

**RESPONSE OF BROILER BIRDS FED *PHYLLANTUS AMARUS* FOR
TREATMENT OF COCCIDIOSIS**

BY

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CERTIFICATION PAGE

This is to certify that this project works has been read and approved at meeting part of the
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DEDICATION

I dedicate this project to the almighty God for is grace, mercy, wisdom and strength giving to me, throughout my academic journey.

This work is also dedicate to my beloved parents who love, support and sacrifice their time for me and make it a success. Lastly to my brother and sister thank you for your encouragement and inspiration.

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TABLE OF CONTENT

Title Page

Certification Page

Dedication

Acknowledgment

Abstract

CHAPTER ONE

Introduction and Background of the study 1-4

Justification 5

Aim and Objectives of the study 5

CHAPTER TWO

Uses and Processing of *Phyllanthus amari* 7

Pharmacological reliance 7

Challenges of Broiler production 10

Economic importance 11

Phyllanthus Amari 12

Origin of Phyllanthus Amari 13

CHAPTER THREE

Site of the experiment, material and Ingredients used 14

CHAPTER FOUR

Results and Discussions	20-24
-------------------------	-------

CHAPTER FIVE

Conclusion and Recommendations	25-26
--------------------------------	-------

References	27-28
------------	-------

Abstract

An experiment was conducted on the effect of Phyllanthus Amarus on broiler birds, 200, 2 weeks old Ross broiler birds was used for the experiments, Eimeria Occyst was applied to their drinking water for three days on the fourth day when signs of coccidiosis are seen then then treatment begins with Phyllanthus Amarus at 5ml and 10ml 0er 4 liters of drinking water and at the end of 3 weeks of the experiment, Analysis of variance (ANOVA) results shows that there was no significant difference between 5ml and 10ml inclusion level and therefore 5ml was recommended.

CHAPTER ONE

INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

Plant materials as sources of medical compounds continue to play a dominant role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of the pharmaceutical industry (Burton et al., 1983). Like any therapeutic agent, when overdosed or incorrectly used they also have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public (Matthews et al., 1999).

In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Several scientific studies conducted throughout the world

have revealed and confirmed the dramatic medicinal properties of plants containing various phytochemicals like flavanoids, carotenoides, alkaloids etc.

Recognition and development of the medicinal and economic benefits of traditional medicinal plants is on the increase in both developing and industrialized countries, although it varies greatly from region to region (WHO-Traditional Medicine (TRM), 1998).

In the last few decades there has been an exponential growth in the field of herbal medicine which is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. World Health Organization (W.H.O) estimates that 80 percent of the world's population uses medicinal plants in primary health needs (WHO, 2003). Since about 80 percent of the 7 billion people of the world are in less developed countries, this means that more than 5.6 billion people are likely to use medicinal plants on a frequent basis. Moreover, the modern medicine contains about 25 per cent of drugs derived from plants. Therefore, there is a need to study medicinal plants for their efficacy, safety and quality, and also to search for potentially valuable medicinal material from which novel curative agents may be created for the benefit of all humankind. A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs, exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many industries (Uniyal et al.,

2006). At present more than 20,000 herbal plants are being used for medicinal purposes at the international level. In India, the home for herbal plants, there are about 7000 medicinal plants, which are in use. Among the estimated source of 3.6 lakhs plant species spread over the earth, 40 per cent are available in our country. Recent survey indicated that about 7195 plant species are being used by 7800 herbal product industries manufacturing 25000 effective plant based formulations. Over 6.5 lakhs practitioners of Indian systems of medicine in the oral and codified streams use these formulations for preventing, promotive and curable application. At the international level the herbal medicine trade has been estimated to be about 36-43 million dollars. India is one of the major exporters of crude drugs and one among the major biodiversity centers in the world. India has been producing herbal plants worth Rs. 2300 crores. Out of this, the quantity available for export amounts to Rs. 500 crores which represent 0.04 per cent of the world trade (Mohideen et al., 2011).

Phyllanthus amarus (*P. amarus*) is a broad spectrum medicinal plant that has received worldwide recognition (Srividiya and Perival, 1995). In Nigeria, it is called “IyinOlobe” in Yoruba (Etta, 2008). *P. amarus* is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as a mild Laxative. *P. amarus* also has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties, and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases (Calixto et al., 1998; Santos et al., 1995).

The plants of the genus *Phyllanthus* are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease (Liu et al., 2003).

Plants contain numerous constituents; some tend to possess some level of toxicity. Cases of this toxicity in plants have been reported (Santos et al., 1995; Shaw et al., 1997; Kaplowitz, 1997). *P. amarus* has been classified among plants with a low potential for toxicity, with an LD₅₀ averaging 2000 mg/kg/day (Krithika and Verma, 2009). The phytochemical analysis of the *P. amarus* extract confirmed the presence of tannins, saponins, flavonoids and alkaloids. The plant extract have been found to contain high levels of saponins, tannins, flavonoids and alkaloids (Fernand, 1998; Naaz, 2007; Krithika and Verma, 2009). *P. amarus*, a distinguished botanical has been used worldwide since many years because of its rich medicinal importance. *P. amarus* is an erect annual herb having large number of phytochemicals that are attributed to its leaves, stems and roots. A wide array of studies conducted revealed the anti-inflammatory, antidiabetic, antimicrobial, antihyperlipidemic, antioxidant, antispasmodic, chemoprotective, antihypercalciuric, antiviral and diuretic properties associated with *P. amarus* (Thyagarajan et al., 1988; Patel et al., 2011). *P. amarus* has been used since ages by the folk because of its rich ethnomedicinal importance. A number of phytochemicals associated with the herb renders it a broad spectrum medicinal valued herb.

Statement of the problem

Coccidiosis is very likely for broiler birds at the age of three weeks and this could wipe the whole birds away on the farm. Most of the available drug for coccidiosis are not very effective again, besides the drug residual effect makes it worst. The world health organization has warn seriously that the use of veterinary drug for poultry birds should be reduced or eradicated completely and then the use of herbal plants is then encouraged.

Justification of the study

The use of herbal plants in poultry production has been encouraged by World health organization and Food Agricultural organization, because of the following reasons residual effect of antibiotics in the body of the chicken for a longer time that those who consume the chicken meat will be taken the drug indirectly. The drug resistance of the chicken makes the drug in effective. No side effect of the herbs. Availability of the herbs all year round it is cost effective.

Objectives of the study

- (i) Carry out both quantitative and qualitative phytochemicals for *Phyllanthus amarus*
- (ii) Evaluate the Antioxidant properties of *Phyllanthus amarus*
- (iii) Study the effect of *Phyllanthus amarus* in treating the coccidiosis

CHAPTER TWO

LITERATURE REVIEW

From past to present, medicinal plants are considerably useful and helpful to cure the human ailments and diseases. Different parts of medicinal herbs are used to prepare different therapeutic medicine in many countries for several centuries. The bioactive substances originated from these plants possess the potential health benefits towards human body. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. In the last few decades there has been an exponential growth in the field of herbal medicine which is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.

P. amarus Schuman important medicinal plant of the tropics, belongs to the family Euphorbiaceae and has long history in traditional system of medicine in every tropical country. *P. amarus* Schum. & Thonn., which is widely spread throughout the tropical and subtropical countries of the world including India, is most commonly used in the Indian Ayurvedic system of medicine in problems of stomach, genitourinary system, liver, kidney and spleen (Zavar, 2011).

P. amarus has long history of usage by the folk because of its rich medicinal values. It has been reported to possess potent anti-inflammatory, antihepatotoxic, analgesic, hypotensive, antispasmodic, antibacterial, antiviral, diuretic, antimutagenic and

hypoglycemic properties (Thyagarajan et al.,1988). The postharvest process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active ingredients in the product sold (Silva & Casali, 2000).

Uses and Processing

Timely and right processing of medicinal plant produce, after it has been harvested is imperative to preserve the quality and enhance the shelf life of the produce. The produce should be properly dried before it is packed for shipping or storage. (NMPB, 2009). Recent surveys indicate that poor postharvest handling and lack of knowledge on suitable packaging systems for herbs as major factors contributing to wastage, poor quality and limited market opportunities. In this background an attempt has been made to develop a suitable drying and storage method for whole plant and extractives of 'Kizharnelli'

PHARMACOLOGICAL RELEVANCE

P. amarus Schum. & Thonn (Euphorbiaceae) is an annual herb, growing as a weed throughout India, commonly known as Jamgli amli, Jaramla, or Bhuiamla. Traditionally, it is useful in treatment of dropsy, jaundice, diarrhoea, dysentery, and intermittent fever, diseases of the urinogenital system, scabies, ulcers, and wounds.

P. amarus is well known for the biologically active compounds it possesses, which include different classes of organic compounds of medicinal importance including alkaloids,

flavanoids, steroids, terpenoids, lignans, lipids and coumarins. Phyllanthin and hypophyllanthin are the important lignans isolated from *P. amarus* (Row et al., 1967).

P. amarus is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977). *P. amarus* is always sold as fresh and dry plant materials in the herb markets. Decoctions are used in herbal baths and after labor, cramps, asthma, uterus complaints, and to treat stomachache (May, 1982). According to Heyde (1990) *P. amarus* extracts are used as blood purifiers, for light malaria fevers and anaemia.

According to Unanader et al. (1991) the aerial parts of the herb *P. amarus* Shum & Thonn have been widely used in folk medicine in India and other tropical countries for the treatment of various diseases and disorders such as jaundice, diarrhoea, constipation, kidney ailments, ulcers, ringworm, malaria, genitourinary infections, hemorrhoids and gonorrhea.

Traditionally, *P. amarus* is useful in treatment of dropsy, jaundice, diarrhoea, dysentery, and intermittent fever, diseases of the urinogenital system, scabies, ulcers and wounds. In a number of countries, the aerial part of *P. amarus* is highly valued in traditional medicine for its healing properties. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. The Spanish name 'chanca piedra' means "stone breaker or shatter stone". In South America, 'chanca piedra' has been used to

eliminate gall bladder and kidney stones, and to treat gall bladder infections. (Foo and Wong, 1992).

The use of *P. amarus* is gaining momentum because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gall bladder stones, for cold, flu, tuberculosis and other viral infections; liver diseases, and disorders including hepatitis, jaundice and liver cancer (Unander et al.,1993). *P. amarus* is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993). Jayarama et al.(1997) reported that *P. amarus* acts against liver cell toxicity and improves the immune system of patients and also found effective against hepatitis A. Infusion of leaves, stem and root of *P. amarus* are used in Brazilian folk medicine for treating kidney problems, intestinal infection and liver problems (Calixito et al.,1998). Different plant parts of *P. amarus* are ethno botanically used in various diseases and disorders e.g. leaves as expectorant and diaphoretic, fruits as carminative, laxative, astringent, diuretic, diaphoretic and tonic to the liver (Kirtikar and Basu, 2001). Khatoon et al. (2006) conducted pharmacognostic studies on *P. amarus* and reported that the plant contains 6.23 percent total ash, 10 percent alcohol extract, 22.25 percent water soluble extract, 0.2 percent phyllanthin, 0.3 percent hypo phyllanthin, 0.7 percent phenols, 1.9 percent sugar and 0.7 percent tannins. Kumaran and Karunakaran (2007) reported that methanol extracts of powdered air dried *P. amarus* showed high antioxidant activities.

Challenges Facing Broiler Production in Nigeria

(i) Poor quality of ingredients used in the manufacture of feed

Many of the raw materials are not properly processed and handled while some others are adulterated. Ademola and Farinu (2006) reported that moisture content, aflatoxin level and other microbial contaminant of raw material are never evaluated prior to purchase and use in the manufacture of poultry feeds. Consumption of such feeds by birds often results into serious health problems, depressed productive (in case of broilers) and reproductive performance (in case of layers). High mortality often occurs in extreme cases of contaminated feeds.

(ii) Supply of poor quality chicks

This is a major source of sub-optimal production and reproductive performance of flocks in the country. The problem is often exacerbated for the fact that poor quality chicks are not always realized early enough until considerable level of cost has been incurred in raising the chicks (Owen *et al.*, 2012). It was observed that there are no strict and compulsory quality control measures in the hatchery or in the market in most developing countries (Araujo *et al.*, 2004).

(iii) High Feed Cost

General knowledge exists that feed constitutes the highest and most expensive input in any livestock farm, especially poultry (Owen *et al.*, 2012). Ani-Okeke and Oni (2012) reported that feed is the major factor militating against intensive animal production in Nigeria.

ECONOMIC IMPORTANCE OF BROILER CHICKEN

Livestock industry in Nigeria is ridden with myriad of problems, which have resulted to a gross shortage of meat and other animal products (Nworgu, 2002). The growth rate of agricultural sector in Nigeria is still below the potentials of the country natural and human resources due to high cost of agricultural inputs, poor funding of agriculture, inadequate functional infrastructural facilities, inconsistencies of government agricultural policies, inadequate private sector participation, poor mechanized farming and little or no adoption of some simple agricultural technologies developed by scientists (Nworgu, 2006). In spite of her numerous human and natural resources, Nigeria still remains among the least consumers of animal protein in Africa. The protein intake of an average Nigerian is about 53.8 g with only 6.0-8.4g/head/day of animal origin (Egbunike, 1997).

CBN (1993) revealed that North America, Western and Eastern European countries consume 66, 39 and 33 g of animal protein per head per day respectively, while an average Nigerian consume 7.5 g which is below the recommended level of 27 g/head/day. To increase protein intake in Nigeria, there is urgent need to increase broiler production at household and commercial holdings. The per capita consumption of broiler meat in Nigeria was below 2.0 kg (Akinwunmi, 1981) as against 12.0 kg to 15.5 kg between 1985 and 1990 in South Africa (Viljoem, 1997). Nigeria presently is food insecure. Food insecurity is a critical variable for understanding the nutritional status of low-income populations in the world. The problem of undernourishment is worst in African

countries where 32% (one out of every three Africans) of the population was under-nourished during 1983-1985 period compared to 22% in far East, 14% in Latin America and 11% in near East (FAO, 1990). This problem still remains the same presently in Nigeria. The WHO (2002) noted that at least 50% of all deaths among the under-age are attributed to malnutrition, while lucky survivors cannot run away from the stark realities of poor health and overall indigent lifestyle.

Phyllanthus Amarus

According to Igwe et al (2007), *P. amarus* was first identified in central and southern India in 18th century but is now found in many countries including Philippine, Cuba, Nigeria among others. It is commonly called 'carry me seed', 'stone breaker', wind breaker' or 'gulf leaf flower'. Among a few potent hepatoprotective phytomolecules reported in the scientific literature, against various types of liver damages, phyllanthin and hypophyllanthin have largely attracted the scientific community (Negi et al., 2008). The extract of *P. amarus* along with a small amount of turmeric is taken orally for 3-5 days to cure jaundice (Prasad et al., 2008). Annamalai and Lakshmi (2009) reported that the interest in *P. amarus* has increased in recent years based on the efficacy of the herb against Hepatitis B virus and all parts of this wonder plant is medicinally important. In the market many drugs which contain *P. amarus* have been released under name like 'Ayurviva' against liver disorders, hepatoprotective, loss of appetite, general debility and convalescence and 'Lovanthin' against Hepatitis-B (Kumar et al., 2010). *P. amarus* has been described in Ayurveda by the Sanskrit name –*Bhoomyaamalakee*. It was described to have the

properties of Rasa, Guna, Veerya and Vipaaka. The Ayurvedic literature has shown its uses as Kaasahara (antitussive), Shwaasahara (antispasmodic, antidyspnoic), Kaphapittahara (which relieves the Kapha Pitta Dosha), Pipaasaaghna (which relieves Polydipsia), Raktapittahara (hemorrhage disease), Paanduhara (antianaemic), Kaamalaahara (which cures jaundice), Kushthaghna (indicated in leprosy), Daahaghna (refrigerant, relieves burning sensation), Kshatakshayaghna (indicated in Trauma) and Mootrarogahara (which cures urinary disorders) (Patel et al., 2011).

Origin of Phyllanthus Amari

Phyllanthus amarus is a leafy herbal plant found in tropical regions in the Americas, Africa, India, China, Sri Lanka and South East Asia. Commons names for this plant include gale of the wind, carry me seed, seed on the leaf, pick-a back, Atlas of Florida Plants (2021).

Phyllanthus amarus is a small, annual plant that grows to a height of 30–60 cm. Its thin branches spread out, and each branch has two rows of small, elliptic-oblong leaves of 5-10mm long that are arranged alternately. Samy and Manickam (2005) Its radial flowers are star-shaped and of about 2mm in size. National Parks Singapore (2021). It grows well in soil of high moisture with light shade, and reaches maturity in 2–3 months.

Phyllanthus amarus contains flavonoids (quercetin-3-O-glucoside and rutin), tannins (geraniin, amariin and gallocatechin) and alkaloids (phyllantine, quinolizidine type, securinine, norsecurinine, isobubbialine and epibubbialine) Samy and Manickam (2005).

Phyllanthus amarus has been used in the traditional medicine of various cultures, including Amazonian tribes for the treatment of gallstones and kidney stones; in Ayurvedic (Sinhala) medicine for bronchitis, anaemia, diabetes; and in Malay traditional medicine for diarrhoea, kidney ailments and gonorrhea Samy and Manickam (2005). More recently there have been preclinical and clinical studies looking into the plant's supposed liver-protective abilities Krithika (2005) and effect on hepatitis B. George, et.al, (2019)

CHAPTER THREE

MATERIALS AND METHODS

The experiment was carried out in the Agricultural garden of Kwara State Polytechnic, Ilorin. Leaves of *P. amarus* were harvested from the Polytechnic campus in Kwara State. The plant materials were dried under shade for one week. The dried leaves were milled to fine powder using a milling machine. The fine powder was placed in a thimble made from thick filter paper which was loaded unto the main chamber of the soxhlet extractor. The solvent (250ml of ethanol) was added to a round bottom flask which is attached to the soxhlet extractor and condenser. The solvent was heated and as it boils its vapors rises up and are condensed by the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. Embazine forth was the conventional anti-coccidial drug used. Extraction of *Eimeria* oocyst About 20g of dry infected droppings was homogenised in distilled water (100mL) and then soaked with 2.5% (W/V) of passions dichromate and allowed to stay for 24hrs. The mixture was filtered using muslin cloth, the residue was discarded and the filtrate was further treated with saturated sodium chloride for precipitation, the mixture was centrifuged at 1500 rpm for 30min. The tubes were removed and the supernatant was discarded. The sediment (occyst) transferred into distilled water for floatation to take place. After floatation, it was then centralized and the supernatant discarded while the residues which are the occyst collected and kept in a plastic material until ready for use. The solution was pipetted to fill both sides of the Mc Master slide and was examined under the microscope at a magnification

of 40X. A total of 200 day old Ross 308 strain chicks were purchased from Agrited farm Kaduna and used for the experiment and Eimeria infection. A week to the arrival of the animals on the experimental site, the pen with the cages was thoroughly washed and cleaned using disinfectant (lysol) and allowed to dry. Heat was provided for the birds day and night for the first two weeks of their age. The birds were fed ad libitum on a proprietary broiler ration without coccidiostat additives and were also given access to water ad libitum. They were routinely vaccinated against Gumboro and Newcastle diseases. At 27day old, the birds were inoculated orally with sporulated oocysts, they were starved with water the night before infection to enhance quick intake of the water. The prepared sporulated Eimeria occyst was added into their drinking water. After a successful infection the birds were transferred to their respective cages and were assigned randomly to treatment groups. Seven days post infection when the birds started to show clinical signs of coccidiosis, the P. amarus treatment was administered to the birds. Split Plot Design was employed and the data were analyzed using statistical package software (SPSS version 23). Differences in mean body weight were tested by least significance difference (LSD) under analysis of variance (ANOVA).

Statistical Methods

(i) Split Plot Design Model

The statistical model for split plot design is as given below:

$$y_{ijk} = \mu + \tau_i + \beta_j + \delta_k + (\beta\delta)_{jk} + \gamma_{ij} + \epsilon_{ijk} \quad (1)$$

$$i = 1, \dots, r; j = 1, \dots, a; k = 1, \dots, b$$

Where, y_{ijk} = the yield associated with k^{th} level of the subplot factor B within the j^{th} levels effect of whole plot factor A and the i^{th} block effect (replication, r)

μ = grand mean to all sub-plot

τ_i = i^{th} block effect

β_j = j^{th} whole plot (A) treatment effect

δ_k = k^{th} sub plot (B) treatment effect

$(\beta\delta)_{jk}$ = interaction due to j^{th} level of whole plot treatment and k^{th} level sub plot treatment

γ_{ij} is random component due to all sub-plots in the $(i, j)^{th}$ whole-plot, and ϵ_{ijk} is a random component peculiar to the sub-plot with the k^{th} level of B in the $(i, j)^{th}$ whole-plot.

Assumptions of Split Plot Design

i. $\sum \tau_i = \sum \beta_j = \sum \delta_k = \sum (\beta\delta)_{jk} = 0$;

ii. $\gamma_{ij} \sim \text{NID}(0, \sigma_w^2)$; and $\epsilon_{ijk} \sim \text{NID}(0, \sigma^2)$; γ_{ij} and ϵ_{ijk} assumed to be independent.

iii. Additivity of block and treatment effects is assumed.

Partitioning of Total Sum of Squares in Split Plot Design

Initially we have,

$$\sum_i \sum_j \sum_k (y_{ijk} - \bar{y}_{...})^2 = b \sum_i \sum_j (y_{ij.} - \bar{y}_{...})^2 + \sum_i \sum_j \sum_k (y_{ijk} - y_{ij.})^2 \quad (2)$$

$$\text{SST} = \text{Whole-plot} + \text{sub-plot}$$

Which represent the whole-plot and within sub-plot, the former is further subdivided as

$$n \sum_i \sum_j (y_{ij.} - \bar{y}_{...})^2 = ab \sum_i (y_{i..} - \bar{y}_{...})^2 + rb \sum_j (y_{.j.} - \bar{y}_{...})^2 + b \sum_i \sum_j (y_{ij.} - y_{i..} - y_{.j.} + \bar{y}_{...})^2$$

$$\text{Whole-plot} = \text{Block} + (A) + \text{Error (a)}$$

The latter also subdivided as

$$\sum_i \sum_j \sum_k (y_{ijk} - y_{ij.})^2 = ra \sum_k (y_{..k} - \bar{y}_{...})^2 + \sum_i \sum_j \sum_k (y_{ijk} - y_{ij.} - y_{..k} + \bar{y}_{...})^2$$

$$\text{Sub-plot} = (B) + \text{Residuals}$$

This residuals may now be partitioned further into

$$\sum_i \sum_j \sum_k (y_{ijk} - y_{ij.} - y_{..k} + \bar{y}_{...})^2 = r \sum_j \sum_k (y_{i.k} - y_{i..} - y_{..k} + \bar{y}_{...})^2$$

$$\text{Residuals} = (AB)$$

$$+ \sum_i \sum_j \sum_k (y_{ijk} - y_{ij.} - y_{i.k} + y_{i..})^2$$

$$\text{Error(b)}$$

Hypothesis H_0 and H_1 can be define for each of whole plot (A), sub plot (B) and interaction

AB $[\beta_j, \delta_k, (\beta\delta)_{jk}]$.

Decision rule: Reject H_0 , if $F_{cal} > F_{table}$; P-value $< \alpha = 0.05$ (level of significance)

(ii) Least Significant Difference (LSD)

Fisher developed the least significant difference test in 1935, which is only used when the null hypothesis is rejected as a result of hypothesis test results. The LSD calculates the smallest significant between two means as if a test had been run on those two means (as opposed to all of the groups together). This enables to make direct comparisons between two means from two individual groups. Any difference larger than the LSD is considered a significant result.

$$\text{LSD} = t * \text{S.E}, \text{ where, } t = t_{[p(n-1), \alpha]} \quad (3)$$

$$S.E = \sqrt{\frac{2MSE}{n}}, \text{ if } n_i=n_j=n$$

$$S.E = \sqrt{MSE \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}, \text{ if } n_i \neq n_j$$

If the observe difference between any two means is greater than the LSD value, then those two means are said to be significantly different.

CHAPTER FOUR

RESULT AND DISCUSSION

Data Analysis and results

The descriptive statistics of the results are shown in the Table 1 below.

Table 1. Descriptive Statistics

Week	Group	Treatment inclusion	Mean	Std. Deviation	N
Week1	Group1	5ml(phyllantus Amarin)	480.0	99.6	15
		10ml(phyllantus Amarin)	423.3	84.2	15
		Total	451.7	95.1	30
	Group2	5ml(phyllantus Amarin)	386.7	93.5	15
		10ml(phyllantus Amarin)	426.7	79.9	15
		Total	406.7	87.8	30
	Total	5ml(phyllantus Amarin)	433.3	106.1	30
		10ml(phyllantus Amarin)	425.0	80.7	30
		Total	429.2	93.6	60
Week2	Group1	5ml(phyllantus Amarin)	470.0	94.1	15
		10ml(phyllantus Amarin)	470.0	106.6	15
		Total	470.0	98.8	30
	Group2	5ml(phyllantus Amarin)	476.7	98.0	15
		10ml(phyllantus Amarin)	466.7	72.4	15
		Total	471.7	84.8	30
	Total	5ml(phyllantus Amarin)	473.3	94.4	30
		10ml(phyllantus Amarin)	468.3	89.5	30
		Total	470.8	91.3	60
Week3	Group1	5ml(phyllantus Amarin)	362.7	101.6	15
		10ml(phyllantus Amarin)	467.3	99.4	15
		Total	415.0	112.2	30
	Group2	5ml(phyllantus Amarin)	380.0	96.5	15
		10ml(phyllantus Amarin)	373.3	96.1	15
		Total	376.7	94.7	30
	Total	5ml(phyllantus Amarin)	371.3	97.7	30
		10ml(phyllantus Amarin)	420.3	107.3	30
		Total	395.8	104.7	60
Week4	Group1	5ml(phyllantus Amarin)	575.3	82.1	15
		10ml(phyllantus Amarin)	587.7	54.6	15
		Total	581.5	68.8	30

	Group2	5ml(phyllantus Amarin)	537.7	64.2	15
		10ml(phyllantus Amarin)	573.3	79.9	15
		Total	555.5	73.5	30
	Total	5ml(phyllantus Amarin)	556.5	74.9	30
		10ml(phyllantus Amarin)	580.5	67.6	30
		Total	568.5	71.8	60

From Table 1, the average weight gain for 5ml inclusion level is $480.0g \pm 99.6g$, while for 10ml inclusion level is $423.3g \pm 84.2g$ with total aggregate weight gain of $451.7g \pm 95.1g$ for the group1 in week1. For the group2 in week 1, the average weight gain for 5ml inclusion level is $386.7g \pm 93.5g$, while for 10ml inclusion level is $426.7g \pm 79.9g$ with total aggregate weight gain of $406.7g \pm 87.8g$. In total, the average weight gain for 5ml inclusion level is $433.3 \pm 106.1g$ and $425.0g \pm 80.7g$ is weight gain for 10ml inclusion level for the week 1.

For the week 2, the average weight gain for 5ml inclusion level is $470.0g \pm 94.1g$, while for 10ml inclusion level is $470.0g \pm 106.6g$ with total aggregate weight gain of $470.0g \pm 98.8g$ for the group1. For the group2, the average weight gain for 5ml inclusion level is $476.7g \pm 98.0g$, while for 10ml inclusion level is $466.7g \pm 72.4g$ with total aggregate weight gain of $471.7g \pm 84.8g$. In total, the average weight gain for 5ml inclusion level is $473.3 \pm 94.4g$ and $468.3g \pm 89.5g$ is weight gain for 10ml inclusion level, respectively.

For the week 3, the average weight gain for 5ml inclusion level is $362.7g \pm 101.6g$, while for 10ml inclusion level is $467.3g \pm 99.4g$ with total aggregate weight gain of $415.0g \pm 112.2g$ for the group1. For the group2, the average weight gain for 5ml inclusion level is $380.0g \pm 96.5g$, while for 10ml inclusion level is $373.3g \pm 96.1g$ with total aggregate weight gain of

376.7g $\pm$ 94.7g. In total, the average weight gain for 5ml inclusion level is 371.3 $\pm$ 97.7g and 420.3g $\pm$ 107.3g is weight gain for 10ml inclusion level.

For the week 4, the average weight gain for 5ml inclusion level is 575.3g $\pm$ 82.1g, while for 10ml inclusion level is 587.7g $\pm$ 54.6g with total aggregate weight gain of 581.5g $\pm$ 68.8g from the group1. For the group2, the average weight gain for 5ml inclusion level is 537.7g $\pm$ 64.2g, while for 10ml inclusion level is 573.3g $\pm$ 79.9g with total aggregate weight gain of 555.5g $\pm$ 73.5g. In total, the average weight gain for 5ml inclusion level is 556.5 $\pm$ 74.9g and 580.5g $\pm$ 67.6g is weight gain for 10ml inclusion level.

In addition, it can be observed that the total average weight gain at week 4 is the highest for both 5ml (556.5g) and 10ml (580.5g), followed by week 2 with 5ml(473.3g) & 10ml(468.3g), then week 1 with 5ml(433.3g) & 10ml(425.0g), and week 3 with 5ml(371.3g) & 10ml(420.3g), in that order respectively.

Table 2. Levene's Test of Equality of Error Variances^a

F	df1	df2	Sig.
1.529	15	224	.096

From Table 2, p-value (0.096 > α = 0.05) suggests that the null hypothesis of equality of error variance is not rejected, hence we conclude that the distribution of error variance is constant and independent, i.e. homoscedasticity.

Table 3. ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.085E6 ^a	9	120503.2	14.7	.000
Intercept	5.214E7	1	5.214E7	6.369E3	.000
Week	1008578.3	3	336192.8	41.07	.000
Group	43470.4	1	43470.4	5.31	.022
Week * Group	19127.9	3	6375.97	.78	.507
Treatment	13350.4	1	13350.4	1.63	.203
Group * Treatment	1.667	1	1.667	.000	.989
Error	1882639.6	230	8185.4		
Total	5.510E7	240			
Corrected Total	2967168.3	239			

From Table 2, the p-value for the Week (0.000) and Group (0.022) are less than $\alpha = 0.05$ (level of significance) which indicate that null hypothesis is rejected and conclude that there is significant difference in the marginal average weight gain of the chicken both within and between the weeks and groups based on the treatment inclusion level. However, there is no significant difference in treatments (p-value=0.203) inclusion level applied as well as interaction effect due to week/group (0.507) or group/treatment (0.989) because the null hypothesis is not rejected since their p-value are greater than $\alpha = 0.05$. This implies that, 5ml or 10ml treatment inclusion level effect is not significantly differ with respect to average weight gain. It is suggested that either 5ml or 10ml inclusion level of treatment (*Phyllanthus Amarus*) is considered adequate for the treatment of chicken with Coccidiosis diseases. Also, the results suggested that treatment based on grouping (blocking) does not have any significant effect on the average weight gain of the chicken with Coccidiosis diseases.

Finally, a multiple comparison (Post hoc) test was performed using least significant difference (LSD) to determine the week with significant average weight gain for the chicken during the Coccidiosis treatment and results is displayed in the Table 4 below.

Multiple Comparisons (Post Hoc) Test

Table 4. Multiple Comparisons (Post Hoc) Test

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) Week	(J) Week				Lower Bound	Upper Bound
LSD	Week1 Week2	-41.6667*	16.51806	.012	-74.2127	-9.1206
	Week1 Week3	33.3333*	16.51806	.045	.7873	65.8794
	Week1 Week4	-139.3333*	16.51806	.000	-171.8794	-106.7873
	Week2 Week1	41.6667*	16.51806	.012	9.1206	74.2127
	Week2 Week3	75.0000*	16.51806	.000	42.4539	107.5461
	Week2 Week4	-97.6667*	16.51806	.000	-130.2127	-65.1206
	Week3 Week1	-33.3333*	16.51806	.045	-65.8794	-.7873
	Week3 Week2	-75.0000*	16.51806	.000	-107.5461	-42.4539
	Week3 Week4	-172.6667*	16.51806	.000	-205.2127	-140.1206
	Week4 Week1	139.3333*	16.51806	.000	106.7873	171.8794
	Week4 Week2	97.6667*	16.51806	.000	65.1206	130.2127
	Week4 Week3	172.6667*	16.51806	.000	140.1206	205.2127

From the Table 4, all the p-value (Sig.) < $\alpha = 0.05$ (level of significance) with (*) indicating that there is significant difference in the average weight gain after the treatment of the chicken affected with Coccidiosis using Phyllanthus Amarus. Thus, average weight gain at week 4 is considered to most significant when compare with other weeks because it yields the highest mean difference.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Summary of the findings

The following are the summary of the findings;

- i. It can observed that the total average weight gain at week 4 is the highest for both 5ml (556.5g) and 10ml (580.5g), follow by week 2 with 5ml(473.3g) & 10ml(468.3g), then week 1 with 5ml(433.3g) & 10ml(425.0g),and week 3 with 5ml(371.3g) & 10ml(420.3g), in that order respectively.
- ii. Results reveals that there is significant difference in the marginal average weight gain of the chicken both within and between the weeks and groups based on the treatment inclusion level.
- iii. The effect of the treatment inclusion level of 5ml or 10ml is not significantly differ with respect to average weight gain. Hence, either 5ml or 10ml inclusion level of treatment (*Phyllanthus Amarus*) is considered adequate for the treatment of chicken with Coccidiosis diseases.
- iv. The results suggested that treatment based on grouping (blocking) does not have any significant effect on the average weight gain of the chicken with Coccidiosis diseases.
- v. There is significant difference in the average weight gain after the treatment of the chicken affected with Coccidiosis using *Phyllanthus Amarus* between the weeks. Thus, average weight gain at week 4 is considered to most significant when compare with other weeks because it yields the highest mean difference.

Conclusion

Based on the analysis, the results shows that the *Phyllanthus amarus* can cure coccidiosis in broiler birds and 5ml inclusion level of *Phyllanthus amarus* extract is very effective for treatment of coccidiosis. Finally, there is significant average weight gain after the treatment of the chicken affected with Coccidiosis using *Phyllanthus Amarus*.

Recommendation

This study suggests *Phyllanthus amarus* should be tried on other farm animals' aside broiler birds.

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