THE UNIVERSITY OF ZAMBIA

SCHOOL OF AGRICULTURE SCIENCES

ANIMAL SCIENCE DEPARTMENT

THE EFFECT OF *Piliostigma thoningii* AQUEOUS LEAF EXTRACTS ON BROILER CARCASS FAT AND ANTIOXIDANT LEVELS.

BY

JONATHAN CHUUKA

UNZA 2010.

THE EFFECT OF *Piliostigma thonningii* AQUEOUS LEAF EXTRACTS ON BROILER CARCASS FAT AND ANTIOXIDANT LEVELS.

BY

JONATHAN CHUUKA

A Research Report Submitted to the School of Agricultural Sciences of the University of Zambia in Partial Fulfillment of the requirements for the Degree of Bachelor of Agriculture Science.

University of Zambia
School of Agricultural Sciences
Department of Animal Science
Lusaka Zambia

May, 2010.

DECLARATION

I, Jonathan Chuuka, declare that this thesis represents my own work and that it has not previously been submitted for a degree at this or any other university. All sources of information have been acknowledged by reference.

JONATHAN CHUUKA

MAY, 2010.

ABSTRACT

Piliostigma thonningii is a plant that has been found to contain a number of phytochemicals, including phenols. Phytochemicals have among other properties cholesterol lowering and anti-oxidant ability. This experiment was aimed at evaluating the effect of including different levels of *Piliostigma thonningii* aqueous leaf extracts on carcass fat and blood phenolic levels of Cobb 500 broilers. The experiment was conducted at the University of Zambia, School of Agricultural Sciences over a period of five (5) weeks.

The experiment was done in a Randomised Complete Block Design with six treatments, of which two were controls and three replicates. Ninety (90) broilers were randomly distributed among the treatments. The birds were given *Piliostigma thonningii* leaf extract-containing drinking water at levels; 100g, 200g, 300g and 400g leaf material per 200Kg bird live weight.

No significant differences ($P<0.05$) were found among the treatments.

Keywords: *Piliostigma thonningii*, Anti-oxidant, Cholesterol (fat).

DEDICATION

To my father Lawrence Chuuka and my brother Kingsley Chuuka. In memory of my late mother Nister Mungile who passed away on 19th July, 2006. To the Almighty God for His unwavering love.

ACKNOWLEDGEMENTS

Heartfelt appreciation goes to my supervisor Ms. Musukwa for the immeasurable support and guidance shown towards the success of the project.

Great appreciation to Mr. Sampa (Biometrician) and Dr. Daura (Head of Animal Science Department), for the technical assistance rendered before and during the course of the project.

Thanks go to Mr. Mungili, Mr. Mambwe, Mrs. Aikayo, Mr. Namushi, Mr. Chota and Ms. Malichi. Thank you for sparing time off your busy schedules and for the edifying advice. Thanks also go to all the other Field Station Animal Science members of staff.

Humble gratitude to my colleagues Lukonde, Duncan, Brian, Raphael and Mathew for the assistance accorded to me during and after the project.

Sincere gratitude goes to my family (the Chuuka and N'gandu families) for their moral support. A debt of gratitude to Sharon Siakanomba for the love and moral support shown during the period.

To God be the Glory.

TABLE OF CONTENTS

TITLE **PAGE**

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
Ca	Calcium
E.U	European Union
Fe	Iron
GST	Glutathione-s-transferase
Mn	Manganese
PAZ	Poultry Association of Zambia
Se	Selenium
U.S	United States
Zn	Zinc

LIST OF TABLES

LIST OF APPENDICES

Page

CHAPTER ONE

1.0 Introduction

Broilers are meat-type chickens. Sometimes they are called fryers or frying chicken. Commercial broilers are marketed at 4-10 weeks of age depending on the desired body weight (Jacqueline et al.). Over the years, the broiler industry has continued to grow. In addition to vertical integration, advances in live production (breeding, nutrition, flock management and health programs) and processing (automatic) have provided an economic advantage for poultry as compared to other meat sources (Bilgiri, 1999). Increases in poultry meat consumption cannot solely be attributed to low cost. Product diversification and wholesomeness has also contributed to increases in broiler meat consumption (Fletcher, 1996). Presently, market forms and distribution of broilers reflect changes in consumer demand for ready-to-cook and ready to eat products. Cut up parts and further processed products account for 46% and 36% of over 8 billion broilers marketed in the United States (US), respectively (Bilgiri, 1999).

Zambia has not been spared by the surge in poultry production. Like in many other countries, production has continued to expand from year to year. Despite the growth however, the short fall in production far outstrips demand. While there are currently many poultry farmers in Zambia, most are small scale farmers raising as little as 100 to 200 birds in their backyards with utterly no knowledge of the field Prominent commercial farmers may only amount to six, namely: Crest chickens, Bokomo, Zamchick, Hybrid, Eureka chickens and Rose breeders. Despite the many small scale poultry producers and notable commercial producers, the demand of broilers far outstrips the supply. Last year (2009) for example, of the expected 32 000 000 broilers, only 22 000 000 were produced (PAZ, 2009).

The shortfall in production can partly be attributed to increased feed costs. The increase in feed costs can be attributed to costly and rather inadequate supply of feed ingredients. The inadequacy of feed ingredients can be attributed to prominent droughts that have in the recent past been recurrent.

Apart from the shortfall in broiler production that has overwhelmed the poultry industry; the quality of most broilers produced has not met the consumers' expectations. In an effort to produce broilers in the shortest possible time in order to meet the demand, producers have resorted to the use of antibiotics.

Antibiotics are medicines that fight bacterial infections. Once used well they are capable of saving human as well as animal life. Despite antibiotics having certain benefits, they also have their disadvantages and one such is that the more they are used the more bacteria becomes resistant to them (U.S. National Library of Medicine and the Natural Institute of health, 2009).

As a result of the growth promoting ability that antibiotics have, livestock farmers started abusing their usage. According to a research by the European Federation of Animal Health (1999), farm animals consumed 4,700 (35%) tonnes of all the antibiotics administered in the European Union (E.U.). Of those, 3,900 (29%) tonnes were given to cure sick animals while 786 (6%) tonnes were used as growth promoters. The use of synthetic drugs in the broiler industry has not only led to further decreased production but also increased product prices. Further still, it has led to production of excess fat-containing broilers. Excess fat apart from being undesirable to the consumer reduces the shelf life of broiler carcasses. Consequently, consumers got concerned and demanded that antibiotics be banned from being used in animal feeds. It is as a result of that that the E.U. through the Agricultural Council decided to ban the use of antibiotics as growth promoters. The E.U. specifically banned the use of Monensia Sodium, Salinomycin Sodium, Avilamcin and Flavophospholipol (Food Production.com., 2003).

Despite the antibiotic ban, it is important that the livestock industry continue to thrive. Thus, to solve that problem it was suggested that phytochemicals replace growth promoters. Phytochemicals are chemical compounds such as beta-carotene that occur naturally in plants (Wikipedia, 2007). Other suggested alternatives include organic acids, feed enzymes and betaine (Frost and Sullivan, 2009; Media Relation, 2005). It can thus be said that organic farming in a way is what is being advocated for. For that to be achieved, there is need that more alternatives to antibiotic growth promoters which will be affordable and without negative health implications be found, especially in Africa and Zambia in particular where the antibiotic ban has not yet been effected.

Piliostigma thonningii (Musekese), a plant that is wide spread on the African continent has been found to possess pharmacological properties. The purpose of this research was thus to evaluate the ability of *P. thonningii's* aqueous leaf extracts' to lower broiler carcass fat while increasing Phytochemical levels.

Main Objective

To investigate the carcass improvement effects of P. thonningii aqueous leaf extracts on broilers.

Specific Objectives

i. To determine the carcass abdominal fat of broilers subjected to various levels of *P. thonningii* aqueous leaf extracts.

ii. To determine the level of antioxidants in the carcasses of broilers subjected to various levels of *P. thonningii* aqueous leaf extracts.

CHAPTER TWO

2.0 Literature Review

2.1 Consumer Food Choice

Poultry meat is an accepted valuable source of nutrients for consumers. In general, consumers are interested in good tasting and healthy food with nutritional physiology. At the same time, they are afraid of potentially harmful ingredients such as drug residues, intoxicants, allergic components and microbial contamination, which may contribute to health problems. On the other hand, consumers are more and more interested in products enriched with beneficial components which will improve their well-being. Therefore, consumers decide to buy food with special " health" contents and, what is more interesting, they are willing to pay more for healthy food (Grashorn, 2007).

2.2 General Carcass Fat

Fat even though essential in human diet is only desired in limited amounts. Excess fat can be described to be a nuisance as it leads to obesity and subsequently is a leading cause of heart attack. There is therefore need to maintain low fat content in the edible parts of poultry carcasses (Sonaiya, 1985). Abdominal fat weight has been found to be a good indicator of total body fat. In a study carried out by Sonaiya (1985), he reviewed that uneviscerated carcass weight could be substituted for abdominal fat weight without a great loss in accuracy. Waldroup et al., (1989) also used abdominal fat weight as an energy indicator in their study to justify whether or not different diets need to be given to male and female chickens.

2.3 Antioxidants

Oxygen is an important atom in nature despite its role in oxidative processes. In organisms, many natural compounds are prone to oxidation (Grashorn, 2007). Oxidation can be explained to be the loss of electrons. It occurs when oxygen is added or when hydrogen is removed from a compound (Raymond and Rose, 1968). The process proceeds as follows: firstly, carbon-centered free radicals are built from a processor molecule. These free radicals then react with oxygen and build peroxyl radicals. Next, a highly reactive peroxyl radical is formed that can attack any

available peroxidizable molecules, resulting in a chain reaction with many potential cycles of peroxidation. Free radicals are highly reactive substances damaging cell membranes; injuring heart, vascular, brain, and nervous and muscular system; impairing immune competence and in this way, they are involved in the occurrence of cancer (Grashorn, 2007).

Antioxidants are capable of prohibiting oxidative processes. Antioxidants also support disease prevention. Inclusion of antioxidants in broiler diet is beneficial, especially with respect to chronic and acute heart disease, because these diseases are related to oxidative stress. The other reason is related to meat quality. Poultry meat is especially prone to oxidative rancidity deterioration due to its high concentration of unsaturated fatty acids. Unsaturated fatty acids have the power to combine with atmospheric oxygen and give a taste that is referred to as "oxidative rancidity". What makes oxidative rancidity even more unpleasant is the concomitant destruction of carotene and vitamin A. Thus oxidative stability can be improved by including antioxidants in the diet (Rathgeber et al, 2008). There are three levels of antioxidant defense and these are:

- Prevention of radical formation (e.g. superoxide dismutase, glutathione peroxidase),
- Prevention and restriction of chain formation and propagation (e.g. vitamin A,E,C, carotene) and
- Excision and repair of damaged parts of molecules (e.g. lipase, peptidases, DNA repair, and enzymes) (Grashorn, 2007).

Examples of antioxidants include Tocopherols, β-carotenoids, Vitamin C, Phenols and Selenium.

2.4 *Piliostgma thonningii* and its Chemical Composition

Piliostigma thonningii is a leguminous plant belonging to the Celsalpinaceae family (Jimol et al., 2005). Its common name in Zambia is Musekese. Preliminary studies carried out by the University of Ilorin, Nigeria on *P. thonningii* showed that the seeds are rich in: crude protein, carbohydrates, and mineral elements. The seeds were further found to be a good source of antioxidant micronutrients such as Iron (Fe), Calcium (Ca), Selenium (Se), Zinc (Zn) and Manganese (Mn). Phytochemical screening of the seed showed the presence of Saponins, Flavonoids, phenols, glycosides, anthraquinones as well as cardiac glycosides (Jimol et al., 2005). The studies further showed that *P.thonningii* protein content in seeds was as high as

30.33% while that of carbohydrates was found to be 23.00%. Among the minerals analyzed Fe was found to be the highest (781.70ppm). Other mineral concentrations were found to be: 3.3ppm for Se; 43,11ppm for Ca; 0.016ppm for Zn; 1.00ppm for Mn and 0.02ppm for P.

In another experiment carried out by Bombardelli et al. (1997), 20% of tannin was recovered from the branches' bark although the composition was not clarified. Some Flavonoids and terpenes such as lambertianic acid and lambertianol were identified in the leaves. The presence of tannins in *P. thonningii* was confirmed confirmed by Fakae et al. (2001) in their research paper when they wrote to say the tree comprises proanthocyanidins (condensed tannins).

2.5 Biochemical Effects of *Piliostigma thonningii* Constituents

2.5.1 Phenols

Phenols are compounds that contain a 6- membered aromatic ring that is directly bonded to a hydroxyl (OH) group. Scalbert and Williamson (2000) reported that polyphenols are the most abundant antioxidants in human diets. Phenols have a number of properties that include:

- Antiseptic properties, this property was made use of by Sir Joseph Lister (1827-1912) in his pioneering technique of antiseptic surgery.
- Their use in the production of drugs such as aspirin, herbicides and synthetic resins.
- Their preference in embalming of bodies for anatomical use and study due to their ability to preserve tissues for extended periods of time.
- Their antioxidant properties.
- Their antimicrobial function (Jurd et al., 1971; Wyman and Van Ether, 1978; and Kubo et al, 1993). Phenol antioxidants exhibit significant antimicrobial activity against bacteria, fungi, viruses and protozoa in a wide variety of food systems and environments (Sherwin, 1990)

2.5.2 Flavonoids

Flavonoids are polyphenols abundantly found in fruits, vegetables, and herbs (e.g. tea, ginger root). Flavonoids are synthesized only in plants. They are a diverse group of phytochemicals, exceeding four thousand in number. From human nutrition perspective, Flavonoids are important

components of a healthy diet because of their antioxidant activity. Nevertheless, the antioxidant potency and specific effect of Flavonoids in promoting human health varies depending on the Flavonoids type (chemical, physical, and structural properties). Among the potent antioxidant Flavonoids types are quercetin, catechins and xanthohumol.

Other activities attributed to Flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral.

2.5.3 Saponnins

Saponins are a group of compounds widely distributed in the plant kingdom characterized by a structure containing a triterpene or steroid aglycone and one or more sugar chains. Consumer demand for natural products coupled with their physiochemical (surfactant) properties and mounting evidence of their biological activity (such as anticancer and anti-cholesterol activity) has led to the emergence of Saponins as commercially significant compounds in expanding applications in food, cosmetics and pharmaceutical sector.

Saponins are able to lower cholesterol levels due to their inhibitory effect on the absorption of cholesterol from the small intestines, or the reabsorption of bile acids.

The non-sugar part of Saponins have also a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases.

Saponins can impact the immune system through their adjuvant activity, their ability to improve effectiveness of orally administered vaccines by facilitating the absorption of large molecules and their immunostimulatory effects. Furthermore, Saponins have toxicity to insects (insecticide activity), parasite worms (anthelmintic activity), molluscs (molluscicidal), and fish (piscidal activity) as well as antifungal, antibacterial and antiviral activity (Gi-studag et al., 2007), and (Jimol et al., 2005).

2.6 Human and Livestock Medicinal uses of *Piliostigma thonningii*

Piliostigma thonningi as a plant has been found to have multiple uses. The plant has both human and livestock uses while some are common to both. Some of the general human uses of the plant include its use as: food, fuel, timber, fiber, tannin or dye and fodder (Agro forestry Tree Database, 2009).

In Tanzania and Zambia, the root bark of the plant is boiled in milk and used in the treatment of cough. The Swahilis people eat the leaves in small amounts to treat pulmonary complications (Bombardelli et al., 1997). Apart from treating cough and pulmonary complications, *P. thonningii* is also used to treat wounds, ulcers, gastric/ heart pain, gingivitis and is also used as an antipyretic (Agro forestry Tree Database, 2009). In Benin for example, Buruli Ulcer (BU) is a skin disease caused by *Mycobacterium ulcerous*. The disease is effectively treated by surgery whether or not associated with specific antibiotic therapy. A research carried out by Yemoa et al. (2005), reviewed that two plants *Jatropha carcaslium* and *Holarrhena floribunda* have antimycobacterial activity against BU and confirmed that *P. thonningii* maybe used by traditional healers to treat symptoms of the disease. They may contain compounds which may have anti-inflammatory, analgesic, anaesthetic, antiseptic or antioedema properties. Aderogba *thonningii* and *P. reticulatum* are both used interchangeably in ethno medicine in Africa to treat wounds, chronic ulcers, diarrhoea, cough, respiratory disorders and toothache.

In Nigeria, *P. thonningii* bark is one of the folklore medicines for the treatment of gastrointestinal helminth infections which have remained a health problem for both man and animals. Fakae et al. (2001) reviewed that the principle constituents of *P.thonningii* are proanthocyanidins (condensed tannins) which are capable of inhibiting recombinant *Ascaris suum* and *Onchocerca volvolus* glutathione-s-transferase (GSTs), yet could discriminate between the mammalian GSTs. *P. thonningii* was found to be comparable in efficacy to Piperazine phosphate, a standard anthelmintic. This study suggested that the inhibition of the GSTs of the worms could, in part, be responsible for the anthelmintic effects of the proanthocyanidins containing medicinal plants and forages. They concluded that it is likely that raising livestock on forages with 'qualified' tannin species would result in sustainable internal nematode without recourse to chemical anthelmintics thus resulting in "organic" produce.

CHAPTER THREE

3.0 Materials and Method

450 Cobb 500 chicks were urchased from Hybrid poultry in Lusaka. The chicks were reared in poultry houses at the Univ ersity of Zambia Animal Science Department Field Station. Upon arrival at the field station, t ıe chicks were divided into two batches with batch one comprising 270 chicks and batch two 1 80 chicks. After brooding for a period of two weeks the birds were transferred to a second poul ry house where randomly selected birds were placed in experimental units and subjected to exper nental treatments.

3.1 Housing and Equipm nt

The first poultry house wa used for brooding and had an east - west orientation. The second poultry house used for rear ng of the birds also had an east - west orientation. Prior to use, the two poultry houses were t oroughly cleaned by sweeping and washing with water and later disinfected with Micro. A ɔrooding area was prepared in the brooding pen. Brooder guards made from wooden frame: and covered with polythene plastic were used. The floor of the brooder pens was covered v ith a layer of wood shavings which were used as litter to protect the chicks from floor cold. The ɔrooder pen was divided into two using brooder guards. The brooder house windows were cover d with plastic drapes and used to control ventilation. A day before the chicks were collected fi ɔm the hatchery electric heaters, bulbs and feed were placed in the brooder pens. A 20 liter co ıtainer was filled with clean tap water and placed near one of the heaters in order to slightly r: ise its temperature.

The rearing house was clear ed in a similar way as the brooding house. Experimental units were made from wooden frames covered with chicken wire. Wood shavings were spread over the floor in each experimental ınit. Each experimental unit was also provided with an infra-red lamp, feeders and drinkers Two bulbs were installed as a light source. The windows were covered with adjustable plas ic drapes.

3.2 Experimental Design Treatments and Units

3.2.1 Experimental Desig n

A Randomized Complete I lock Design (RCBD) was used with three replicates, each replicate having six experimental un s. Factors considered when making the blocks were:

- Direct rays of the s n in the morning and late afternoon. Birds in Blocks I and III were expected to be affec ed by direct sun rays and heat while the birds in Block II which was mid way between I nd II were not.
- Sound from activiti s around the field station was the other blocking factor. Birds in Block III were expe ted to be the most affected due to the high level of activity near their wall followed by bi ls in Block I.

3.2.2 Treatments

Treatments were given to fc ur experimental units of each block. The experimental units in each block were designated by th treatment codes which represented different *Piliostigma thonningii* leaf extract concentrations. The concentrations were changed every week based on the average weight of the birds under given treatment. Apart from the four treatments, each block also contained two controls whic were designated C_1 and C_2. That is:

Treatments were assigned tc the experimental units using random numbers.

Table 3.1: Experimental L yout

B_1 T_2	B_1 T_3	B_1 T_1	C_1	B_1 T_4	C_2
B_2 T_3	C_2	B_2 T_1	B_2 T_4	B_2 T_2	C_1
C_2	C_1	B_3 T_1	B_3 T_4	B_3 T_3	B_3 T_2

Key

Where B_1, B_2, and B_3 represe nt Blocks 1, 2 and 3
T_1-treatment 1 (100g crushe *P. thonningii leaves* per 200Kg live weight)
T_2-treatment 2 (200g crushe *P. thonningii leaves* per 200Kg live weight)
T_3-treatment 3 (300g crushe *P. thonningii leaves* per 200Kg live weight)
T_4-treatment 4 (400g crushe *P. thonningii leaves* per 200Kg) live weight)
C_1-control 1(Commercial Ti er Feed, no *P.thonningii* leaf and vaccinated)

C_2-control 2(Field station compounded feed, no *P. thonningii leaf,* not vaccinated)

3.2.2.1 Treatment Preparation

Piliostigma thonningii leaves were collected from around the University every morning. The leaves were washed with water after which they were pound using a mortar and pestle. Clean water was then drawn into four containers. Different amounts of the pound leaves were then measured according to the desired concentration to be made for the different treatments and put in the water buckets. After 30 minutes the leaves were sieved out and the mixture distributed to the various experimental units.

Table 3.2: Pound Leaf Material used per week per Treatment

Week	Treatment	Leaf Material (g)
1 (12/12/09)	T_1	4.244
	T_2	8.427
	T_3	13.188
	T_4	16.344
2 (19/12/09)	T_1	7.815
	T_2	15.946
	T_3	24.299
	T_4	30.938
3 (26/12/09)	T_1	9.905
	T_2	19.225
	T_3	30.976
	T_4	39.976
4 (02/01/10)	T_1	12.368
	T_2	24.394
	T_3	38.892
	T_4	49.998
5 (09/01/10)	T_1	13.283
	T_2	27.477
	T_3	44.595

	T_4	60.608

The amount of pound leaf material was calculated based on the average live weight on the birds in each treatment relative to the standard treatment concentrations which are shown below.

Key

T_1-treatment 1 (100g pounded *P.thonningii leaves* per 200Kg live weight)
T_2-treatment 2 (200g pounded *P.thonningii leaves* per 200Kg live weight)
T_3-treatment 3 (300g pounded *P.thonningii leaves* per 200Kg live weight)
T_4-treatment 4 (400g pounded *P.thonningii leaves* per 200Kg live weight)

3.2.3 Experimental Units

Randomly selected birds were placed in experimental units of five (5) birds each. Each treatment and control had three replicates, giving a total of eighteen (18) experimental units.

3.3 Bird Management

450 Day old chicks were collected from Hybrid poultry on the morning of the 24th of November, 2009. On arrival at the field station, the boxes containing the birds were placed in one of the brooder pens and left for about an hour in order for them to rest from the journey stress as well as adapt to the room temperature. Drinking water was added with anti-stress pack and mixed before distributing it into the drinkers. An hour also after arrival, the birds were divided into two batches with batch one comprising 270 chicks and batch two 180 chicks after which 10% of the chicks from each group were weighed to get their average weight. The two batches were put in different brooding pens but in the same brooding house.

3.3.1 Feed

The chicks in batch one were given field station compounded starter feed while batch two were given Commercial Tiger feed. A Three Phase Feeding Regime in which the Starter feed was given from the first day till the third week, Grower feed from the third week to the fourth week and finisher feed from the fourth week until slaughter was used. Throughout this Regime, birds in control one were given commercial Tiger feed, while the birds from batch one with the experimental treatments of 100g,200g, 300g and 400g pounded *P. thonningii* leaf and C2 birds were given field station formulated feed.

3.3.4 Slaughter Procedure

Upon terminating the experiment on the 16th of January, three birds from each experimental unit were randomly selected and slaughtered. At slaughter, blood from each bird was drawn into two vacutainers. The carcasses were then taken for plucking and evisceration.

3.3.5 Carcass Dressing

The eviscerated carcasses were obtained by removing the head, feet, abdominal fat, gizzard, liver and all other abdominal and thoracic contents. The eviscerated carcass and abdominal fat were measured and recorded.

3.3.6 Serum Preparation

The collected blood was taken to the school of Veterinary medicine where it was cooled for about an hour and then centrifuged. Serum from each bird's blood was collected and put in a new vacutainer. The vacutainers were then put in a freezer before analyzing for phenols.

3.4.0 Data Collection

3.4.1 Carcass Weight and Abdominal Fat Weight

The eviscerated carcass and abdominal fat weights were recorded.

3.4.2 Phenolic Determination

Phenols were determined as described by Okwu (2005). 5 ml of serum was measured using a measuring cylinder and placed in a 50 ml flask into which 10 ml of distilled water was added, followed by 2 ml of ammonium hydroxide and 5 ml of amyl alcohol. The mixture was then left to react for 30 minutes after which absorbance of the solution was read using a spectrophotometer at 505 nm wavelength.

3.5 Statistical Analysis

3.5.1 Analysis of Variance

Analysis of variance was done for carcass weight, abdominal fat and absorbance to find out significant differences (if any) among the treatment means. The parameters were analyzed using Genstat Discovery Edition 3 statistical package. Least significant differences were used to separate means. Bar charts were plotted using Microsoft Excel.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Mortalities

In the sixth week a condition that was later diagnosed to be a respiratory distress was observed in treatment 1 and 2. The condition however was not observed two days later. In the sixth and seventh weeks a total of five deaths were recorded from treatments 1, 2 and 3 and control 1. Carcasses were taken to the school of Veterinary medicine for postmortem and showed signs characteristic to those of the disease caused by Salmonella.

4.2 General Performance of the Birds

Analysis of variance (ANOVA) was used to analyze treatment means for significant differences. ANOVA tables for all the parameters measured did not show any significant differences ($P<0.05$) among the treatments. The non significant results could be attributed to:

- The differences in the treatment concentrations may have been too small to produce significant effects.

4.2.1 Mean Carcass Weight

Analysis of variance for mean carcass weight of birds at different levels of *Piliostigma thonningii* aqueous leaf extracts showed no significant differences ($P<0.05$) among all the treatments. Values for mean carcass weight are presented in table 4.

Table 4.1: Mean Carcass Weight

Treatment	Mean Carcass Weight (Kg)
T_1	1.503^a
T_2	1.549^a
T_3	1.598^a
T_4	1.578^a
C_1	1.757^a
C_2	1.628^a
GRAND MEAN	**1.602**
Coefficient of Variation %	**14.6**
Standard error %	**11.06**

Means in the same column followed by the same superscript are non significantly different from each other ($P<0.05$).

Key

T_1-treatment 1 (100g crushed *P. thonningii leaves* per 200Kg live weight)
T_2-treatment 2 (200g crushed *P. thonningii leaves* per 200Kg live weight)
T_3-treatment 3 (300g crushed *P. thonningii leaves* per 200Kg live weight)
T_4-treatment 4 (400g crushed *P. thonningii leaves* per 200Kg live weight)
C_1-control 1 (Commercial Tiger Feed, vaccinated and no *P. thonningii leaf)*
C_2-control 2 (Field station compounded leaf, no *Piliostigma thonningii* and not vaccinated)

4.2.2 Mean Abdominal Fat Weight

Analysis of variance for mean abdominal fat weight of birds at different levels of *Piliostigma thonningii* aqueous leaf extracts showed no significant differences ($P<0.05$) among all the treatments. Values for mean abdominal fat weight are presented in table 5.

Table 4.2: Mean Abdominal Fat Weight and Dressing Out Percentage (DCP).

Treatment	Mean Abdominal Fat Weight (g)	DCP (%)
T_1	59.1^a	71.03^b
T_2	61^a	71.45^b
T_3	53^a	72.89^b
T_4	47.1^a	66.90^b
C_1	56.3^a	71.71^b
C_2	47.4^a	66.07^b
GRAND MEAN	**54**	
Coefficient of Variation %	**36.7**	
Standard error %	**9.34**	

Means in the same column followed by the same superscript are non significantly different from each other ($P<0.05$).

Key

T_1-treatment 1 (100g crushed *P. thonningii leaves* per 200Kg live weight)
T_2-treatment 2 (200g crushed *P. thonningii leaves* per 200Kg live weight)
T_3-treatment 3 (300g crushed *P. thonningii leaves* per 200Kg live weight)
T_4-treatment 4 (400g crushed *P. thonningii leaves* per 200Kg live weight)
C_1-control 1 (Commercial Tiger Feed, vaccinated and no *P. thonningii leaf)*
C_2-control 2 (Field station compounded leaf, no *Piliostigma thonningii* and not vaccinated)

4.2.3 Mean Absorbance of Phenols

Analysis of variance for absorbance of bird blood serum at different levels of *Piliostigma thonningii* aqueous leaf extracts showed no significant differences ($P<0.05$) among all the treatments. Values for absorbance are presented in table 6.

Table 4.3: Mean Absorbance of Phenols

Treatment	Mean Absorbance (nm)
T_1	1.755^a
T_2	1.733^a
T_3	1.704^a
T_4	1.666^a
C_1	1.663^a
C_2	1.610^a
GRAND MEAN	**1.688**
Coefficient of Variation %	**8.4**
Standard error %	**6.67**

Means in the same column followed by the same superscript are non significantly different from each other ($P<0.05$).

Key

T_1-treatment 1 (100g crushed *P. thonningii leaves* per 200Kg live weight)
T_2-treatment 2 (200g crushed *P. thonningii leaves* per 200Kg live weight)
T_3-treatment 3 (300g crushed *P. thonningii leaves* per 200Kg live weight)
T_4-treatment 4 (400g crushed *P. thonningii leaves* per 200Kg live weight)
C_1-control 1 (Commercial Tiger Feed, vaccinated and no *P. thonningii leaf)*
C_2-control 2 (Field station compounded leaf, no *Piliostigma thonningii* and not vaccinated)

4.3 Conclusion

From the results obtained and analyzed, it can be concluded that *Piliostigma thonningii* aqueous leaf extracts have no significant effect on the antioxidant levels and fat content in broiler carcass at the treatment levels: 100g crushed *P.thonningii* per 200Kg live weight; 200g *P.thonningii* per 200Kg live weight, 300g *P.thonningii* per 200Kg live weight and 400g *P.thonningii* per 200Kg live weight to which the birds were subjected.

4.4 Recommendations

The research should be red ɔne taking the following aspects into consideration.

- The birds to be ˢubjected to *Piliostigma thonningii* plant extracts should be given treatments as early as possible before the immune system is affected.
- Higher quantities o *Piliostigma thonningii* should be used.
- Other plant parts o ner than leaves should be used, for example pods.
- A quantitative an lysis other than a qualitative one be carried out for colorimetric Phytochemical det rmination.

6.0 References

Aderogba M.A., Okoh E.K., Adelanwa T.A., Obuotor E.M., 2004. Antioxidant Properties of the Nigerian Piliostigma Species. Boilogical Sciences Vol. (44),pp.501-503.

Agricultural Marketing Service, 1999. Poultry Grade Yield Report, poultry Grading Branch USDA, Washington DC.

Bilgili S.F., 1999. Broiler Carcass Quality, Auburn University, Auburn.

Bombardelli e., Morazzoni P., Mustich G., 1997. Extracts of Piliostigma thonningii, the use there of the formulations containing them, United States Patents, USA.

Fakae B.B., Borphy P.M., 2001. Differential Inhibition of Parasitic Nematodes and Mammalian Glutathione S- Transferases (GSTs) by Extracts from Piliostigma thonningii, Virtual Library Nigeria.

Fletcher D.L., 1996. Processing to Meet Quality Expectations in a Changing market, World's Poultry Science Assoc. Meeting, Delhi, India.

Grashorn M.A., 2007. Functionality of Poultry Meat, Poultry Science Association Vol.16:pp. 99-106.

Jabbar A., Igbal Z., Khan M.N., 2006. In vitro Anthelmintic Activity of Trachyspermum ammi seed Pharmacogsy Magazine Vol.2 (6), pp.126-129.

Jacob J.P., Mather F.B., 2009. The Home Broiler Chicken Flock, University of Florida, Florida.

Jimol F.O., Oladiji A.T., 2005. Preliminary studies on Piliostigma thonningii seeds: Proximate Analysis, Mineral Composition and Phytochemical Screening, Biotechnology Vol. 4(12), pp. 1439-1442.

John T.A., 1961. Animal Nutrition and Veterinary Dietetics, 4th Edition, W. Green and Sons, Limited Edinburgh.

Jurd, L., J. Corse, AD. King, H. Bayne, K. Mihara. 1971. Antimicrobial properties of 6,7-dihydroxy- and 7 8-dihydroxy-, 6-hydroxy- and 8-hydroxycoumarins. Phytochemistry. 10:2971-2974.

Kubo, I., Muroi, and M. Hinejima. 1993. Antibacterial activity against *Streptococcus mutans* of Of mate tea flavor components. *Journal of Agricultural and Food Chemistry.* 41: 107-111.

Lalchhandama K., 2008. Nematodal Effects of Piperazine and the Extract of Acacia oxyphlla stem Barkon the Poultry Nematode, Ascaridia galli, Pachhunga University College, India.

Little T.M., Hills F.J., 1978 Agricultural Experimentation Design and Analysis, John Wiley and Sons, Newyork.

Media Relations, 2005. Feed Enzymes and Betaine help to replace Antibiotics Growth Promoters.

Rathgeber B., Rupasinghe V., 2008. Research Results for the Poultry Industry, Poultry Industry Council, Ontario.

Raymond P.M., Rose A., 1968. Chemistry of Life Processes, Harcourt, Bruce and Worll Inc., USA.

Scalbert, A., and G. Williamson. 2000. Dietary intake of polyphenols. *Journal of Nutrition.* 130: 2073S – 2085S.

Sonaiya L.B., 1985. Abdominal Fat Weight and Thickness as Predictors of Total Body Fat in Broilers, British Poultry Science Vol. 26, pp. 453-458.

United States Natural Libr ry of Medicines and the Natural Institute of Health, 2009.
Waldroup P.W., Tidwell N M., Izat A.L., 1989. The Effects of Energy and Amino Acid Levels of performance a nd Carcass Quality of Male and Female Broilers Grown Separately, University of Ar ansas, Arkansas.
Yemoa A.L., Gbenon J.D., Johnson R.C., Djego J.G., Zinson C., Martin A., Moudachiron M., Bigot A., Portael F., Quetin-Leclercq J., 2005. Indentification and Chemical Study of Plants Used in th Traditional Treatment of Buruli Ulcer I Benin, Universite' Catholique de Lo uvain, Belgium.

6.0 APPENDICES

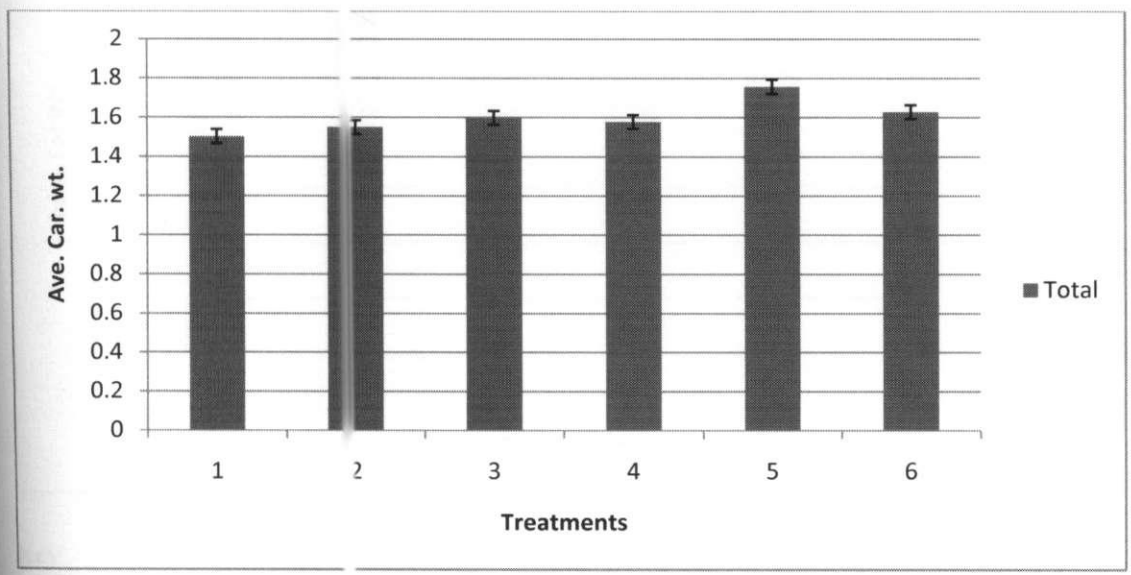
Appendix A
Table AI Analysis of Variance for Mean Carcass Weight

Carcass Weight

Source of Variation	Degrees of freedom	Sum of Squares	Mean Squares	Calculated F Value	Tabulated	
					5%	1%
Treatment	5	0.34101	0.06820	0.312^{ns}	19.30	99.30
Rep	2	0.79613	0.39807	0.002^{ns}	4.10	7.56
Trt.Rep	10	0.52947	0.05295	0.492^{ns}	2.11	2.86
Residue	36	1.98218	0.05506			
TOTAL	**53**	**3.64879**				
Least significant difference at 5 %			**0.2243**			

Key
Rep- Replicate
Trt- Treatment

Figure AI: Mean Carcass Weight at Different Levels of *P. thonningii*



Key
1- Treatment 1. 2- Treatment 2. 3- Treatment 3
4- Treatment 4 5- Control 1 6- Control 2

Appendix B

Table BI: Analysis of Variance for Mean Abdominal Fat Weight

Mean Abdominal Fat Weight

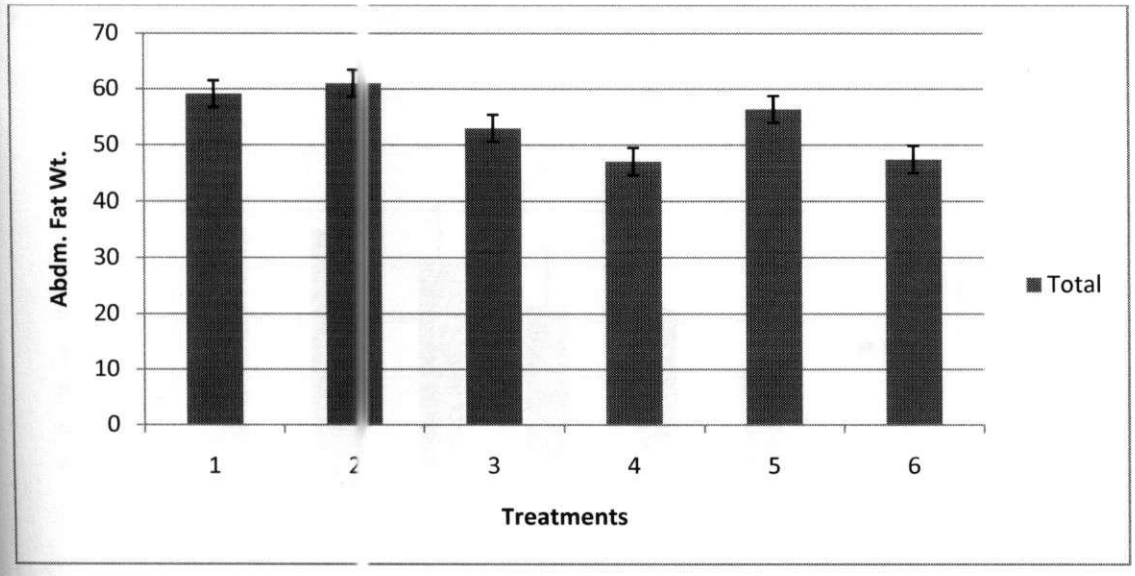
Source of Variation	Degrees of freedom	Sum of Squares	Mean Squares	Calculated F Value	Tabulated	
					5%	1%
Treatment	5	1548.0	309.6	0.565^{ns}	19.30	99.30
Rep	2	1796.8	898.4	0.116^{ns}	4.10	7.56
Trt.Rep	10	2657.2	265.7	0.738^{ns}	2.11	2.86
Residue	36	14134.0	392.6			
TOTAL	**53**	**20136.0**				
Least significant differences at 5%			**32.81**			

Key

Rep- Replicate

Trt- Treatment

Figure BI: Mean Abdominal Fat Weight at Different Levels of *P.thonningii*



Key

1- Treatment 1. 2- Treatment 2. 3- Treatment 3

4- Treatment 4 5- Control 1 6- Control 2

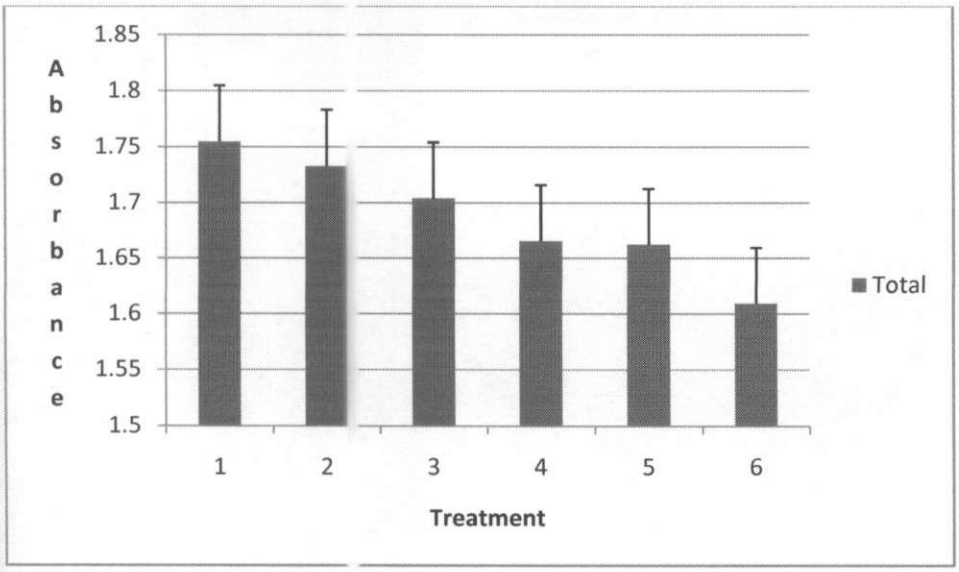
Appendix C
Table CI: Analysis of Variance for Absorbance

Mean Absorbance

Source of Variation	Degrees of freedom	SS	MS	Calculated F Value	Tabulated	
					5%	1%
Treatment	5	0.12639	0.02528	0.301^{ns}	19.30	99.30
Rep	2	0.03188	0.01594	0.459^{ns}	4.10	7.56
Treatment.Replication	10	0.16697	0.01670	0.600^{ns}	2.11	2.86
Residue	30	0.72114	0.02003			
TOTAL	53	**1.04637**				
Least significant differences at 5 %			**0.2344**			

Key
Rep- Replicate
Trt- Treatment

Figure CI: Mean Absorbance at different Levels of *P. thonningii*



Key
Rep- Replicate
Trt- Treatment