid at the dietary levels of 0.0125% to 0.10 % negatively impacted growth performance, haematological indices and plasma iron status of pigs, and linearly reduced faecal coliform bacteria count. However, the same authors found that feeding 0.0125% of tannic acid had no effect on growth performance, but negatively affected blood haematology and plasma iron status when pigs were fed iron deficient diets. It was also observed that total anaerobic bacteria, *Clostridium spp.* and coliforms were decreased but *Bifidobacterium spp.* and *Lactobacillus spp.* were increased by 0.0125% tannin acid (Lee *et al.,* 2009).

### 2.6.4 Other Sources of Tannins

A few studies assessed several other sources of tannins for monogastric animals. Iji *et al*. (2004) reported that addition of mimosa (*Mimosa pudica*) tannin extract (CT) to broiler diets at the levels of 0.50 %, 1.50 %, 2.00 %, 2.50 % reduced feed intake and body weight gain but improved feed efficiency at the levels less than 1.50 %. Hydrolysable tannin at the dietary level of 0.516 tannic acid equivalent/kg diets did not affect feed intake or gastric mucosa but improved feed efficiency. Red quebracho (*Schinopsis lorentzii*) CT was assessed for its effects on decreasing coccidiosis in *Eimeria tenella* challenged broilers (Cejas *et al.*, 2011). The study revealed that addition of 10% quebracho CT extract increased body weight gain of challenged birds, increased crypt:villi ratio of the intestine and decreased oocyst excretion. This study suggest that quebracho CT could be a potential prophylactic anti-coccidials agent. Zotte and Cossu (2009) also found that 1% and 3% of red quebracho tannins improved significantly weight gain and feed conversion of rabbits in a 6-week feeding trial.

## 2.7 Biological Activity of Tannins

Tannins have shown numerous biological activities and some of them, which are most important to the modern animal production, are summarized below.

### 2.7.1 Antimicrobial Property

The antimicrobial activities of tannins have long been recognized and the toxicity of tannins to bacteria, fungi and yeasts has been reviewed (Scalbert, 1991). The mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, direct action on microbial metabolism through inhibition of oxidative phosphorylation, metal ions deprivation or formation of complexes with the cell membrane of bacteria causing morphological changes of the cell wall

and increasing membrane permeability (Scalbert, 1991; Liu *et al*., 2013). Evidences have shown that the microbial cell membrane is the primary site of inhibitory action by tannins (Mcallister *et al*., 2005; Liu *et al*., 2013) through cell aggregation and disruption of cell membranes and functions. Although protein precipitation is a universal property for all tannins, anti-microbial activity of tannins is microbial species-specific and is closely related to the chemical composition and structure of tannins. Generally, antimicrobial activity of tannins against Gram-positive bacteria has been reported to be greater than against Gram-negative bacteria (Ikigai *et al*., 1993), because Gram-negative bacteria possess an outer membrane that consists of a lipid bilayer structure which is composed of an outer layer of lipopolysaccharide and proteins and an inner layer composed of phospholipids. However, tannins especially condensed tannin (CT) isolated from several plants have been shown to possess strong activity against Gram-negative bacteria.

It is worth noting that pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella, Shigella, Staphylococcus*, *Pseudomonas* and *Helicobacter pylori* were all sensitive to tannins (Funatogawa *et al.*, 2004; Doss *et al.*, 2009; Banso and Adeyemo, 2010; Liu *et al*., 2013). Wang *et al*. (2013) compared 12 tannins and found only CT isolated from purple prairie clover (*Dalea purpurea Vent*) and Phlorotannins (PT) from brown alga (*Ascophyllum nodosum*) possessed strong anti-*E. coli* and anti-*E. coli* O157:H7 activity. Phlorotannins also have greater antimicrobial activity than condensed tannin (CT) and hydrolysable tannin (HT) (Wang *et al*., 2009). It has been shown that the number of hydroxyl groups and liberation of hydrogen peroxide upon oxidation of tannins are two important factors responsible for the antimicrobial properties of tannins (Akagawa *et al*., 2003; Smith and Mackie 2004; Mueller-Harvey, 2006). It

has been proposed that flavonols with a trihydroxy B ring (gallocatechin) have a greater inhibitory effect on *Streptococcus, Clostridium, Proteus* and *Staphylococcus species* than catechin with a dihydroxy B ring (Sakanaka *et al*., 1989). Similarly, the toxicities of epi-catechin gallate and epigallocatechin gallate towards *Clostridium botulinum* were greater than those of their ungallated counterparts-epicatechin and epigallocatechin (Okuda *et al*., 1985). Due to the vast sources of tannins, which results in great diversity in their antimicrobial activities, screening and identification of tannins that are effective and specific to target microbes would continuously be a research endeavor.

### 2.7.2 Anti-Parasitic Property

Anti-parasitic properties of tannins have been demonstrated by both in vitro and in vivo studies. Condensed tannins extracted from legume tanniferous forages such as sainfoin (*Onobrychis viciifolia*), big trefoil (*Lotus pedunculatus*), birdsfoot trefoil (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*) reduced the proportion of *Trichostrongylus colubriformis* hatched eggs (Molan *et al*.,2002). Four tropical tanniniferous plant extracts have shown anthelmintic effect on *Haemonchus contortus* and *Trichostrongylus colubriformis*, which mainly interfered with the process of larval exsheathment (Alonso-Diaz *et al*., 2008a, 2008b). Tannins extract from chicory (Molan *et al.,* 2003) and green tea (Molan *et al.,* 2004) significantly inhibited the larval migration in a dose dependent manner. These results suggest that the anti-parasitic effect of tannins occurred throughout different stages of life-cycle of parasite.

The anthelmintic mechanisms of plant tannins have been suggested through “direct” action of tannins on parasite cells by:

1. Reducing establishment of the infective third-stage larvae in the host thereby reducing the host invasion.

2. Reducing excretion of nematodes eggs by the adult worms.

3. Reducing development of eggs to third-stage larvae (Hoste *et al.,* 2012) and through "indirect" action by improving the host's resistance to nematodes (Min and Hart 2003). However, similar to their antimicrobial activities, the anthelmintic effects of tannins vary greatly depending on chemical composition and structure of tannins, the parasite species or growth stages and/or the hosts' species (Hoste *et al.*,2012).

### 2.7.3 Antioxidant Property

Naturally occurring phenolic compounds have long been recognized as effective antioxidants (Rice-Evans *et al.,* 1995, 1996). The antioxidant property of tannins has wide application in food industry and medical field to prevent oxidative stress related diseases such as cardiovascular disease, cancer or osteoporosis (Hollman and Katan, 1999; Scalbert *et al.,* 2005). It has been shown that CT and HT of relatively high molecular weight exhibited greater antioxidant activities than simple phenolics (Hagerman *et al.,* 1998). The number of hydroxyl groups and the degree of polymerization of tannins are considered to be correlated with their abilities to scavenge free radicals (Ariga and Hamano, 1990). Tannins with the most hydroxyl groups are most easily oxidized (Hodnick *et al.,* 1988) and therefore possess greatest antioxidant activity. Ricci *et al.* (2016) demonstrated that effectiveness of tannins as natural antioxidants is due to their complex combinations of reducing and redox activities, which also contributes to their abilities to scavenge radicals. The potential of tannins as biological antioxidants has been indicated in many in vitro studies (Ho *et al.,* 1999; Lin *et al.,* 2001; Beninger and Hosfield, 2003;

Barreira *et al.,* 2008). It has been speculated that dietary tannins may spare other nutritive antioxidants during digestive process or they may protect proteins, carbohydrates, and lipids in the digestive tract from oxidative damage during digestion (Marshall and Roberts, 1990). However, the antioxidant mechanism of tannins in animal tissues is unknown. Further research in this area is needed, especially because enhancing antioxidant status is suggested to be one of the most benefits of feeding tannins to animal wellbeing and performance.

### 2.7.4 Anti-Inflammatory Property

Tannins possess varying anti-inflammatory activities (Mota *et al.,* 1985; Terra *et al.,* 2007; Sugiura *et al.,* 2013; Park *et al.,* 2014) that are positively associated with their antioxidant activities (Gonçalves *et al.,* 2005; Park *et al.,* 2014). Anti-inflammatory activity of CT extracted from black raspberry seeds was demonstrated by its ability to inhibit lipopolysaccharide induced RAW 264.7 cells in producing nitric oxide (NO), a pro-inflammatory mediator that induces inflammation (Park *et al.,* 2014). Hydrolyzable tannins from Myricaria bracteata showed a significant anti-inflammatory effect on croton oil-induced ear edema in mice and on collagen-induced arthritis in DBA/1 mice (Liu *et al.,* 2015). The authors speculated that the mechanism of anti-inflammatory effects was related to the potent ability for scavenging free radicals rather than inhibitory effects of HT on NO and pro-inflammatory cytokines production. Phlorotannins from*Ascophyllum nodosum* and *Ecklonia cava* also exhibited potent anti-inflammatory effects based on their ability to inhibit cytokines release (Dutot *et al.,* 2012). It needs to be pointed out that most of the studies in this area were conducted using in vitro models. The efficacy of the anti-inflammatory action of tannins in animal body after digestion needs to be evaluated further in in vivo model.

## 2.8 Dietary Zinc (Zn) For Poultry Production

In poultry, Zn deficiency leads to decreased feed intake and decreased collagen formation, which in turn leads to lesions on the skin, delayed wound healing, long bone malformation, and poor feathering (Kienholz *et al.*, 1961, Park *et al.,* 2004, Starcher *et al.,* 1980)**.** The National Research Council (NRC, 1994) recommended 40 ppm for broiler chickens, which appeared to be based on the results that considered growth performance as the only criterion (Feng*et al.,* 2010, Sunder *et al.,* 2008). However, there are several reports that demonstrate that higher Zn levels (60-180 ppm) produce better immune, growth performance and intestinal function of broiler chickens (Wenqiang *et al.,* 2011, Tang*et al.,* 2014).

Zn can come from organic or inorganic sources. Currently, there are two inorganic feed grade zinc sources commercially used by the poultry feed industry (Wedekind and Baker 1990, Batal *et al.,* 2001):zinc oxide (ZnO: 72% Zn) and zinc sulphate monohydrate ($ZnSO_4 \cdot H_2O$: 36% Zn). Of the supplemental zinc feed, 80–90% is ZnO, which is less bioavailable for poultry than reagent-grade or feed-grade Zn sulphate(Fosmire, 1990; Sandoval*et al*., 1997; Edwards and Baker 2000).However, the sulphate (acid salt) is highly water soluble, allowing reactive metal ions to promote free-radical formation, which can facilitate reactions that lead to the breakdown of vitamins and ultimately to the degradation of fats and oils, decreasing the nutrient value of the diet (Batal *et al.,* 2001). Oxide is less reactive, but also less bioavailable (Batal *et al.,* 2001). Dietary zinc is relatively non-toxic to animals and humans; both exhibit considerable tolerance to high intakes of zinc (Fosmire 1990). The organic forms of zinc include: amino acid chelates, bioplexes, proteins, as well as lactates and acetates.

### 2.8.1 Effect of Zinc on Growth Performance of Broiler Chickens

The earliest observed effect of zinc deficiency was reduced feed intake by animals (Quarterman *et al.,* 1969), which may be related to the role of zinc in inducing appetite (Berger, 2002). Ao *et al.* (2006) showed that feed intake was increased quadratically with increasing levels of dietary zinc up to 10mg $kg^{-1}$, and increased linearly when zinc was supplied as zinc sulphate ($ZnSO_4$) at 40mg$kg^{-1}$ in broiler chicks, after which no further increase occurred. Batal *et al.* (2001) also reported that weight gain, feed intake, and feed efficiency (feed to gain) responded quadratically to graded levels of supplemental zinc up to 20 mg $kg^{-1}$. Huang *et al*. (2007) reported that weight gain and feed intake of broiler chicks were significantly increased with dietary zinc level, and the maximum weight gain and feed intake were observed in the diet supplemented with 20mg $kg^{-1}$ of zinc (equates to 48.37 mg $kg^{-1}$, total dietary zinc). Since zinc functions mostly in enzyme systems, it was generally agreed that deficiency in some enzyme activity was involved in this loss of appetite and taste.

Progressive addition of zinc in an organic form (Rossi *et al.,* 2007) or an inorganic form (Kim and Patterson, 2004), and in combination of both as a complex form (Burrell *et al.,* 2004) to the basal diet did not affect the feed efficiency of broilers. On the other hand, diets supplemented with zinc from zinc-amino acid complexes (ZnAA) improved feed efficiency in broilers. Hess *et al.* (2001) supplemented practical broiler diets (55mg $kg^{-1}$ zinc from $ZnSO_4$) with 40mg $kg^{-1}$ zinc from three different ZnAA. Feed efficiency was improved from 0 to 35 days and from 0 to 42 days of age when supplemental ZnAA were provided to female broilers. Sanford and Kawchumnong (1972) reported an improved feed efficiency of broilers when dietary zinc was supplemented as zinc-methionine rather than zinc oxide (ZnO). Similarly, Nollet *et al.* (2007) indicated that feeding organic minerals replacing inorganic sources may have benefits in feed

efficiency in young broilers. These combined data suggest that both zinc concentration and zinc source influence the feed efficiency in broiler chickens.

Growth retardation is universally observed in zinc deficiency, perhaps because of impairment of nucleic acid biosynthesis and amino acid utilisation or protein synthesis (O'Dell, 1981). Batal *et al*. (2001) reported the positive effect of zinc on the growth of broiler in optimum management system but other reports show no significant effect of zinc in broiler growth. Rossi *et al.* (2007) showed that body weight gain and carcass yield were not influenced by the addition of increasing levels of dietary organic zinc in broiler diets. Earlier studies with inorganic zinc (Wang *et al.*, 2002), and with organic zinc (Hudson *et al.*, 2004), indicated that growth performance, leg abnormalities, and meat yields were unaffected when dietary zinc were provided in excess of the NRC (1994) recommendations of 40mg $kg^{-1}$.

By contrast, many investigators have added zinc in inorganic form (Edwards and Baker, 2000), or in organic form (Johnson and Fakler, 1998; Burrell *et al.*, 2004; Yu *et al.*, 2005), to diets of broilers and observed an improvement in growth performance. Hess *et al.* (2001) reported that an overall growth rate of broilers was 56.7 g $d^{-1}$ (the average growth rate of male broiler in USA is 54.6 g $d^{-1}$) and showed significant improvement in body weight of male broilers at 21 days of age, but not at 42 and 49 days observed when birds fed zinc-methionine and zinc-lysine as complexed zinc products. Similarly, Mohanna and Nys (1999) showed that chick's body weight significantly increased with the dietary zinc content until supplementation with 25 $mgkg^{-1}$ zinc (45 $mgkg^{-1}$ total dietary zinc). They also indicated that no additional response was observed at higher zinc concentration in the diet of broiler chicks. The lack of consistent effects of dietary zinc on performance of birds may be due to the amount and sources of zinc present in the basal diet (Leeson and Summers, 2005).

### 2.8.2 Dietary Zinc and Intestinal Health of Broiler Chickens

The strength and dynamics of gut of poultry is highly dependent on diet (Choct, 2009). The manner of poultry feeding and quality of the feed can havebeneficial effects on the health of the birds, mainly by influencingthe immune system to ensure the maintenance of homeostasisand protection against infections induced by pathogenicmicrobes (Takahashi *et al.,* 1995, Takahashi *et al.,* 2002, Shira *et al.,* 2005, Buyse *et al.,* 2007). Supplementation of Zn in diets improves intestinal morphology by increasing the villus height and reducing the crypt depth in animals (Burrell *et al.,* 2004, Payne *et al.,* 2006). Zinc is also known to influence the intestinal morphology and improve absorptive capacity, and enhance growth performance (Katouli *et al.,* 1999; Feng *et al.,* 2010).

The three major components of intestinal mucosa, epithelial cells, mucus secreting goblet cells (GC) and intra-epithelial lymphocytes (IELs), provide a barrier for entrance of harmful microbes from luminal contents to underlying capillary network (Deplancke and Gaskins, 2001). The internal surface of chicken intestine contains broad, finger-like projections called villi, which increase its absorptive surface area (Yazdani *et al.,*2013). Elongated villus indicates greater surface area for absorption of nutrients (Choct, 2009). From duodenum to ileum, goblet cell number and resultantly secretion of mucus increases while villus height decreases (Choct, 2009). Intestinal architecture is influenced with Zinc supplementation by increasing the villus height (Li *et al.,* 2001).

Immune activity in poultry is influenced bymicro- and macronutrients, particularly zinc, which is includedin poultry diets as feed additives. Zinc also play a key role in the development of immune system and helps to improve both cellular and humoral immune responses (Moghaddam and Jahanian, 2009). In particular, zincincreases the activation and proliferation of lymphocytes,

mainlyT and natural killer (NK) cells, and stimulates cellular defensemechanisms (Sunder *et al.,* 2008; Jarosz *et al.,* 2017). The immune modulatory effect of zinc alsoresults in an increase in the activity of thymocytes, macrophages,and heterophils, as well as increased antibody production,which enhances the potential of the humoral response (Wellinghausen *et al.,* 1997).Dietary Zn might also influence the immune system indirectly by interaction with growth and infectivity of organisms that are pathogens to animals. Chickens have hypozincemia when infected with the Newcastle disease virus (Squibb *et al.,* 1971) or *Escherichia coli*endotoxin (Butler and Curtis, 1973). Zinc concentrations in the liver are increased by *E. coli*endotoxin infection (Klasing, 1984). Temporal and quantitative changes in zinc concentrations in immune tissues might be important in the response to infection because the host uses zinc as a cofactor for enzymes involved in defense against pathogens (Klasing, 1984).

Zinc concentrations in serum and plasma are initially depressed when birds are infected with *Salmonella gallinarum* (Hill, 1989), *E. coli* (Tufft *et al.,* 1988), or *E. coli* endotoxin (Butler and Curtis, 1973). The effectiveness of Zn inhibition of bacterial growth results from changing the active transport system and impeding the initial phase of bacterial mating (Sobocinski *et al.,* 1977).

The basic components of poultry feed do not contain enough zinc to ensure that these physiological processes proceed correctly (Ao *et al.,* 2011). In addition, endogenous losses of zinc are linked to disturbances in intestinal absorption and increased excretion of the element. Zinc deficiency in poultry manifests as impaired resistance and thus increased susceptibility to disease and can lead to debilitation and death (Jarosz *et al.,* 2017). Supplementation of poultry feed with various forms of zinc, usually inorganic forms such as sulphates and oxides, has been recommended (Cao *et al.,* 2002).

## 2.9 Environmental Implications of Zinc Supplementation in Poultry Production

Modern broiler producers are faced with many challenging issues in reference to sustainable agriculture. Of these, environmental issues have begun to make an impact on production practices. One of the major environmental concerns associated with the poultry industry is emission of ammonia ($NH_3$), which increases atmospheric acid deposition (Moore, 1998). Many studies have demonstrated that high levels of $NH_3$ on the farm could reduce feed efficiency, growth rate, and egg production (Charles and Payne, 1966; Reece *et al*., 1980); damage the respiratory tract (Nagaraja *et al*., 1983); and impair immune responses (Nagaraja *et al*., 1984). Thus, reduction of $NH_3$ emission is very important to maintain human and animal health and a clean environment. Kim and Patterson (2003) reported that $ZnSO_4$ significantly inhibited the activity of microbial uricase,reduced $NH_3$ emission, and increased nitrogen retentionin broiler manure when manure was mixed withup to 2% $ZnSO_4$. Similarly, Kim and Patterson(2004) indicated that the zinc treatments significantlyreduced nitrogen loss in poultry manure, and $ZnSO_4$could be a better zinc source to prevent nitrogen lossto the atmosphere without any detrimental effect ongrowth performance.

Large amounts of dietary supplements relative to therequirements of poultry production are often used toprovide well balanced trace elements in foodstuffs andthis practice may increase the risk of soil pollution. Areduction in dietary zinc content decreased zincconcentration in broiler manure by 75% (Mohannaand Nys, 1999). Mohanna and Nys (1997) showedthat under normal commercial dietary conditions, 94%of the zinc ingested was excreted by broiler chicks. Thelow percentage of body retention (6%) may result firstly, from the high amount of zinc ingested, andsecondly, from the low utilisation of this element.Therefore, the excretion of zinc can be

reduced bylowering the dietary zinc concentration or by using thesources of zinc with higher availability.

Burrel *et al*. (2004) designed a study to evaluate thepotential reduction in zinc excretion by using differentsources and levels of zinc supplementation to broilers. They concluded that increased supplemental zinc to the broiler diet significantly increased zinc excretion but this excretion reduced when diet was supplemented with a combination of ZnAA+$ZnSO_4$. The reduction of zinc excretion may be due to increased zinc absorption, which by supplying both organic and inorganic sources of zinc could involve more absorption sites or transporters in the intestine, hence increasing zinc retention. Dozier *et al.* (2003) conducted two experiments to know the early growth and environmental implications of dietary zinc and copper concentrations and sources on broiler chicks and reported that decreasing dietary zinc concentration from 120 to 40 $mgkg^{-1}$ significantly reduced zinc excretion by 50%. They also suggested that dietary manipulation of zinc and copper concentrations can potentially decreased the accumulation of heavy metals in the environment without compromising bird performance. Bao *et al.* (2007) and Nollet *et al.* (2007) reported that lower levels of organic minerals in broiler chicken diets resulted in significantly lower ($P<0.05$) concentrations of minerals in manure, compared with birds fed inorganic minerals. They also reported that ground water pollution can be reduced by proper utilisation of organic minerals in poultry diets. In addition, a better adjustment of dietary zinc requirements for chick may considerably reduce the risk of soil phytotoxicity.

Calculated estimates of the balance between the mineral composition of poultry manure and soil mineral requirements showed that the amount of zinc in poultry manure was 6.6 times in excess of plant requirements when supplied the recommended European rate of 170 kg Nitrogen $ha^{-1}year^{-1}$ (Mohanna and Nys, 1997). This excess amount of zinc from poultrymanure has

generated environmental concerns andreduced crop yields. The symptoms caused from zinctoxicity with sorghum were evidenced by the result ofinadequate plant growth (Ohki, 1984). Increasing theapplication rate of zinc from 90 to 360mg $kg^{-1}$decreased the yield of bush beans from 876 to33 kg $ha^{-1}$ (Giordano *et al*., 1975). Therefore, using asmaller safety margin when formulating broiler dietsmay be an efficient way of reducing the risk of zincaccumulation in the soil and contribute to delaying theappearance of soil phytotoxicity in areas of intensivebroiler farming.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Experimental site

The experiment was conducted at the Livestock Section, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Zaria is located in the Northern Guinea Savannah Ecological Zone on longitude 11° 09' 01.78'N and latitude 7°39' 14.79' E, 671m above sea level. The climate is characterized by a well-defined dry and wet seasons with annual rainfall ranging from 700-1400mm.The maximum temperature varies from 26-32$^0$C depending on the season while the mean relative humidity during the dry and wet season are 21 and 72% respectively (IAR, 2016).

### 3.2 Feeding Trials

Two feeding trials were conducted.The first was to evaluate the performance of broiler chickens fed graded levels of Chestnut *(Castenea sativa)* phytobiotics while the second evaluated the performance of broiler chickens fed graded levels of zinc sulphate.

#### 3.2.1 Experiment 1: Performance evaluation of broiler chickens fed diets containing graded levels of Chestnut *(Castenea sativa)* phytobiotics as eubiotics

3.2.1.1 Experimental design and management of birds

Three hundred (300) day old broiler chicks from Olam hatcheries Kaduna state were allocated to five dietary treatments with 3 replicates of 20 birds each in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices. Feed and water were provided *ad libitum* for the 7 weeks period of the experiment.

3.2.1.2 Experimental diets

Five treatment diets were formulated as shown in Tables 3.1 and 3.2 for starter and finisher chickens respectively. The phytobiotics additives were added as non-inclusive part of the diets as follows:

Diet 1: (Control diet) – without phytobiotics.

Diet 2: 100g of phytobiotics/100 Kg diet

Diet 3: 125g of phytobiotics/100 Kg diet

Diet 4: 150g of phytobiotics/100 Kg diet

Diet 5: Oxytetracycline at 111g/100Kg diet (as recommended by manufacturer).

Recommended dosage for phytobiotics is 1.0-1.5kg/metric tonne of feed.

3.2.1.3 Growth Study

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

3.2.1.4 Haematological Study

At day 56 of the feeding trial, 2.0 ml of blood samples was taken from three birds per treatment into sterilized sample bottles containing ethylenediamine tetraacetic acid (EDTA) and taken to the clinical pathology laboratory of the Ahmadu Bello University Teaching Hospital for haematological study. The samples were analyzed for packed cell volume (PCV), Haemoglobin (Hb) count, red blood cell (RBC), white blood cell (WBC) and differential counts of the WBC for the various cell types incuding lymphocytes, monocytes, heterophils, eosinophils, basophils,

mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) using an auto haemo-analyser (Lamb, 1991).

3.2.1.5 Serum Biochemical Analysis

At day 56 of the feeding trial, 2.0 ml of blood samples was taken from three birds per treatment into sterilized sample bottles without anticoagulant to allow for clotting and wasused for the blood biochemical analysis. Samples were taken to the clinical pathology laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for determination of parameters related to liver function, including total protein (TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), albumin and glucose using standard laboratory procedures (Lamb, 1991). Globulin values was obtained by subtracting albumin values from corresponding values of total protein (Coles, 1986). Also, parameters related to lipid profile (total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein) were determined using standard laboratory procedures (Lamb, 1991).

3.2.1.6 Digestibility Study

Digestibility trial was carried out at the end of the experiment, Three (3) birds were randomly selected from each treatment and kept in individual cages for total faecal collection. The birds were allowed a period of 3 days to adjust to the cage environment and thereafter offered a known amount of experimental diet. Total faecal droppings wascollected for five consecutive days, weighed and oven-dried at 65 °C for 24 hours. The dried faecal samples were assayed for their nutrient contents using the methods described by AOAC (1990) at the Biochemical Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria. Nutrient digestibility was determined for crude protein, ether extract, crude fibre and nitrogen-free extract using the formula:

% Digestibility of Nutrient = $\frac{\textit{Nutrient in feed - Nutrient in feaces}}{\textit{Nutrient in feed}} \quad X\ 100$

3.2.1.7 Intestinal Bacterial Count

At the end of the starter and finisher phases, bacterial cell numbers in the ileum was determined for *Escherichia coli, Lactobacilli spp, Clostridium spp, Salmonella spp, Bacillus spp* using different selective media for isolation of bacteria groups and characterization based on sugars fermentation using Microbact 12E kit and conventional biochemical methods. (O.M.P[abc] 2015). This was carried out at the clinical pathology laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.2.1.8 Villi Morphometric

Intestinal segment samples (approximately 2 cm in length) ileum was taken from birds after slaughter to ascertain villi morphometry. The morphometry indices that was evaluated include villus height, from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio as described by Zhang *et al*.(2009). This wascarried out at the Histology Laboratory of the Department of Anatomy, Ahmadu Bello University Zaria.

3.2.1.9 Economic Indices

Economic analysis cost of production was calculated based on some specific items such as chicks, feed, vaccine, test ingredients and other miscellaneous expenses. The prevailing market price was ascertained before the computation. This was used to determine whether the inclusions of Chestnut phytobiotics in the diets of broiler chicken have economic advantage. The following parameters were determined; feed cost/Kg, mean feed intake, mean feeding cost, cost of chicks, mean final weight, cost of chicken, mean yield cost and net profit.

### 3.2.2 Experiment 2: Performance evaluation of broiler chickens fed diets containing graded levels of zinc sulphate.

3.2.2.1 Experimental design and management of birds

As in experiment 1

3.2.2.2 Experimental diets

Five diets wereformulated as shown in Tables 3.3 and 3.4. Zinc was added as non-inclusive part of the diets.

Diet 1: (Control diet) – without Zinc

Diet 2: 10mg of Zinc/100 Kg diet

Diet 3: 20mg of Zinc/100 Kg diet

Diet 4: 30mg of Zinc/100 Kg diet

Diet 5: Oxytetracycline at 111g/100Kg diet

3.2.2.3 Growth Study

Same as in experiment 1

3.2.2.4 Haematological Study

Same as in experiment 1

3.2.2.5 Serum Biochemical Analysis

Same as in experiment 1

3.2.2.6 Digestibility Study

Same as in experiment 1

3.2.2.7 Intestinal Bacterial Count

Same as in experiment 1

3.2.2.8 Villi Morphometric

Same as in experiment 1

3.2.2.9 Carcass Analysis

At the end of the finisher phase, 3 chickens were randomly selected from each treatment, representing the average weight of the group for carcass evaluation. The selected birds were starved of feed overnight, bled by severing the jugular vein and then scalded in warm water to remove the feathers. Live weight for each chicken was taken before slaughter. Dressing percentage, weight of organs and the standard prime cut parts were measured. The organs were expressed as a percent of live weight while the cut parts were expressed as percentage of dressed weight.

3.2.2.10 Economic Indices

Same as in experiment 1

**Table 3.1: Composition of Broiler Starter Diets Supplemented with Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives. (Experiment 1)**

| **Levels of Phytobiotics** | | | | | |
|---|---|---|---|---|---|
| **Ingredients (%)** | **0g** | **100g** | **125g** | **150g** | **Oxytet** |
| Maize | 57.00 | 57.00 | 57.00 | 57.00 | 57.00 |
| GNC | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| SBC | 23.55 | 23.55 | 23.55 | 23.55 | 23.55 |
| Bone Meal | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Limestone | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vit/min Premix[A] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | **100** | **100** | **100** | **100** | **100** |
| ***Calculated Analysis*** | | | | | |
| ME Kcal/kg | 2,909 | 2,909 | 2,909 | 2,909 | 2,909 |
| Crude Protein (%) | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 |
| Crude Fibre (%) | 3.84 | 3.84 | 3.84 | 3.84 | 3.84 |
| Ether Extract (%) | 4.64 | 4.64 | 4.64 | 4.64 | 4.64 |
| Calcium (%) | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 |
| Avail. Phos (%) | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 |
| Lysine (%) | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 |
| Methionine (%) | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Feed cost (₦ /Kg) | 119.89 | 122.69 | 123.39 | 124.09 | 125.44 |

Vitamix Premix Provided per Kg of diet: 10000i.u Vit. A; 2000i.u Vit. D3; 20i.u Vit. E; 2.25mg Vit. K; 1.75mg Vit. $B_1$; 5mg Vit. $B_2$; 2.75mg Niacin; 0.015mg Vit. $B_{12}$; 7.5mg Panthotenic acid; 7.5mg Folic acid; 0.05mg Biotin; 0.4g Choline chloride; 125mg Antioxidant; 80mg Manganese; 50mg Zinc; 20mg Iron; 5mg Copper; 1.2mg Iodine; 0.2mg Selenium; 0.2mg Cobalt. Oxytet= Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake.

**Table 3.2: Composition of Broiler Finisher Diets Supplemented with Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives. (Experiment 1)**

| **Levels of Phytobiotics** | | | | | |
|---|---|---|---|---|---|
| **Ingredients (%)** | **0g** | **100g** | **125g** | **150g** | **Oxytet** |
| Maize | 57.85 | 57.85 | 57.85 | 57.85 | 57.85 |
| Maize offal | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| GNC | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| SBC | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Bone Meal | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 |
| Limestone | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Lysine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vit/min Premix[A] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | **100** | **100** | **100** | **100** | **100** |
| ***Calculated Analysis*** | | | | | |
| ME Kcal/kg | 2,936 | 2,936 | 2,936 | 2,936 | 2,936 |
| CrudeProtein (%) | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Crude Fibre (%) | 4.15 | 4.15 | 4.15 | 4.15 | 4.15 |
| Ether Extract (%) | 4.45 | 4.45 | 4.45 | 4.45 | 4.45 |
| Calcium (%) | 1.21 | 1.21 | 1.21 | 1.21 | 1.21 |
| Phosphorus (%) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Lysine (%) | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 |
| Methionine (%) | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 |
| Feed cost (₦ /Kg) | 113.00 | 115.00 | 116.00 | 117.00 | 118.00 |

Vitamix Premix Provided per Kg of diet: 10000i.u Vit. A; 2000i.u Vit. D3; 20i.u Vit. E; 2.25mg Vit. K; 1.75mg Vit. $B_1$; 5mg Vit. $B_2$; 2.75mg Niacin; 0.015mg Vit. $B_{12}$; 7.5mg Panthotenic acid; 7.5mg Folic acid; 0.05mg Biotin; 0.4g Choline chloride; 125mg Antioxidant; 80mg Manganese; 50mg Zinc; 20mg Iron; 5mg Copper; 1.2mg Iodine; 0.2mg Selenium; 0.2mg Cobalt. Oxytet= Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake.

**Table 3.3: Composition of Broiler Starter Diets Supplemented with Different Levels of ZincSulphate.(Experiment 2)**

| | Levels of Zinc | | | | |
|---|---|---|---|---|---|
| **Ingredients (%)** | **0mg** | **10mg** | **20mg** | **30mg** | **Oxytet** |
| Maize | 57.00 | 57.00 | 57.00 | 57.00 | 57.00 |
| GNC | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| SBC | 23.55 | 23.55 | 23.55 | 23.55 | 23.55 |
| Bone Meal | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Limestone | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vit/min Premix[A] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | **100** | **100** | **100** | **100** | **100** |
| ***Calculated Analysis*** | | | | | |
| ME Kcal/kg | 2,909 | 2,909 | 2,909 | 2,909 | 2,909 |
| Crude Protein (%) | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 |
| Crude Fibre (%) | 3.84 | 3.84 | 3.84 | 3.84 | 3.84 |
| Ether Extract (%) | 4.64 | 4.64 | 4.64 | 4.64 | 4.64 |
| Calcium (%) | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 |
| Avail. Phos (%) | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 |
| Lysine (%) | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 |
| Methionine (%) | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Zinc (mg) | 78.00 | 88.00 | 98.00 | 108.00 | 78.00 |
| Feed cost (₦ /Kg) | 122.80 | 122.80 | 122.80 | 122.80 | 127.20 |

Vitamix Premix Provided per Kg of diet: 10000i.u Vit. A; 2000i.u Vit. D3; 20i.u Vit. E; 2.25mg Vit. K; 1.75mg Vit. $B_1$; 5mg Vit. $B_2$; 2.75mg Niacin; 0.015mg Vit. $B_{12}$; 7.5mg Panthotenic acid; 7.5mg Folic acid; 0.05mg Biotin; 0.4g Choline chloride; 125mg Antioxidant; 80mg Manganese; 50mg Zinc; 20mg Iron; 5mg Copper; 1.2mg Iodine; 0.2mg Selenium; 0.2mg Cobalt. Oxytet= Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake.

**Table 3.4: Composition of Broiler Finisher Diets Supplemented with Different Levels of Zinc Sulphate. (Experiment 2)**

| | Levels of Zinc | | | | |
|---|---|---|---|---|---|
| **Ingredients (%)** | **0mg** | **10mg** | **20mg** | **30mg** | **Oxytet** |
| Maize | 57.85 | 57.85 | 57.85 | 57.85 | 57.85 |
| Maize offal | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| GNC | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| SBC | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Bone Meal | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 |
| Limestone | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Lysine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vit/min Premix[A] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | **100** | **100** | **100** | **100** | **100** |
| ***Calculated Analysis*** | | | | | |
| ME Kcal/kg | 2,936 | 2,936 | 2,936 | 2,936 | 2,936 |
| CrudeProtein (%) | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Crude Fibre (%) | 4.15 | 4.15 | 4.15 | 4.15 | 4.15 |
| Ether Extract (%) | 4.45 | 4.45 | 4.45 | 4.45 | 4.45 |
| Calcium (%) | 1.21 | 1.21 | 1.21 | 1.21 | 1.21 |
| Phosphorus (%) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Lysine (%) | 1.12 | 1.12 | 1.12 | 1.12 | 1.12 |
| Methionine (%) | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 |
| Zinc (mg) | 75.00 | 85.00 | 95.00 | 105.00 | 75.00 |
| Feed cost (₦ /Kg) | 117.10 | 117.10 | 117.10 | 117.10 | 121.50 |

Vitamix Premix Provided per Kg of diet: 10000i.u Vit. A; 2000i.u Vit. D3; 20i.u Vit. E; 2.25mg Vit. K; 1.75mg Vit. $B_1$; 5mg Vit. $B_2$; 2.75mg Niacin; 0.015mg Vit. $B_{12}$; 7.5mg Panthotenic acid; 7.5mg Folic acid; 0.05mg Biotin; 0.4g Choline chloride; 125mg Antioxidant; 80mg Manganese; 50mg Zinc; 20mg Iron; 5mg Copper; 1.2mg Iodine; 0.2mg Selenium; 0.2mg Cobalt. Oxytet= Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake.

## 3.3 Statistical Analysis

All data obtained from the feeding trials was statistically analysed using the General Linear Model Procedure of Statistical Analysis Systems (SAS, 2002). Significant difference between treatments means was separated using Duncan's Multiple Range Test in the SAS package (SAS, 2002).

### 3.3.1 Experimental Model

The linear model for the experiment is:

$Yij = \mu + Ti + e_{ij}$

Yij = dependent variable

μ= Overall mean

Ti= $i^{th}$ effect of treament

$e_{ij}$= Random Error.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Experiment 1: Performance Evaluation of Broiler Chickens Fed Diets Containing Graded Levels of Chestnut Phytobiotics as Eubiotics

#### 4.1.1 Growth Performance of Broiler Chicks Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives (0-4 weeks)

Table 4.1 shows the growth performance of broiler chicks fed diets containing different levels of Chestnut (*Castenea sativa*) Phytobiotics feed additives. There were significant ($P<0.05$) differences in final weight, weight gain, feed intake, feed conversion ratioand feed cost per kilogram gain across the treatments. This study revealed that addition of chestnut phytobiotic to broiler diets at the levels of 125g, 150g reduced feed intake. This may be attributed to tannin which is present in the chestnut. Tannins are known to have a bitter or astringent taste which reduces palatability, and hence will negatively affect voluntary feed intake. This agrees with the reports of Maass *et al* (2005); Roth-Maier *et al* (2005); Jamroz *et al* (2009) and Ebrahim *et al* (2015) who reported that addition of phytobiotics to the diet of broiler chickens resulted in depression in feed intake.

Feed intake was significantly higher for birds placed on oxytetracycline than the rest of the other treatment groups. Feed cost per kilogram gain was best in the control, followed by phytobiotics, however higher feed cost per kilogram gain was recorded for birds fed with AGP (Oxytetracycline). Broiler chicks fed diet supplemented with 100g phytobiotics were significantly ($P<0.05$) higher and showed best performance in terms of final weight and weight gain.

**Table 4.1: Growth Performance of Broiler Chicks Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (0-4 weeks)**

| Levels of Phytobiotics | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| Initial weight (g/b) | 45.83 | 45.83 | 45.83 | 45.83 | 45.83 | 0.00 |
| Final weight (g/b) | 1129.67$^{a}$ | 1170.67$^{a}$ | 1086.67$^{c}$ | 1108.33$^{bc}$ | 1107.67$^{b}$ | 11.11 |
| Weight gain (g/b) | 1083.84$^{b}$ | 1124.84$^{a}$ | 1040.84$^{c}$ | 1062.50$^{bc}$ | 1061.84$^{bc}$ | 11.11 |
| Feed intake (g/b) | 1620.33$^{c}$ | 1709.00$^{b}$ | 1590.67$^{c}$ | 1562.67$^{c}$ | 1776.33$^{a}$ | 33.15 |
| Feed conversion ratio | 1.49$^{a}$ | 1.51$^{a}$ | 1.52$^{a}$ | 1.47$^{a}$ | 1.67$^{b}$ | 0.03 |
| Feed cost/gain (₦/Kg gain) | 179.32$^{a}$ | 186.34$^{ab}$ | 188.65$^{b}$ | 182.52$^{ab}$ | 209.84$^{c}$ | 3.78 |
| Mortality (%) | 0.00 | 0.67 | 0.33 | 0.33 | 0.67 | 0.47 |

a,b,c; Means with different superscripts on the same row are significantly different (P<0.05). SEM; Standard Error of Means
Oxytet; Oxytetracycline

There was significant (P<0.05) differences in the feed conversion ratio. This disagrees with the report of Jamroz *et al.*(2009) who reported that chestnut tannin extracts have no effect on feedconversion ratio. There were no significant (P>0.05) differences in the feed cost and mortality.

### 4.1.2 Growth Performance of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.2 shows the growth performance of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. The result shows non-significant (P>0.05) difference in feed intake and feed cost, whereas significant (P<0.05) differences were observed in final weight, weight gain, feed conversion ratio, feed cost per kilogram gain and mortality. Final weight was best in birds fed control diet (2884.3g) and diet containing oxytetracycline (2890.7g). Birds fed diet containing oxytetracycline were best in terms of weight gain (1783.2g), however there was an improvement in values for birds placed on phytobiotics. This work agrees with the report of Gessica *et al* (2019) who reported that the use of tannin in the diet of broilers enhanced weight gain. Feed conversion ratio was significantly (P<0.05) different across the treatment groups which was best in treatment that had oxytetracycline and the control diet followed by birds on diet with phytobiotics. Francesco *et al* (2011) revealed that generally, chestnut tannin seems to play a more favourable role in the younger than in older broilers.

There was significant (P<0.05) difference in terms of feed cost per Kg diet with the control having lower value which implies that addition of feed additives increased the feed cost per Kg. Mortality was significantly lower in diet containing 100g phytobiotics, control and

oxytetracycline but statistically similar with birds placed on diet with 125g phytobiotic, followed by diet containing 150g phytobiotics.

**Table 4.2: Growth Performance of Broiler Finisher Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| Initial weight (g/b) | 1129.67 $^{a}$ | 1170.57 $^{b}$ | 1086.67$^{c}$ | 1108.33$^{bc}$ | 1107.53$^{bc}$ | 11.18 |
| Final weight (g/b) | 2884.27$^{a}$ | 2851.33$^{ab}$ | 2843.40$^{ab}$ | 2759.63$^{b}$ | 2890.73$^{a}$ | 50.18 |
| Weight gain (g/b) | 1754.60$^{ab}$ | 1680.77$^{bc}$ | 1756.73$^{ab}$ | 1651.30$^{c}$ | 1783.20$^{a}$ | 50.86 |
| Feed intake (g/b) | 3458.33 | 3461.67 | 3471.33 | 3454.33 | 3479.00 | 58.51 |
| Feed conversion ratio | 1.97$^{ab}$ | 2.06$^{bc}$ | 1.98$^{b}$ | 2.09$^{c}$ | 1.96$^{a}$ | 0.05 |
| Feed cost/gain (₦/Kg gain) | 222.61$^{a}$ | 236.90$^{b}$ | 229.68$^{ab}$ | 244.53 $^{b}$ | 231.28$^{ab}$ | 6.00 |
| Mortality (%) | 0.33$^{a}$ | 0.00$^{a}$ | 0.67$^{ab}$ | 1.00$^{b}$ | 0.33$^{a}$ | 0.25 |

a,b,c; Means with different superscripts on the same row are significantly different (P<0.05) SEM; Standard Error of Means
Oxytet; Oxytetracycline

### 4.1.3 Haematological Indices of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.3 shows the haematological indices of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) as phytobiotics feed additives. There were significant ($P<0.05$) differences in the values of Hb, RBC, WBC, Heterophils, Lymphocytes, Monocytes, Eosinophils, Basophils, MCV, MCH except for the value of PCV which was non-significant but fell within the normal range of 24.00-44.00%. The heamoglobin count of 10.47-11.65g/dL in this study is within the normal range of 9.10-13.90g/dL as reported by Mitruka and Rawnsley (1997) for healthy chickens; indicating that the birds had sufficient blood pigment for proper transportation of oxygen.

The values for RBC, Lymphocytes, Monocytes, MCV and MCH obtained in this study, though differed significantly among treatment groups but were within the normal range for RBC (2.00-3.00X$10^6$ /µL), Lymphocytes (40-100%), Monocytes (1.00-7.00%), Basophils (0.00-2.00%), MCV (90-140fL) and MCH (33-47pg) for healthy broiler chickens as reported by Jain, (1993) andNanbol *et al*(2016). The results revealed that the health of the birds was not compromised. However, the values for WBC (91.90x$10^3$/µL) and Heterophils (20.63%) were significantly ($P<0.05$) higher in birds on diets containing oxytetracycline than the other treatment groups. Birds fed diets with 100g phytobiotics had significantly ($P<0.05$) higher values for RBC, Monocytes, Eosinophils and Basophils. The results obtained for RBC is in agreement with the work done by Gessica *et al* (2019) who reported that broiler chickens fed with an extract containing tannins showed higher RBC counts.

**Table 4.3: Haematological Indices of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** | **REF. V** |
| PCV (%) | 34.80 | 38.07 | 36.47 | 32.80 | 38.07 | 2.64 | 24.00-44.00$^{+}$ |
| Hb (g/dL) | 11.25$^{b}$ | 11.63$^{ab}$ | 10.47$^{c}$ | 11.05$^{b}$ | 11.90$^{a}$ | 0.31 | 9.10-13.90$^{+}$ |
| RBC (x10$^{6}$/µL) | 2.01$^{c}$ | 2.93$^{a}$ | 0.61$^{d}$ | 2.27$^{bc}$ | 2.53$^{b}$ | 0.18 | 2.00-3.00$^{++}$ |
| WBC (x10$^{3}$ /µL) | 84.75$^{bc}$ | 82.07$^{c}$ | 83.07$^{c}$ | 90.00$^{ab}$ | 91.90$^{a}$ | 2.88 | - |
| Heterophils (%) | 12.55$^{b}$ | 11.00$^{b}$ | 7.93$^{c}$ | 14.45$^{b}$ | 20.63$^{a}$ | 2.10 | - |
| Lymphocytes (%) | 79.95$^{b}$ | 76.00$^{bc}$ | 87.27$^{a}$ | 77.50$^{b}$ | 72.67$^{c}$ | 2.32 | 40-100$^{++}$ |
| Monocytes (%) | 4.00$^{b}$ | 7.07$^{a}$ | 3.07$^{b}$ | 4.15$^{b}$ | 4.27$^{b}$ | 1.05 | 1.00-7.00$^{++}$ |
| Eosinophils (%) | 2.75$^{b}$ | 4.13$^{a}$ | 1.03$^{c}$ | 2.35$^{b}$ | 2.07$^{b}$ | 0.61 | 1.50-6.00++++ |
| Basophils (%) | 0.80$^{b}$ | 1.80$^{a}$ | 0.70$^{b}$ | 0.55$^{b}$ | 0.33$^{b}$ | 0.47 | 0.00-2.00$^{+++}$ |
| MCV (fL) | 131.15$^{a}$ | 130.73$^{a}$ | 58.56$^{b}$ | 129.40$^{a}$ | 130.80$^{a}$ | 8.80 | 90-140$^{++}$ |
| MCH (pg) | 42.90$^{b}$ | 41.83$^{b}$ | 90.90$^{a}$ | 42.65$^{b}$ | 43.50$^{b}$ | 16.26 | 33-47$^{++}$ |

a,b,c,d; Means with different superscripts on the same row are significantly different (P<0.05) SEM; Standard Error of Means Oxytet; Oxytetracycline, PCV=Packed Cell Volume, RBC=Red Blood Cell, WBC= White Blood cell, Hb=Haemoglobin concentration, MCV=Mean Corpuscular Volume, MCH=Mean corpuscular haemoglobin, REF.V= Reference value, $^{+}$Mitruka and Rawnsley, 1997, $^{++}$Jain 1993, $^{+++}$Nanbol *et al* 2016, ++++Simrak *et al* 2004.

### 4.1.4 Serum Biochemical Composition of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.4 shows the serum biochemical composition of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives at finisher phase. There were significant ($P<0.05$) differences in all the parameters measured. The values for ALP show that birds placed on phytobiotics, oxytetracycline and control diet were within the normal range of 10-106µ/L for healthy birdsas reported by Bounous and Stedman (2000) except for AST which were below the reference value of 100-400 µ/L (LAVC, 2009).ALT was significantly ($P<0.05$) higher in the treatment diets with 100g, 125g and 150g phytobiotics which may signify liver injury as reported by WebMed (2016). However, this did not reflect on the haematology parameters as injurious to the birds.

Albumin was similar across the treatment group and the values are statistically similar. Serum albumin is a strong predictor of health, a lower albumin concentration is a sign of poor health and predictor of poor outcome (Kastow, 2009). The higher the values of albumin the higher the clothing ability of blood, hence preventing haemorrhage. However, the values obtained for total protein, albumin and globulin fell within the nomal range as reported by (LAVC, 2009; Ross *et al.*, 1976).

Glucose (206.51mg/dL) was significantly higher in treatment diet that contained oxytetracycline. The birds from this treatment were more lively and agile. This could be attributed to higher

available energy (glucose) present in the blood. Total protein was significantly higher in treatment that contained phytobiotics (150g) which indicate good health.

**Table 4.4: Serum Biochemical Composition of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| **Levels of Phytobiotics** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** | **REF. V** |
| Glucose (mg/dL) | 97.04$^{c}$ | 127.41$^{b}$ | 127.97$^{b}$ | 111.87$^{b}$ | 206.51$^{a}$ | 10.39 | - |
| Total Protein (g/dL) | 4.00$^{bc}$ | 3.60$^{c}$ | 3.79$^{c}$ | 5.83$^{a}$ | 5.37$^{ab}$ | 0.73 | 3.60-5.50+ |
| Albumin (g/dL) | 1.66$^{a}$ | 1.58$^{a}$ | 1.46$^{ab}$ | 1.62$^{a}$ | 1.60$^{a}$ | 0.08 | 1.10-2.20+ |
| Globulin (g/dL) | 2.35$^{b}$ | 2.03$^{b}$ | 2.33$^{b}$ | 4.21$^{a}$ | 3.77$^{a}$ | 0.70 | 1.20-3.20++ |
| AST **(**µ/L**)** | 63.45$^{a}$ | 65.73$^{a}$ | 54.47$^{b}$ | 67.75$^{a}$ | 58.30$^{a}$ | 5.05 | 100-400++ |
| ALT **(**µ/L**)** | 12.70$^{b}$ | 14.07$^{a}$ | 14.80$^{a}$ | 14.65$^{a}$ | 11.77$^{b}$ | 0.64 | - |
| ALP **(**µ/L**)** | 78.50$^{a}$ | 79.67$^{a}$ | 80.77$^{a}$ | 71.65$^{b}$ | 79.63$^{a}$ | 1.92 | 10-106+++ |

a,b, c; Means with different superscripts on the same row are significantly different (P<0.05): Aspartate amino transferase (AST): Alanine amino transferase (ALT): Alkaline Phosphatase (ALP): Albumin(ALB): Globulin (GLB): Oxytet; Oxytetracycline; SEM: Standard error of mean, $^{+}$Ross *et al* (1976), $^{++}$LAVC (2009), $^{+++}$Bounous and Stedman (2000).

### 4.1.5 Lipid Profile of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.5 shows the lipid profile of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives at the finisher phase. There were significant ($P<0.05$) differences in all the parameters measured. This study revealed that inclusion of chestnut phytobiotics reduced total cholesterol values from 151.13 - 96.55mg/dL. This work is in agreement with the report of Gessica*et al* (2019) who reported similar trend. Sturkie (2000) reported that the concentration of cholesterol is influenced by physical and nutritional status of the bird. Low cholesterol reduces the occurrence of cardiovascular disease. However, the values obtained from this study is within the normal range (75.30-196.00mg/dl) for a healthy chicken as reported by (Gessica *et al*.,2019). Triglycerides was significantly ($P<0.05$) different across the treatment groups with phytobiotics at 150g having the least value (36.87g/dL), followed by oxytetracycline (60.20 g/dL) and phytobiotics at (125g) while phytobiotics at 100g (83.70g/dL) and control (78.74g/dL) were higher and similar. There was a significant ($P<0.05$) decrease in the level of triglyceride in the birds fed diets containing phytobiotics (100g, 125g, 150g). This result shows that addition of chestnut phytobiotics reduces the levels of triglyceride in the blood. Triglycerides are the major form of energy stored in the body. High density lipoprotein and low density lipoprotein were statistically different; they are the major transporter of triglyceride in the system.

**Table 4.5: Lipid profile of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| TCHOL (mg/dL) | $97.99^{b}$ | $151.13^{a}$ | $126.40^{a}$ | $96.55^{b}$ | $112.27^{b}$ | 14.76 |
| Triglyceride (g/dL) | $78.74^{ab}$ | $83.70^{a}$ | $60.81^{b}$ | $36.87^{c}$ | $60.20^{b}$ | 9.70 |
| HDL (mg/dL) | $54.85^{b}$ | $79.58^{a}$ | $80.64^{a}$ | $71.42^{ab}$ | $71.25^{ab}$ | 7.70 |
| LDL (mg/dL) | $33.88^{a}$ | $51.51^{a}$ | $49.47^{a}$ | $11.36^{b}$ | $43.52^{a}$ | 11.20 |

a,b, c; Means with different superscripts on the same row are significantly different (P<0.05): Total Cholesterol (TCHOL): High Density Lipoprotein (HDL): Low Density Lipoprotein (LDL): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.1.6 Total Tract Apparent Nutrient Digestibility of Broiler Chicken Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.6 shows the total tract apparent nutrient digestibility of broiler chicken fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. The results show non-significant ($P>0.05$) differences in the digestibility of ether extract, crude protein, crude fibre and nitrogen free extract. Significant ($P<0.05$) difference was observed in crude protein digestibility. Birds fed diets containing 100g phytobiotics were significantly ($P<0.05$) better in crude protein digestibility (76.15%) compared to that of control (66.54%) but were however, statistically similar to the birds that fed 125g (73.71%), phytobiotics, 150g (72.77%), and oxytetracycline (75.43%) supplementation. This present work is in agreement with the report of Murugesan*et al* (2015) who reported that supplementation of either AGP or PFA (phytobiotics) to the basal diet significantly increased the apparent total tract digestibility of CP when compared to the control group.

**Table 4.6: Apparent Total Tract Percent Nutrient Digestibility of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| Crude Protein (%) | $66.54^{b}$ | $76.15^{a}$ | $73.71^{ab}$ | $72.77^{ab}$ | $75.43^{ab}$ | 4.12 |
| Ether Extract (%) | 67.56 | 76.09 | 71.18 | 70.71 | 73.15 | 4.45 |
| Crude Fibre (%) | 40.50 | 56.81 | 41.00 | 44.38 | 50.37 | 8.91 |
| Nitrogen Free Extract (%) | 67.29 | 73.40 | 68.52 | 68.03 | 69.06 | 4.65 |

a,b: Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.1.7 Villi Morphometry of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.7 shows the villi morphometry of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. There were significant ($P<0.05$) differences in all the parameters measured except for crypt depth. Villi area, perimeter, height, width and villi height/crypt depth ratio were higher for birds fed 125g of phytobiotics than other treatment groups except for birds in the control and oxytetracycline group, which were similar in villi height/crypt depth ratio. Villi area, perimeter, height, width, crypt depth and villi height/crypt depth ratio values were similar for birds fed control and oxytetracycline based diets. The morphology of intestinal villi and crypts has been associated with intestinal function and growth in chickens. Higher intestinal villi are associated with increased absorptive surface area of the intestine and thus, an increased absorptive capacity with resultant higher body weight gain (Kanduri *et al*., 2013). A lower villus/crypt ratio has been associated with the presence of toxins, poor nutritive absorption and increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue (Xu *et al*., 2003). The result obtained from this study indicates that the gut morphology may be responsible for the growth performance of the broiler chickens.

The photomicrograph of the villi in the control group, phytobiotics and AGPs are presented in Figures 4.1, 4.2 and 4.3 respectively. The representative photomicrograph of villi has shown that the crypt depths in the ileum of birds fed the control diets insignificantly ($P>0.05$) decreased while the villi were unevenly distributed in the intestinal lumen. Birds fed diets containing phytobiotics were significantly ($P<0.05$) higher in villus height, villus width and insignificantly

(P>0.05) higher in crypt depth while birds fed diets containing oxytetracycline significantly (P<0.05) increased villus width and insignificantly (P>0.05) increased the crypt depth.

**Table 4.7: Villi Morphometry of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| Area $(\mu m)^2$ | $26231^{b}$ | $19447^{b}$ | $45696^{a}$ | $16585^{b}$ | $25346^{b}$ | 6678.52 |
| Perimeter (µm) | $939.22^{ab}$ | $696.85^{b}$ | $1071.28^{a}$ | $693.68^{b}$ | $861.71^{ab}$ | 106.80 |
| Villi height (µm) | $411.85^{ab}$ | $287.36^{b}$ | $458.83^{a}$ | $277.99^{b}$ | $368.21^{ab}$ | 56.16 |
| Villi width (µm) | $138.08^{ab}$ | $127.44^{ab}$ | $160.05^{a}$ | $123.86^{b}$ | $124.60^{ab}$ | 18.05 |
| Crypt depth (µm) | 68.57 | 75.58 | 71.08 | 71.85 | 69.04 | 12.20 |
| Villi height/crypt (µm) | $5.94^{a}$ | $3.85^{b}$ | $6.40^{a}$ | $3.90^{b}$ | $5.93^{a}$ | 0.64 |

a,b; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

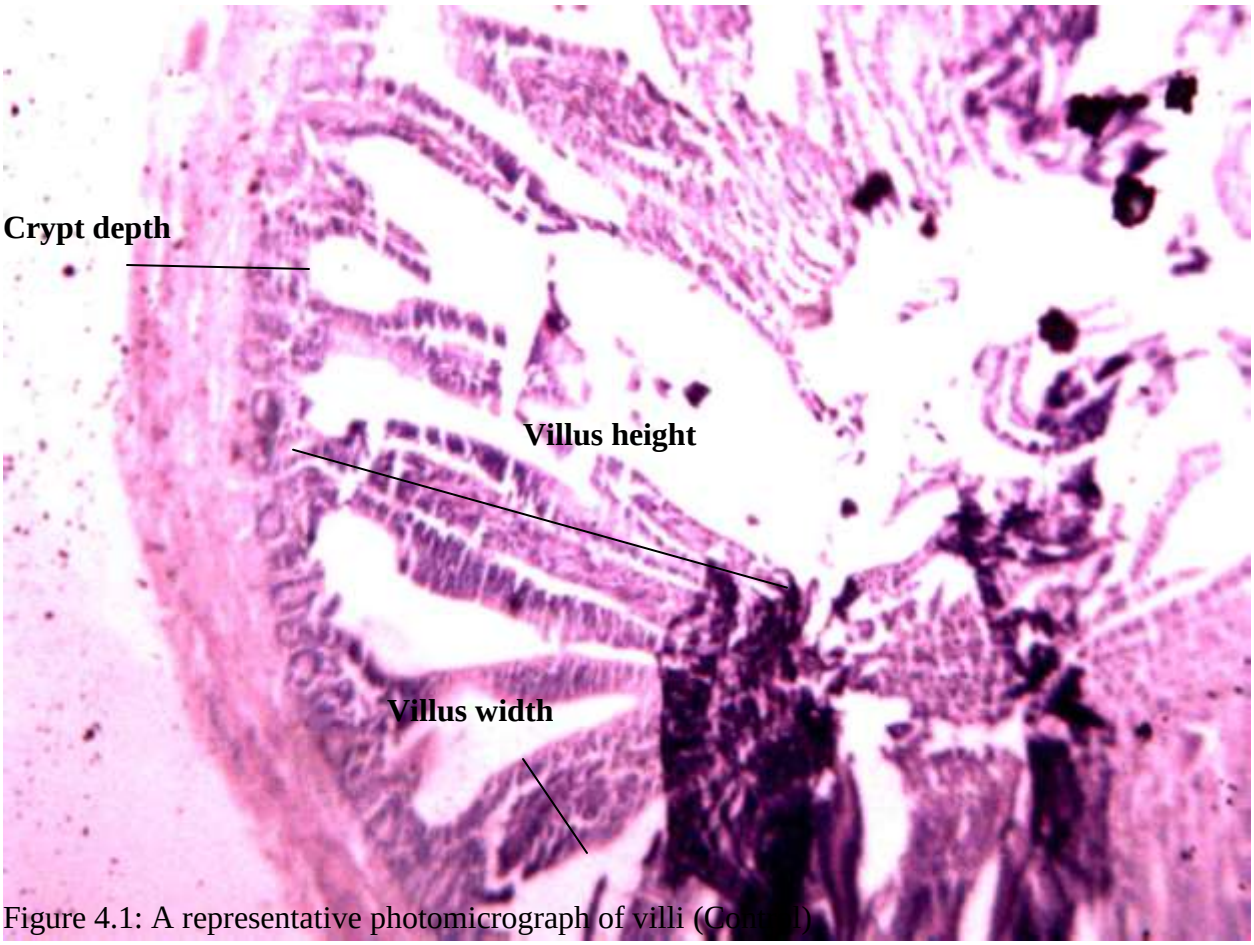

Figure 4.1: A representative photomicrograph of villi (Control)

Figure 4.2: A representative photomicrograph of villi (Phytobiotics)


Figure 4.3: A representative photomicrograph of villi (AGPs)

### 4.1.8 Intestinal Bacteria Count of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics Feed Additives at Finisher Phase (5-7 weeks)

Table 4.8 shows the intestinal bacteria count of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. There were significant (P<0.05) differences for values of *Lactobacillus spp* and *Bacillus spp*, whereas non-significant (P>0.05) differences were observed for *Escherichia coli*, *Clostridium spp* and *Salmonella spp*. The results showed that there was no presence of *Clostridium spp* among the treatment groups. Lactobacillus spp was significantly (P<0.05) higher and best in birds on diet containing 100g phytobiotics ($15.33x10^3$cfu/g) when compared to that of the control ($5.00x10^3$cfu/g) but similar to that fed oxytetracycline ($10.67x10^3$cfu/g). However, the results showed a decrease in the *Lactobacilli spp* count ($15.33\ x10^3$cfu/g, $8.00\ x10^3$cfu/g, $4.00\ x10^3$cfu/g) as the levels of phytobiotics increases (100g, 125g, 150g). The results reveals that inclusion of phytobiotics at 100g improved the beneficial bacteria (*Lactobacillus spp*). Supplementation with phytobiotics also increased the concentration of *Bacillus spp*, which is also a beneficial bacteria. Significant (P<0.05) differences were observed in birds fed diets containing 125g phytobiotics ($9.67\ x10^3$cfu/g) when compared to the control ($4.00\ x10^3$cfu/g) but similar to those fed diets containing 100g phytobiotics ($6.33\ x10^3$cfu/g) and oxytetracycline ($6.33\ x10^3$cfu/g). *Lactobacillus spp* and *Bacilllus spp* are beneficial harmless microbes in the microbiota. Phytobiotics have shown positive effects by increasing the population of beneficial bacteria and displacing the pathogenic bacteria. The colonization of the gastro intestinal tract(GIT) by the beneficial bacteria (*Lactobacillus spp* and *Bacillus spp*) suppresses the activity and habitation of potentially pathogenic species (Rinttila and Apajalahti, 2013). The results revealed that chestnut phytobiotics do not act in bacteriocidal manner but in a bacteriostatic manner, which means thatit

helps to colonize the GIT with beneficial bacteria and reduces the virulence of pathogenic bacteria.

This result is in agreement with the report of Murugesan *et al* (2015) who reported that phytogenic feed additive (phytobiotics) significantly reduced the cecal population of coliforms and fortified the gut microbiota with beneficial bacteria, such as *Lactobacillus spp.* Once the *Lactobacillus spp.* are established, they might selectively exclude the pathogens from adhering due to their fast colonization, proliferation, and acidification properties in the GIT (McReynolds *et al*., 2009). However, Gessica *et al* (2019) reported that diets containing tannin caused positive effects on the immune system, and exerted potent bactericidal and coccidiostatic properties in broiler chickens.

**Table 4.8: Intestinal Bacteria Count of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa)* Phytobiotics feed additives (5-7 weeks)**

| | Levels of Phytobiotics | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** ($10^3$cfu/g) | **0g** | **100g** | **125g** | **150g** | **Oxytet** | **SEM** |
| *Escherichia coli* | 12.66 | 13.67 | 13.67 | 11.00 | 10.33 | 4.45 |
| *Lactobacilli spp* | $5.00^{bc}$ | $15.33^{a}$ | $8.00^{b}$ | $4.00^{c}$ | $10.67^{ab}$ | 3.06 |
| *Clostridium spp* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella spp* | 1.00 | 2.00 | 1.00 | 2.00 | 2.67 | 1.33 |
| *Bacillus spp* | $4.00^{b}$ | $6.33^{ab}$ | $9.67^{a}$ | $3.00^{b}$ | $6.33^{ab}$ | 2.80 |

a,b,c ; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.1.9 Economic Indices of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics feed additives (1-7 weeks)

Table 4.9 shows the economic indices of broiler chickens fed diets containing different levels of chestnut (*Castenea sativa*) phytobiotics feed additives. The feed cost/Kg, mean feed intake, total expenses and mean yield cost were higher in birds placed on phytobiotics and oxytetracycline based diets. This can be attributed to the cost of added levels of chestnut phytobiotics and oxytetracycline. The mean yield cost decreased as the level of phytobiotics increased. Birds placed on oxytetracycline (₦2023.00) and control (₦2016.00) diets were higher in mean yield cost while control diets was best in net profit (₦1004.43) than other treatment groups. This maybe attributed to the additional cost of additives. This underlines the importance of using cost of feed consumed to obtain a unit of product as a basis for recommending feeds to farmers (Ukachukwu and Anugwa, 1995). However, slight difference exist between those birds placed on phytobiotics and other treatment groups although gut welfare was the key focus.

**Table 4.9: Economic Indices of Broiler Chickens Fed Diets Containing Different Levels of Chestnut (*Castenea sativa*) Phytobiotics feed additives (1-7 weeks)**

| Parameter | Levels of Phytobiotics | | | | |
|---|---|---|---|---|---|
| | 0g | 100g | 125g | 150g | Oxytet |
| Feed cost/kg (₦/Kg) | 116.45 | 118.85 | 119.70 | 120.55 | 121.72 |
| Mean feed intake (Kg/b) | 5.08 | 5.17 | 5.06 | 5.02 | 5.26 |
| Mean feeding cost (₦) | 591.57 | 614.45 | 605.68 | 605.16 | 640.25 |
| Cost of chicks (₦) | 220.00 | 220.00 | 220.00 | 220.00 | 220.00 |
| Other expenses (₦) | 200.00 | 200.00 | 200.00 | 200.00 | 200.00 |
| Total expenses (₦) | 1011.57 | 1034.45 | 1025.68 | 1025.16 | 1060.25 |
| Mean final wt. (Kg) | 2.88 | 2.85 | 2.84 | 2.76 | 2.89 |
| Cost of Chicken/Kg (₦) | 700 | 700 | 700 | 700 | 700 |
| Mean yield cost(₦) | 2016.00 | 1995.00 | 1988.00 | 1932.00 | 2023.00 |
| Net profit(₦) | 1004.43 | 960.55 | 962.32 | 906.84 | 962.75 |

Oxytet; Oxytetracycline, Other expenses = Cost of multi-vitamins, repairs etc; Mean yield cost = Cost of chicken/Kg x Mean final weight (Kg); Net pofit = Mean yield cost – Total expenses.

## 4.2 Experiment 2: Performance Evaluation of Broiler Chickens Fed Diets Containing Graded Levels of Zinc Sulphate.

### 4.2.1 Growth Performance of Broiler Chicks Fed Diets Containing Different Levels of Zinc Sulphate (0-4 weeks).

Table 4.10 shows the growth performance of broiler chicks fed diets containing different levels of zinc. There were significant (P<0.05) differences in final weight, weight gain, feed conversion ratio and feed cost/Kg gain, whereas non-significant differences were observed for feed intake, feed cost/Kg and mortality. Birds placed on diets with 10mg inclusion of zinc were significantly (P<0.05) higher in final weight (783.33g/bird) and weight gain (742.16g/bird). This result agrees with the findings of Huang *et al* (2007) who reported that weight gain and feed intake of broiler chicks were significantly increased with dietary zinc level. The results revealed that inclusion of zinc at low level significantly (P<0.05) improved the final weight (783.33g/bird), weight gain (742.16g/bird), feed conversion ratio (1.49) and feed cost per kg gain (183.56₦/kg gain). Although, there was no particular trend in the level of inclusion. Feed conversion ratio was significantly lower and best in birds placed on diets with 10mg zinc (1.49) followed by birds placed on diets with 30mg zinc (1.52), control (1.51), 20mg zinc (1.6) and oxytetracycline (1.61) respectively. Feed cost per kg gain was significantly lower in birds placed on diets with 10mg zinc (183.56₦/kg gain) and 30mg of zinc (186.46₦/kg gain), followed by the control group (193.19₦/kg gain).

**Table 4.10: Growth Performance of Broiler Chicks Fed Diets Containing Different Levels of Zinc Sulphate (0-4 weeks)**

| Levels of Zinc inclusion | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| Initial weight (g/b) | 41.17 | 41.17 | 41.17 | 41.17 | 41.17 | 0.00 |
| Final weight (g/b) | 730.66 $^{b}$ | 783.33$^{a}$ | 719.33 $^{b}$ | 753.00$^{ab}$ | 720.33 $^{b}$ | 15.70 |
| Weight gain (g/b) | 689.49 $^{b}$ | 742.16 $^{a}$ | 678.16 $^{b}$ | 711.83 $^{ab}$ | 679.16 $^{b}$ | 15.70 |
| Feed intake (g/b) | 1084.67 | 1108.67 | 1084.67 | 1080.33 | 1090.67 | 15.24 |
| Feed conversion ratio | 1.57$^{ab}$ | 1.49$^{a}$ | 1.60$^{b}$ | 1.52$^{ab}$ | 1.61$^{b}$ | 0.04 |
| Feed cost/gain (₦/Kg gain) | 193.19$^{ab}$ | 183.56 $^{a}$ | 196.83$^{b}$ | 186.46$^{a}$ | 204.59$^{b}$ | 4.92 |
| Mortality (%) | 0.33 | 0.67 | 0.33 | 0.33 | 0.67 | 0.42 |

a,b; Means with different superscripts on the same row are significantly different (P<0.05). SEM; Standard Error of Means
Oxytet; Oxytetracycline

### 4.2.2 Growth Performance of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.11 shows the growth performance of broiler finisher chicken fed diets containing different levels of zinc. Significant ($P<0.05$) differences were observed for final weight, weight gain, feed intake, feed conversion ratio, feed cost/kg gain and mortality whereas non-significant ($P>0.05$) difference was observed for feed cost/Kg. No particular trend was observed for birds fed diet with different levels of zinc sulphate except in the final weight. The result indicates that oxytetracycline treatment group had significantly ($P<0.05$) higher final weight (2756.67g/bird), weight gain (2036.33g/bird) and feed intake (3566.00g/bird) compared to other treatment groups but, statistically similar to zinc treatment group (10mg, 30mg) for final weight, weight gain and feed intake. Feed intake was statistically similar among zinc dietary levels. This result disagrees with the findings of Ao *et al* (2006), who reported that feed intake was increased quadratically with increasing levels of dietary zinc in broiler chickens. In addition, Batal *et al*(2001) reported that weight gain, feed intake, and feed efficiency responded quadratically to graded levels of supplemental zinc in broiler chickens. Feed conversion ratio was significantly lower and best in birds placed on diets with oxytetracycline (1.75) and 10mg zinc (1.79). This result reveals that zinc at lower level (10mg) and oxytetracycline have similar effect on feed conversion. The cost per kg gain was significantly lower in birds placed on 10mg zinc (209.92₦/kg gain) compared to the control group. Mortality was significantly lower in birds fed diets containing zinc levels but statistically similar with birds placed on control and oxytetracycline diets.

**Table 4.11: Growth Performance of Broiler Finisher Chickens Fed Diets Containing Different Levels of**

**Zinc Sulphate (5-8 weeks)**

| **Levels of Zinc inclusion** | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| Initial weight (g/b) | 730.66 [b] | 783.33 [a] | 719.33 [b] | 753.00 [ab] | 720.33 [b] | 15.70 |
| Final weight (g/b) | 2507.67[b] | 2730.67 [ab] | 2580.33[b] | 2669.67 [ab] | 2756.67 [a] | 72.03 |
| Weight gain (g/b) | 1777.00[b] | 1947.33 [ab] | 1861.00[b] | 1916.67 [ab] | 2036.33 [a] | 70.91 |
| Feed intake (g/b) | 3361.00[b] | 3489.67[ab] | 3442.00[ab] | 3483.67[ab] | 3566.00[a] | 98.00 |
| Feed conversion ratio | 1.89 [b] | 1.79 [a] | 1.84 [b] | 1.82 [ab] | 1.75 [a] | 0.04 |
| Feed cost/gain (₦/Kg gain) | 221.79[b] | 209.92[a] | 216.44[ab] | 213.24[ab] | 213.19[ab] | 4.48 |
| Mortality (%) | 3.00[ab] | 1.00[a] | 2.00[a] | 1.33[a] | 2.33[ab] | 0.61 |

a,b; Means with different superscripts on the same row are significantly different (P<0.05). SEM; Standard Error of Means
Oxytet; Oxytetracycline

### 4.2.3 Haematological Indices of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.12 shows the haematological indices of broiler chickens fed diets containing different levels of zinc. There were significant ($P<0.05$) difference in WBC, lymphocytes, monocytes, basophils, MCV and MCH whereas there were nosignificant ($P>0.05$) differences in the values for PCV, Hb, RBC, heterophils and eosinophils. Birds which had access to 20mg ($93.33x10^3/\mu L$), 30mg zinc and oxytetracycline were significantly ($P<0.05$) higher in WBC when compared to the control group ($59.40x10^3/\mu L$) but statistically similar to those of the birds placed on 10mg ($72.97x10^3/\mu L$) zinc. Report by Mitruka and Rawnsley (1977) had shown that higher value of white blood cells depicts better health of the bird. However, too high or too low white blood cells may be an indication of infection. There was significant difference in lymphocytes which ranges from 83.20-90.27% with the control group having the highest value whereas 30mg zinc inclusion was least. However, all the values for lymphocytes fall within the normal range of 40-100% as reported by Jain, (1993). There was significant ($P<0.05$) difference in monocytes which range from 4.40-6.20% with birds on diets with 20mg zinc inclusion having the highest (6.2%), followed by 30mg zinc, oxytetracycline, 10mg zinc and the control group being the least. Birds placed on diets with 10mg zinc inclusion were significantly ($P<0.05$) different in the values of MCV (147.10fl) and MCH (46.67pg). However, significant difference was observed in MCV (145.83fl) and in birds placed on control diet (145.83fl). The immunomodulatory effect of zinc also resulted in an increase in the activity of thymocytes, macrophages,and heterophils, as well as increased antibody production,which enhances the potential of the humoral response (Wellinghausen *et al.,* 1997).

**Table 4.12: Haematological Indices of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| Levels of Zinc inclusion | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** | **REF. V** |
| PCV (%) | 28.00 | 25.13 | 35.97 | 35.35 | 37.87 | 7.43 | 24.00-44.0$^{+}$ |
| Hb (g/dL) | 8.50 | 7.73 | 11.33 | 10.90 | 11.53 | 2.23 | 9.10-13.90$^{+}$ |
| RBC (x10$^{6}$/uL) | 1.93 | 1.74 | 2.51 | 2.57 | 2.70 | 0.52 | 2.00-3.00$^{++}$ |
| WBC (x10$^{3}$ /uL ) | 59.40$^{b}$ | 72.97$^{ab}$ | 93.33$^{a}$ | 92.00$^{a}$ | 90.83$^{a}$ | 14.92 | - |
| Heterophils (%) | 1.90 | 1.97 | 4.60 | 4.55 | 2.40 | 1.18 | - |
| Lymphocytes (%) | 90.27$^{a}$ | 89.10$^{ab}$ | 85.70$^{bc}$ | 83.20$^{c}$ | 90.00$^{ab}$ | 2.32 | 40-100$^{++}$ |
| Monocytes (%) | 4.40$^{b}$ | 5.57$^{ab}$ | 6.20$^{a}$ | 5.70$^{ab}$ | 4.87$^{ab}$ | 0.74 | 1.00-7.00$^{++}$ |
| Eosinophils (%) | 2.67 | 2.43 | 2.73 | 2.90 | 1.73 | 0.62 | - |
| Basophils (%) | 0.77$^{b}$ | 0.93$^{b}$ | 0.77$^{b}$ | 1.40$^{a}$ | 1.00$^{b}$ | 0.12 | 0.00-2.00$^{+++}$ |
| MCV (fL) | 145.83$^{a}$ | 147.10$^{a}$ | 143.27$^{ab}$ | 138.10$^{c}$ | 140.5$^{bc}$ | 1.93 | 90-140$^{++}$ |
| MCH (pg) | 45.87$^{ab}$ | 46.67$^{a}$ | 45.10$^{ab}$ | 42.70$^{b}$ | 42.83$^{b}$ | 1.60 | 33-47$^{++}$ |

a,b; Means with different superscripts on the same row are significantly different (P<0.05) SEM; Standard Error of Means
Oxytet; Oxytetracycline, PCV=Packed Cell Volume, RBC=Red Blood Cell, WBC= White Blood cell, Hb=Haemoglobin concentration, MCV=Mean Corpuscular Volume, MCH=Mean corpuscular haemoglobin, REF.V= Reference value, $^{+}$Mitruka and Rawnsley 1977, $^{++}$Jain 1993, $^{+++}$Nanbol *et al*., 2016.

### 4.2.4 Serum Biochemical Parameters of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.13shows the serum biochemical parameters of broiler chickens fed diets containing different levels of zinc. There were significant ($P<0.05$) difference in all the parameters measured which comprises of glucose, total protein, albumin, globulin, aspartate amino transferase (AST), alalnine amino transferase (ALT) and alkaline phosphatase (ALP). Glucose was significantly ($P<0.05$) higher in birds on the treatment diet that contained 20mg zinc (87.71mg/dL) compared to those with 10mg zinc but however, similar with other treatment groups. The result for total protein, albumin and globulin were significantly ($P<0.05$) influenced by the dietary treatments. The result for albumin presented similar trend with globulin in broiler chicken on diets containing 10mg, 20mg and 30mg zinc. Albumin was significantly ($P<0.05$) higher in birds placed on control diet,10mg zinc (1.73g/dL) and 20mg zinc followed by 30mg zinc (1.17g/dL) and oxytetracycline (1.21g/dL). Birds placed on control diets had significantly ($P<0.05$) higher valueof total protein(2.84g/dL). The value of globulin for birds on diets with 30mg zinc (1.07g/dL) and control (1.06g/dL) were higher ($P<0.05$) when compared to other treatment groups. However, the improved total protein, albumin and globulin levels as observed in this experiment suggested that the diets are capable of supplying the protein needed by the birds. The result for AST, ALT and ALP were significantly ($P<0.05$) influenced by the dietary treatment that resulted to an increase in the amount of these enzymes which were produced by the liver. Control diet had significantly($P<0.05$) higher value for AST and ALT while 30mg zinc had significantly ($P<0.05$) higher value also for ALT. This work is in accordance with the report of Idowu *et al* (2011) who observed significant difference in the levels of serum alkaline phosphatase (ALP) and serum zinc concentrations between control and the other treatments and

opined that due to zinc binding capacity of serum, alkaline phosphatase (ALP) acts as good indicator of zinc status. Furthermore, zinc is a cofactor for hepatic enzymes such as ALP, AST, and ALT (Bennett *et al*., 2001) and interferes in many metabolic and enzymatic functions (Prasad *et al*., 2009). Das *et al*., (2014) reported that these hepatic enzymes activity were affected by organic Zn in diets. ALP is an important protein marker in bone for osteoblast differentiation (Graneli *et al.,* 2014). High ALP activity usually is symptoms of either liver damage or increased-activity of cell bone (Sarac and Saygili, 2007). However, the higher levels of ALP observed in birds which received 30mg zinc also reflected in significant increase in the weight of the legs (4.22%) under carcass characteristics. Although, it did not affect the birds negatively. It could be concluded that higher ALP level in the birds which received the diet supplemented with 30mg zinc occurred because of higher bone growth and development and this was evident in weight of legs of the birds.

**Table 4.13: Serum Biochemical Parametersof Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| Parameter | Levels of Zinc inclusion | | | | |
|---|---|---|---|---|---|
| | 0g | 10mg | 20mg | 30mg | OxytetSEMREF. V |
| Glucose (mg/dL) | 83.67$^{ab}$ | 69.70$^{b}$ | 87.71$^{a}$ | 84.89$^{ab}$ | 78.85$^{ab}$5.11 - |
| Total Protein (g/dL) | 2.84$^{a}$ | 2.41$^{ab}$ | 2.71$^{ab}$ | 2.24$^{b}$ | 2.04$^{b}$0.22 3.60-5.50+ |
| Albumin (g/dL) | 1.78$^{a}$ | 1.73$^{a}$ | 1.69$^{a}$ | 1.17$^{b}$ | 1.21$^{b}$0.06 1.10-2.20+ |
| Globulin (g/dL) | 1.06$^{b}$ | 0.67$^{b}$ | 1.02$^{b}$ | 1.07$^{b}$ | 22.61$^{a}$9.70 1.20-3.20++ |
| AST **(**µ/L**)** | 47.71$^{a}$ | 39.95$^{b}$ | 38.50$^{b}$ | 39.52$^{b}$ | 41.28$^{b}$1.97100-400++ |
| ALT **(**µ/L**)** | 18.24$^{a}$ | 17.46$^{ab}$ | 13.86$^{b}$ | 15.41$^{ab}$ | 15.82$^{ab}$2.11 - |
| ALP **(**µ/L**)** | 80.65$^{ab}$ | 79.25$^{ab}$ | 77.44$^{b}$ | 85.97$^{a}$ | 73.80$^{b}$3.33 10-106+++ |

a,b; Means with different superscripts on the same row are significantly different (P<0.05): Aspartate amino transferase (AST): Alanine amino transferase (ALT): Alkaline Phosphatase (ALP): Albumin(ALB): Globulin (GLB): Oxytet; Oxytetracycline; SEM: Standard error of mean. $^{+}$Ross *et al* (1976), $^{++}$LAVC (2009), $^{+++}$Bounous and Stedman (2000).

### 4.2.5 Lipid Profile of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.14 shows the lipid profile of broiler chickens fed diets containing different levels of zinc. There were significant ($P<0.05$) differences in for all the parameters measured. Oxytetracycline inclusion in the diets of the chickens resulted to a significant ($P<0.05$) increase in the total cholesterol (90.63mg/dL) whereas 20mg zinc was the least (74.61mg/dL). Triglyceride was significantly ($P<0.05$) different across the treatment groups, with birds on diet with 30mg zinc (95.49g/dL) having the highest value while those on diets with 20mg zinc had the least (24.49g/dL). This work contradicts the report by Herzig *et al* (2009) who reported that there was a significant decrease of plasma cholesterol when broilers were fed with high amount of Zn in the diet. This result did not follow a particular trend; however, addition of 20mg zinc drastically reduced the triglyceride level in the blood. Low cholesterol reduces the occurrence of cardiovascular disease. High density lipoprotein and low density lipoprotein were not statistically different; they are the major transporters of glycerides in the system.

**Table 4.14: Lipid profile of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| Levels of Zinc inclusion | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| TCHOL (mg/dL) | $80.04^{bc}$ | $86.13^{ab}$ | $74.61^{c}$ | $79.60^{bc}$ | $90.63^{a}$ | 4.73 |
| Triglyceride (g/dL) | $78.12^{ab}$ | $77.77^{ab}$ | $24.49^{c}$ | $95.49^{a}$ | $63.81^{b}$ | 14.25 |
| HDL (mg/dL) | $25.19^{c}$ | $52.63^{a}$ | $39.83^{abc}$ | $32.97^{bc}$ | $47.09^{abc}$ | 9.31 |
| LDL (mg/dL) | $39.58^{a}$ | $23.84^{b}$ | $27.46^{ab}$ | $27.53^{ab}$ | $28.04^{ab}$ | 7.78 |

a,b,c; Means with different superscripts on the same row are significantly different (P<0.05): Total Cholesterol (TCHOL): High Density Lipoprotein (HDL): Low Density Lipoprotein (LDL): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.2.6 Total Tract Apparent Nutrient Digestibility of Broiler Chicken Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.15 shows the apparent total tract percent nutrient digestibility of broiler chickens fed diets containing different levels of zinc. There were significant ($P<0.05$) differences in crude protein digestibility with that group of birds fed diets with 10mg (76.47%), 20mg (78.95%), 30mg (80.66%) and oxytetracycline (76.87%) level having higher values compared to that of the control (64.18%). The inclusion of zinc tends to increase the apparent nutrient digestibility of crude protein across the treatment. Significant ($P<0.05$) differences were observed for the birds placed on diets with 10mg, 20mg, 30mg zinc and oxytetracycline having higher values for crude protein, ether extract and nitrogen free extract whereas the control has the least values for the parameters measured. The result obtained shows that the control diet was not efficiently utilized. There was significant ($P<0.05$) difference in crude fibre digestibility with the group fed diets containing 30mg dietary zinc level having higher means (73.30%) compared to the other treatment groups but similar to those fed diets containing 20mg (63.58%) dietary zinc level. The results from this work indicates that crude protein, ether extract, crude fibre and nitrogen free extract increased with an increase in the levels of zinc. High significant ($P<0.05$) difference were observed in the values of nitrogen free extract in birds fed diets containing 10mg (72.68%), 20mg (74.45%), 30mg (78.07%) zinc and oxytetracycline (72.50%) whereas the control has the least value (57.82%). The results reveal that zinc and oxytetracycline inclusion greatly improved soluble carbohydrate digestibility and utilization. These results are in harmony with other studies (Sahin and Kucuk, 2003; Sahin *et al*., 2009) which noted that increasing supplemental Zn (0, 30 and 60 mg/kg) linearly increased digestibility of dry matter, organic matter, crude protein and ether extract.

**Table 4.15: Apparent Total Tract Percent Nutrient Digestibility of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks).**

| | Levels of Zinc inclusion | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| Crude Protein (%) | $64.18^{b}$ | $76.47^{ab}$ | $78.95^{ab}$ | $80.66^{a}$ | $76.87^{ab}$ | 3.92 |
| Ether Extract (%) | $64.44^{b}$ | $75.21^{ab}$ | $77.70^{ab}$ | $80.53^{a}$ | $75.71^{ab}$ | 4.09 |
| Crude Fibre (%) | $53.04^{b}$ | $61.69^{b}$ | $63.58^{ab}$ | $73.30^{a}$ | $60.71^{b}$ | 5.73 |
| Nitrogen Free Extract (%) | $57.82^{b}$ | $72.68^{ab}$ | $74.45^{ab}$ | $78.07^{a}$ | $72.50^{ab}$ | 4.61 |

a,b; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.2.7 Villi Morphometry of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.16 shows the villi morphometry of broiler chickens fed diets containing different levels of zinc. The results show significant ($P<0.05$) differences in villi area, width, height/crypt depth ratio while non-significant ($P>0.05$) differnces were observed in perimeter, height, crypt depth. Villi area (29919µm$^2$), width (164.36µm), height/crypt depth ratio (5.03µm) were higher in birds fed the control diet than other treatment groups except for oxytetracycline which was similar with villi height/crypt depth ratio (5.20µm). The value for villi width and height/crypt depth ratio decreased with an increase in the levels of zinc. This reveals that dietary zinc at lower level improved the villi height, width and height/crypth depth ratio. This work is in agreement with the study conducted by Levkut *et al* (2017) who demonstrated that inorganic zinc source ($ZnSO_4$) increased the height of villi and surface area of villi after supplementation of feed with a low dose of zinc. The height of villi and their area can influence the source of supplemented zinc in diets (Lonnerdal, 2000). Zinc is also known to influence the intestinal morphology and improve absorptive capacity, and enhance growth performance (Katouli *et al.,* 1999; Feng *et al.*, 2010). The morphology of intestinal villi and crypts has been associated with intestinal function and growth in chickens.

The photomicrograph of the villi in the control, zinc supplemented and AGPs groups are presented in Figures 4.4, 4.5 and 4.6 respectively. The representative photomicrograph of villi has shown that the villus height in the ileum of birds fed the control diets insignificantly($P>0.05$) increased while the villus width were significantly($P<0.05$) the highest when compared to other treatment. Birds fed diets containing zinc were insignificantly($P>0.05$) high in villus height and

crypt width while the villus width were significantly($P<0.05$)high. Birds fed diets containing oxytetracycline insignificantly($P>0.05$) increased the villus height, crypt depth and significantly ($P<0.05$)increased the villus width.

**Table 4.16: Villi Morphometry of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| Parameter | Levels of Zinc inclusion | | | | | |
|---|---|---|---|---|---|---|
| | 0g | 10mg | 20mg | 30mg | Oxytet | SEM |
| Area $(\mu m)^2$ | $29919^{a}$ | $18149^{b}$ | $22512^{ab}$ | $15478^{b}$ | $23948^{ab}$ | 5245.62 |
| Perimeter (µm) | 858.28 | 648.54 | 791.04 | 611.78 | 890.94 | 142.29 |
| Villi height (µm) | 351.17 | 258.88 | 338.19 | 268.10 | 391.05 | 66.84 |
| Villi width (µm) | $164.36^{a}$ | $122.61^{ab}$ | $113.56^{b}$ | $85.76^{b}$ | $111.66^{b}$ | 21.91 |
| Crypth depth (µm) | 70.35 | 70.69 | 92.01 | 90.79 | 74.25 | 14.48 |
| Villi height/crypt depth (µm) | $5.03^{a}$ | $3.63^{b}$ | $3.49^{b}$ | $3.22^{b}$ | $5.20^{a}$ | 0.52 |

a,b, c; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

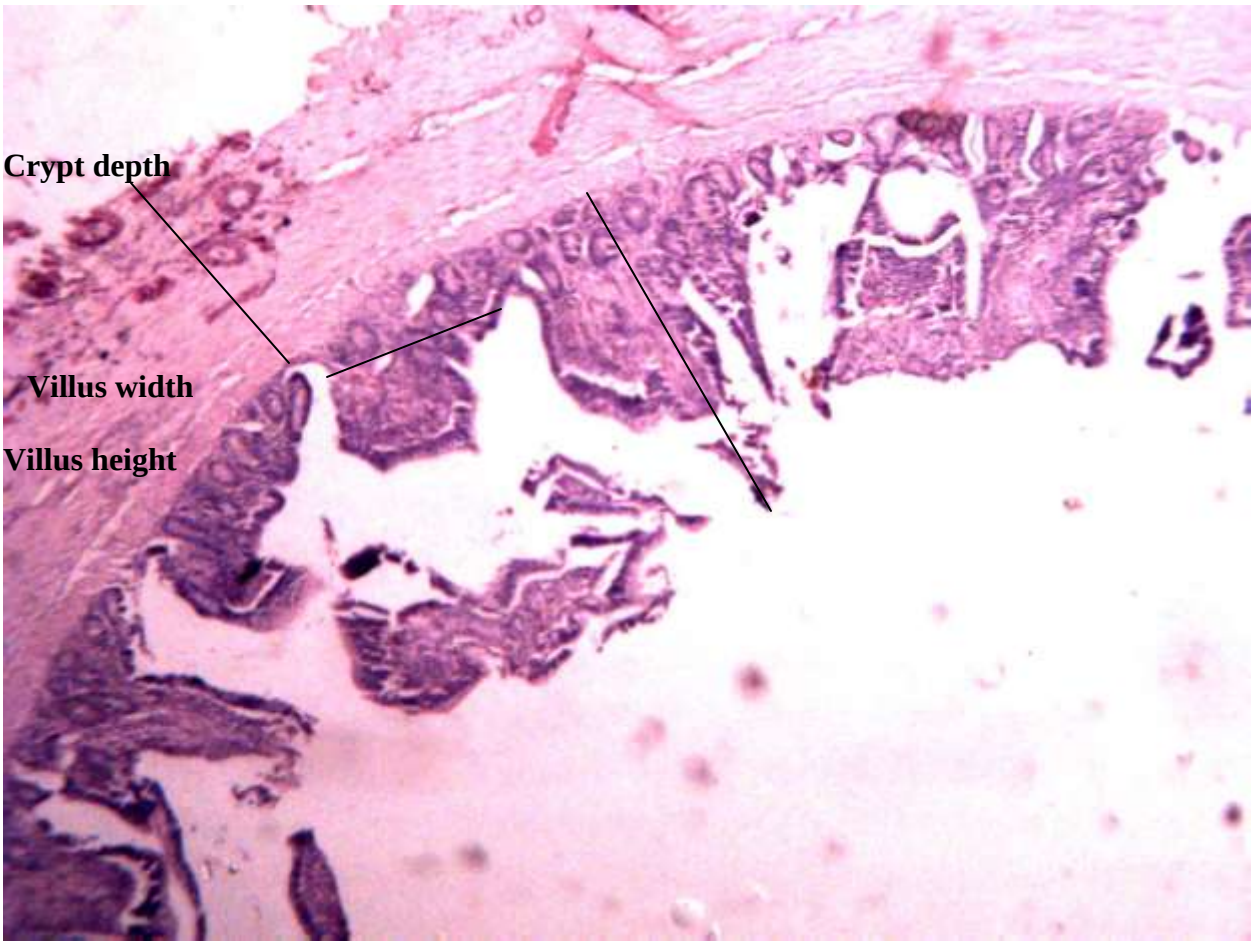


Figure 4.4: A representative photomicrograph of villi (Control)

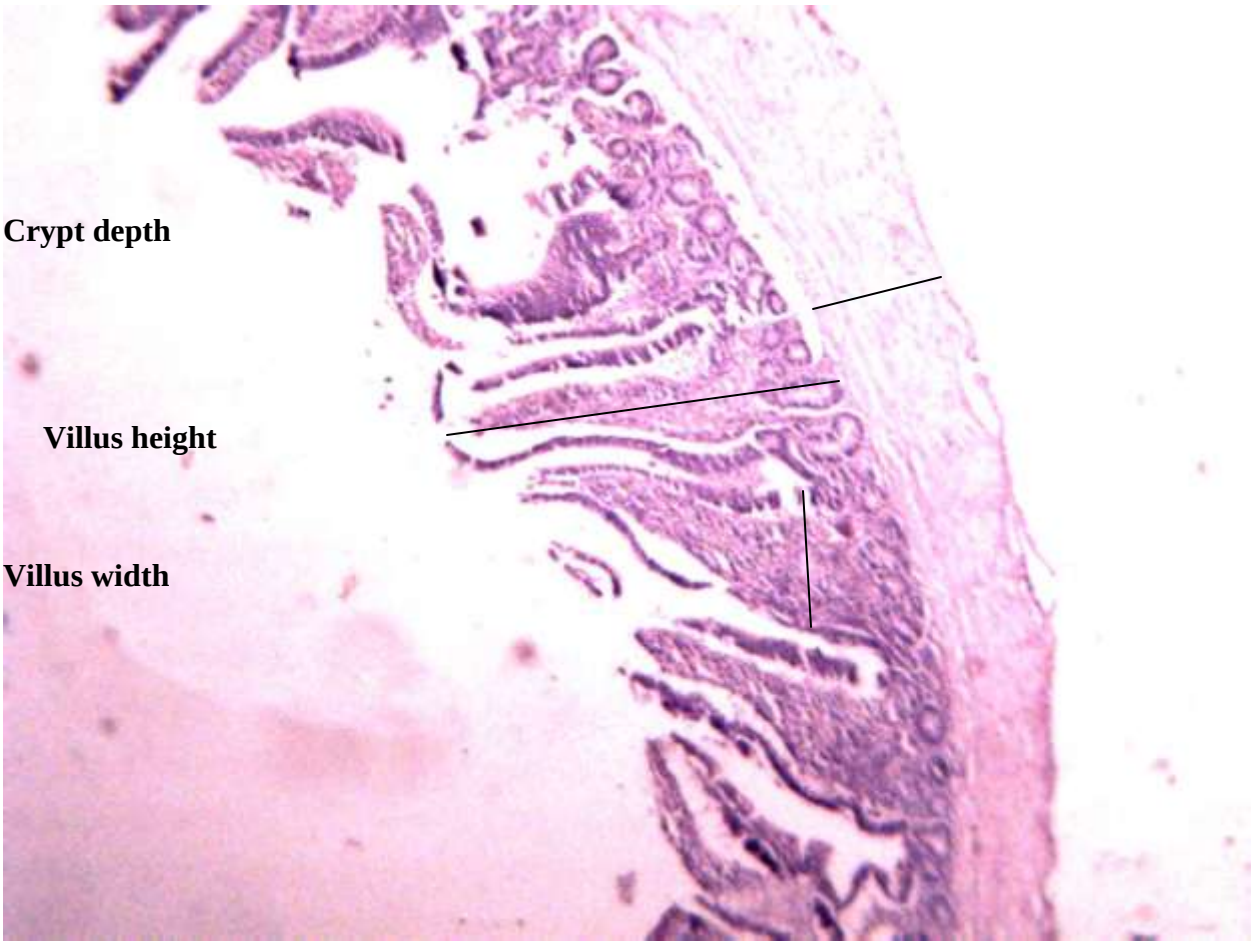


Figure 4.5: A representative photomicrograph of villi (Zinc)

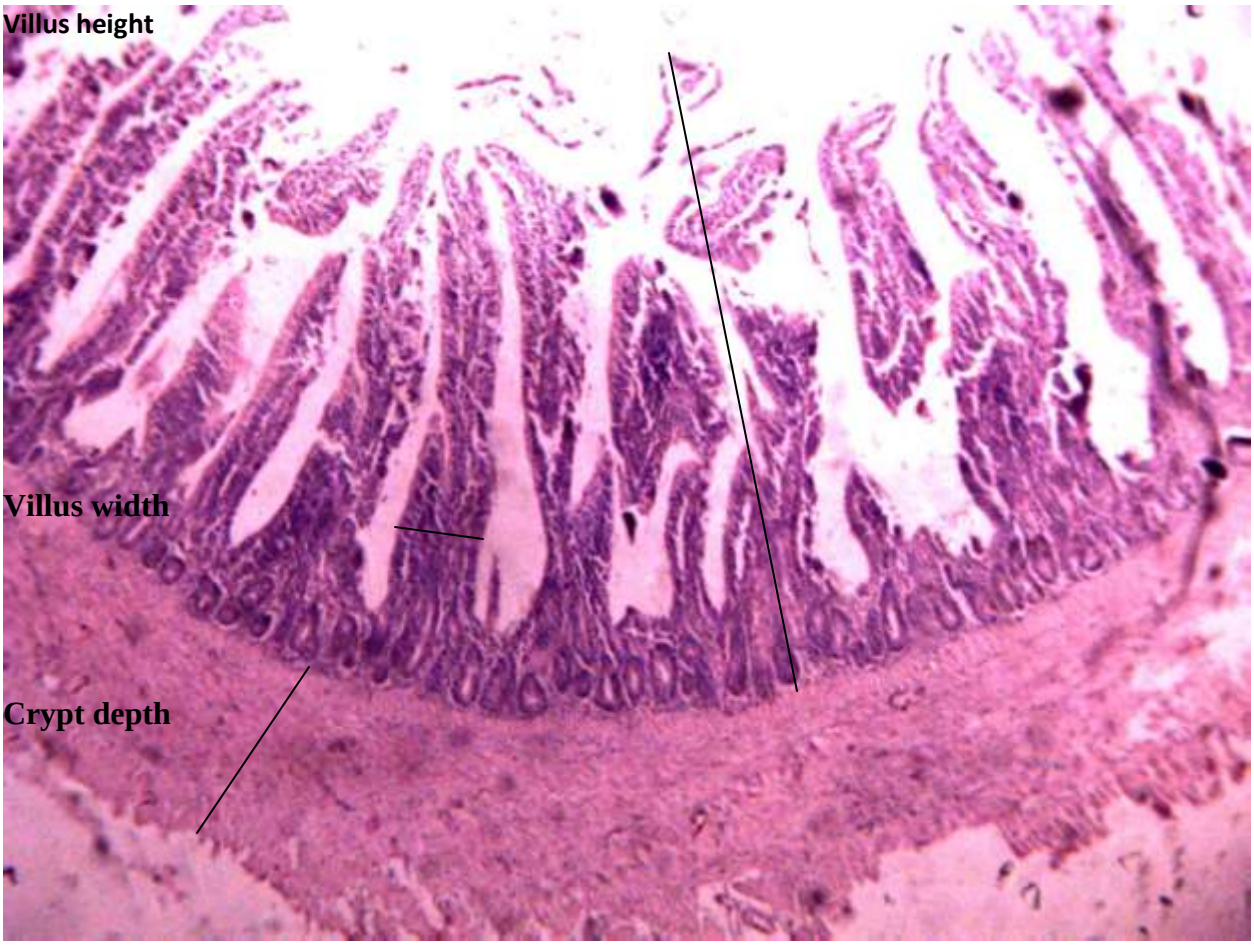


Figure 4.6: A representative photomicrograph of villi (AGPs)

### 4.2.8 Intestinal Bacteria Count of Broiler Chickens Fed Diets Containing Different Levels of Zinc sulphate (5-8 weeks)

Table 4.17 shows the intestinal bacteria count of broiler chickens fed diets containing different levels of zinc. The results show significant ($P<0.05$) differences in *Lactobacilli spp* and *Salmonella spp* whereas non-significant($P>0.05$) differences were observed in *Escherichia coli*, *Clostridium spp* and *Bacillus spp.* The results show that there was no presence of *Clostridium spp* among the treatment groups. *Lactobacilli spp* was significantly ($P<0.05$) higher and best in birds placed on the control diet ($15.00x10^3$cfu/g), followed by those on diets with 20mg zinc ($9.67x10^3$cfu/g) dietary level. *Salmonella spp* was significantly ($P<0.05$) lower in birds which had access to diets with 20mg zinc ($0.00x10^3$cfu/g) but statistically similar with those on the control diet ($1.67x10^3$cfu/g) and diets containing 30mg zinc ($2.67x10^3$cfu/g). This result reveals that dietary zinc at 20mg level was able to eliminate *Salmonella spp* in the gut. Dietary level at 20mg zinc regulated the microbial community by increasing the number of beneficial *Lactobacillus* bacteria ($9.67x10^3$cfu/g), and eliminating the number of *Salmonella spp* ($0.00x10^3$cfu/g) drastically. This report is in agreement with the report of Shao *et al* (2014) who reported that lower concentration of Zn restored the cecal microbial community balance after *Salmonella typhimurium* challenge by increasing *Lactobacillus* and reducing *Salmonella* colonization. Similar report by Zhang *et al* (2012) showed that supplementation with zinc in the diet was very effective in improving growth performance and enhancing intestinal barrier function against *Salmonella* infection. Zinc was shown to have an important function in resisting diseases, improving wound healing and maintaining epithelial tissue integrity (Vallee and Falchuk, 1993).

**Table 4.17: Intestinal Bacteria Count of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| | Levels of Zinc inclusion | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** ($10^3$cfu/g) | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| *Escherichia coli* | 17.33 | 7.67 | 7.67 | 10.33 | 9.67 | 5.58 |
| *Lactobacilli spp* | 15.00$^{a}$ | 3.33$^{b}$ | 9.67$^{ab}$ | 2.33$^{b}$ | 4.00$^{b}$ | 3.70 |
| *Clostridium spp* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Salmonella spp* | 1.67$^{ab}$ | 4.67$^{a}$ | 0.00$^{b}$ | 2.67$^{ab}$ | 4.00$^{a}$ | 1.90 |
| *Bacillus spp* | 4.33 | 2.33 | 7.33 | 6.00 | 4.33 | 3.82 |

a,b; Means with different superscripts on the same row are significantly different (P<0.05): Oxytet; Oxytetracycline; SEM: Standard error of mean.

### 4.2.9 Carcass Characteristics of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)

Table 4.18 shows the carcass characteristics of broiler chickens fed diets containing different levels of zinc. The results revealed non-significant (P>0.05) difference in the wings, gizzard, heart and liver whereas significant (P<0.05) differences were observed in other parameters. Birds placed on diet containing 10mg zinc and oxytetracycline were significantly (P<0.05) higher in live weight, dressed weight and dressing percent whereas birds placed on oxytetracycline was significantly (P<0.05) higher in kidney (1.54%). Birds placed on diets with 10mg zinc were significantly (P<0.05) higher in breast (33.89%), back (19.14%) whereas those on 20mg dietary zinc were significantly (P<0.05) higher in drumstick (12.09%) and head (2.77%); also those on diets with 30mg dietary zinc were significantly (P<0.05) higher in thigh (14.32%), legs (4.22%) and lungs (0.70%). The results observed for organ weight were not consistent with those observed by Hernandez *et al* (2004) who did not observe differences among the control treatment and those containing antibiotic for weights of 42 day-old broilers. Carcass is an important measurement for meat-type birds. Jahanian *et al* (2008) observed that the breast meat yield increased with organic zinc inclusion (40, 80 or 120 mg/kg). Organic supplementations with zinc led to a significant increase in liver, breast and carcass weight percentages (Abd El-Hack*et al.*,2017).

**Table 4.18: Carcass Characteristics of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (5-8 weeks)**

| Levels of Zinc inclusion | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** | **SEM** |
| Live wt. (g/bird) | 2665.33$^{b}$ | 2791.33$^{a}$ | 2682.33$^{b}$ | 2684.67$^{b}$ | 2756.67$^{a}$ | 26.54 |
| Dressed wt. (g/bird) | 2026.67$^{b}$ | 2270.00$^{a}$ | 2026.67$^{b}$ | 2116.67$^{ab}$ | 2243.33$^{a}$ | 64.57 |
| Dressing percent (%) | 76.54$^{ab}$ | 81.32$^{a}$ | 75.54$^{b}$ | 78.83$^{ab}$ | 81.41$^{a}$ | 2.74 |
| **Cut parts expressed as percentage of dressed weight (%)** | | | | | | |
| Breast | 33.21$^{ab}$ | 35.89$^{a}$ | 32.36$^{b}$ | 35.13$^{ab}$ | 31.04$^{b}$ | 1.65 |
| Back | 17.57$^{ab}$ | 19.14$^{a}$ | 18.17$^{ab}$ | 15.75$^{b}$ | 16.00$^{b}$ | 0.96 |
| Thigh | 12.74$^{b}$ | 12.28$^{b}$ | 12.87$^{ab}$ | 14.32$^{a}$ | 13.10$^{ab}$ | 0.77 |
| Drumstick | 12.01$^{a}$ | 10.90$^{b}$ | 12.09$^{a}$ | 11.93$^{ab}$ | 10.55$^{b}$ | 0.48 |
| Wings | 9.26 | 8.74 | 8.85 | 9.51 | 8.58 | 0.54 |
| Neck | 6.09$^{a}$ | 5.21$^{b}$ | 5.91$^{ab}$ | 5.50$^{ab}$ | 5.03$^{b}$ | 0.40 |
| Head | 2.44$^{b}$ | 2.17$^{c}$ | 2.77$^{a}$ | 2.44$^{b}$ | 2.47$^{b}$ | 0.11 |
| Legs | 3.97$^{ab}$ | 3.69$^{b}$ | 4.03$^{ab}$ | 4.22$^{a}$ | 3.58$^{b}$ | 0.22 |
| **Organ weights expressed as percentage of live weight (%)** | | | | | | |
| Gizzard | 1.76 | 1.91 | 1.91 | 1.87 | 1.98 | 0.12 |
| Heart | 0.29 | 0.36 | 0.40 | 0.37 | 0.33 | 0.03 |
| Lungs | 0.51$^{b}$ | 0.63$^{ab}$ | 0.50$^{b}$ | 0.70$^{a}$ | 0.53$^{b}$ | 0.08 |
| Liver | 0.51 | 0.63 | 0.50 | 0.70 | 0.53 | 0.18 |
| Kidney | 1.20$^{b}$ | 1.31$^{b}$ | 1.49$^{ab}$ | 1.38$^{b}$ | 1.54$^{a}$ | 0.06 |

a,b,c; Means with different superscripts on the same row are significantly different (P<0.05) SEM; Standard Error of Means
Oxytet; Oxytetracycline.

### 4.2.10 Economic Indices of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (1-8 weeks)

Table 4.19 shows the economic indices of broiler chickens fed diets containing different levels of zinc. The result shows that feed cost/Kg (119.95₦/Kg) was the same across the treatment groups except for the group on diets with oxytetracycline (124.35₦/Kg) which had a higher value. This indicates that antibiotic group promoter (AGP) increases the feed cost/Kg of diet. The control group had the least value for cost of mean feed intake (₦532.28). Birds placed on diets with phytobiotics and oxytetracycline were higher in mean feeding cost. This could be attributed to the increase in the feed intake that was caused by increase appetite because of the additives. However, a slight difference was observed in the total expenses with birds on diet with antibiotic growth promoter having the higher value (₦998.23) and control group (₦952.58) being the least. Birds placed on 10mg dietary zinc and oxytetracycline were higher in mean yield cost and net profit than other treatment groups.This underlines the importance of using cost of feed consumed to obtain a unit of product as a basis for recommending feeds to farmers (Ukachukwu and Anugwa, 1995).

**Table 4.19: Economic Indices of Broiler Chickens Fed Diets Containing Different Levels of Zinc Sulphate (1-8 weeks)**

| Levels of Zinc inclusion | | | | | |
|---|---|---|---|---|---|
| **Parameter** | **0g** | **10mg** | **20mg** | **30mg** | **Oxytet** |
| Feed cost/kg (₦/Kg) | 119.95 | 119.95 | 119.55 | 119.55 | 124.35 |
| Total feed intake (Kg/b) | 4.44 | 4.59 | 4.52 | 4.56 | 4.65 |
| Cost of TFI. (₦) | 532.58 | 550.57 | 540.37 | 545.15 | 578.23 |
| Cost of chicks (₦) | 220.00 | 220.00 | 220.00 | 220.00 | 220.00 |
| Other expenses (₦) | 200.00 | 200.00 | 200.00 | 200.00 | 200.00 |
| Total expenses (₦) | 952.58 | 970.57 | 960.37 | 965.15 | 998.23 |
| final wt. (Kg) | 2.51 | 2.73 | 2.58 | 2.67 | 2.76 |
| Cost of Chicken/Kg (₦) | 700 | 700 | 700 | 700 | 700 |
| Mean yield cost(₦) | 1757.00 | 1911.00 | 1806.00 | 1869.00 | 1932.00 |
| Net profit(₦) | 804.42 | 940.43 | 845.63 | 903.85 | 933.77 |

Oxytet; Oxytetracycline, Other expenses = Cost of multi-vitamins, repairs etc; Mean yield cost = Cost of chicken/Kg x Mean final weight (Kg); Net pofit = Mean yield cost – Total expenses.

# CHAPTER FIVE

## 5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Summary

Two Experiments were conducted to evaluate the performance of broiler chickens fed diets supplemented with graded levels of Chestnut (*Castenea sativa*) Phytobiotics and Zinc respectively, as Eubiotics. In each feeding trial, 300-day-old cobb 500 broiler chicks were allotted in a completely randomized design to five (5) dietary treatments each replicated thrice, with 20 chicks per replicate for starter phase and 20 chickens at the finisher phase.

In Experiment 1, Chestnut Phytobiotics was included at 0g, 100g, 125g and 150g/100 Kg diet whileOxytetracycline was included at 111g/100kg diet. In Experiment 2, Zinc was supplemented at 0mg, 10mg, 20mg and 30mg/100 Kg diet while Oxytetracycline was included at 111g/100kg diet.

In the two Experiments, data collected included the initial and final weights at both starter and finisher phases, weight gain and feed intake were recorded on weekly basis and feed/gain ratio, feed cost, feed cost/Kg gain and mortality were computed. Haematological parameters, liver function indices, lipid profiles and villi morphometric were evaluated. Digesta from the ceacum were taken and subjected to microbial analysis. Carcass evaluation (Experiment 2), nutrient digestibility study and were done at the end of finisher phase. Data collected were subjected to analysis of variance and significant differences among treatment means were compared using the Duncan's Multiple Range Test of significance.

In Experiment 1, result for the starter phase showed birds on the 100g phytobiotics had significantly ($P<0.05$) higher final weight and weight gain than other birds on 125g, 150g

phytobiotics, oxytetracycline and control. Feed intake was significantly (P< 0.05) higher for the birds on oxytetracycline than other treatment groups. Weight gain was significantly (P<0.05) higher on oxytetracycline (1783g) for finisher chickens however, there was an improved values for birds placed on phytobiotics. Haematological indices showed significant (P<0.05) difference in birds on diets with 100g phytobiotics for white blood cell ($91.90x10^3$/µL) and Heterophils (20.63%) while liver function indices showed that birds placed on diets with phytobiotics, oxytetracycline and control diet resulted into an increased amounts of these enzymes ALT, AST and ALP which were produced by the liver and the amounts present in the blood is indicative of the integrity of the liver. Chestnut phytobiotics reduced total cholesterol values from 151.13 - 96.55mg/dL when compared to other treatment groups. Apparent crude protein digestibility was significantly (P<0.05) higher in birds fed diets with 100g phytobiotics (76.15%) when compared to other treatment groups.

Villi morphometric showed significant (P<0.05) differences in all the parameters measured except for crypt depth. Villi area, perimeter, height, width and villi height/crypt depth ratio which were higher in birds fed diets containing 125g of phytobiotics than other treatment groups except for the control and oxytetracycline groups, which were similar in villi height/crypt depth ratio. Villi area, perimeter, height, width, crypt depth and villi height/crypt depth ratio values were similar for the birds fed control and oxytetracycline supplemented diets. Intestinal bacteria count revealed that *Lactobacillus spp* was significantly (P<0.05) higher and best in diets containing 100g phytobiotics ($15.33x10^3$cfu/g) when compared to that of the control ($5.00x10^3$cfu/g) but similar to that of birds fed diets containing oxytetracycline ($10.67x10^3$cfu/g). Supplementation with phytobiotics also increased the concentration of Bacillus spp, which is

also a beneficial bacteria. The results show that there was no presence of *Clostridium spp* among the treatment groups. *Lactobacillus spp* and *Bacilllus spp* are beneficial harmless microbes in the microbiota. The revenue/bird decreased as the level of phytobiotics increased.

In experiment 2, results for the starter phase showed significant ($P<0.05$) differences in all the growth parameters measured except feed intake and mortality. Birds placed on diets with 10mg inclusion of zinc were significantly ($P<0.05$) higher in final weight (783.33g/bird) and weight gain (742.16g/bird). Feed cost per kg gain was significantly lower in birds placed on diets with 10mg zinc (183.56₦/kg gain) and 30mg of zinc (186.46₦/kg gain), followed by the control group (193.19₦/kg gain). No particular trend was observed for birds fed different levels of zinc except in final weight at the finisher phase. There were significant ($P<0.05$) differences in WBC, lymphocytes, monocytes, basophils, MCV and MCH whereas there were non-significant ($P>0.05$) differences in the values for PCV, Hb, RBC, heterophils and eosinophils. Birds which had access to 20mg of zinc ($93.33X10^3/\mu L$), 30mg of zinc and oxytetracycline were significantly ($P<0.05$) higher in WBC when compared to the control group ($59.40x10^3/\mu L$) but statistically similar to the birds placed on diets with 10mg ($72.97X10^3/\mu L$) zinc.

The result for AST, ALT and ALP were significantly ($P<0.05$) influenced by the dietary treatment that resulted to the increase in the amount of these enzymes which were produced by the liver. Control diet had higher ($P<0.05$) value for AST and ALT while birds on diets with 30mg zinc had higher ($P<0.05$) value also for ALT. Oxytetracycline inclusion resulted to a significant ($P<0.05$) increase in the total cholesterol (90.63mg/dL) whereas birds on diets with 20mg zinc had the least values (74.61mg/dL). Triglyceride was significantly ($P<0.05$) different

across the treatment groups. Birds fed diets with 30mg zinc (95.49g/dL) having the higher value while those on diets with 20mg zinc had the least values (24.49g/dL). There was significant ($P<0.05$) difference in crude protein with group fed diets with 10mg (76.47%), 20mg (78.95%), 30mg (80.66%) of zinc and oxytetracycline (76.87%) level having higher values compared to that of the control (64.18%). The inclusion of zinc tends to increase the crude protein content across the treatments. Significant difference ($P<0.05$) were observed in the values of nitrogen free extract in birds fed diets with 10mg (72.68%), 20mg (74.45%), 30mg (78.07%) zinc and oxytetracycline (72.50%) whereas the control was the least (57.82%). The results reveal that zinc and oxytetracycline inclusion greatly improved soluble carbohydrate digestibility and utilization. Villi area (29919$\mu m^2$), width (164.36µm), height/crypt depth ratio (5.03µm) were higher in birds fed control diet than other treatment groups except for oxytetracycline which was similar with villi height/crypt depth ratio (5.20µm). The value for villi width and height/crypt depth ratio decreased with an increase in the levels of zinc. There were significant ($P<0.05$) differences in the values of *Lactobacillus spp* and *Bacillus spp*, whereas non-significant ($P>0.05$) differences were observed for *Escherichia coli*, *Clostridium spp* and *Salmonella spp. Lactobacilli spp* was significantly ($P<0.05$) higher and best in birds placed on the control diet ($15.00x10^3$cfu/g), followed by birds on diets with 20 mg zinc ($9.67x10^3$cfu/g) dietary level. *Salmonella spp* was significantly ($P<0.05$) lower in birds which had access to 20mg zinc ($0.00x10^3$cfu/g) but statistically similar with those on the control diet ($1.67x10^3$cfu/g) and diets with 30mg zinc ($2.67x10^3$cfu/g). Birds placed on diets with 10mg zinc and oxytetracycline were significantly higher in live weight, dressed weight and dressing percent. Birds placed on diets with 10mg zinc were significantly ($P<0.05$) higher in breast (33.89%), back (19.14%) whereas those on 20mg dietary zinc were ($P<0.05$) significantly higher in drumstick (12.09%) and head (2.77%); also

birds on diets with 30mg dietary zinc were significantly (P<0.05) higher in thigh (14.32%), legs (4.22%) and lungs (0.70%).

## 5.2 Conclusions

From the results obtained, it could be concluded that,Chestnut (*Castenea sativa*) Phytobiotic, a natural growth promoter:

1. Did not significantly improve feed intake above the AGPs for starters but however, improved the weight gain, feed conversion ratio and feed cost/Kg gain for both starter and finisher chickens.
2. Improved lipid profile, villi morphometry, nutrient digestibility and intestinal microbiota. It also improved the activities of beneficial bacteria in the ileum of broiler chickens thereby making more nutrients especially energy and protein available from the feed consumed.
3. Decreased the revenue per bird as the level of phytobiotics increased. Birds placed on diets with oxytetracycline (₦2023.00) and control (₦2016.00) diets were higher in mean yield cost while control diets was best in net profit (₦1004.43) than other treatment groups.

Zinc Sulphate, a gut modulating essential mineral and growth promoter:

1. Did not significantly improve villi height/crypt depth ratio of broiler chickens, but significantly lowered mortality, feed conversion ratio and feed cost per kg gain than the control group of finisher broilers.

2. Improved liver health, lipid profile, nutrient digestibility, intestinal microbiota, and carcass characteristics. It also enhanced intestinal barrier function against pathogenic bacteria hence, improving the immune system of broiler chickens.

### 5.3 Recommendations

Poultry farmers can use:

Chestnut (*Castenea sativa*) Phytobiotics at 100g and 125g/100 Kg feed as replacement for antibiotics in broiler chicken production. These levels of inclusion significantly improved final weight and weight gain for both starter and finisher and significantly improved *Lactobacillus spp and Bacillus spp* which are the beneficial bacteria.

Zinc Sulphate can be used at 10mg/100 Kg and 20mg/kg diet as replacement for antibiotics in broiler production for best performance. This level of inclusion significantly improved final weight, weight gain and low cost of production but significantly lowered *Salmonella spp*, mortality, feed conversion ratio and feed cost per kg gain control of finisher broilers.

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