THE UNIVERSITY OF ZAMBIA

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF ANIMAL SCIENCE

ANTIOXIDANT EFFECT OF SELECTED EDIBLE PLANT EXTRACTS ON PERFORMANCE OF BROILERS

BY

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UNZA JUNE, 2012

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A THESIS SUBMITTED TO THE SCHOOL OF AGRICULTURAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCES

UNZA, LUSAKA

JUNE, 2012

DECLARATION

This thesis represents original research and has not been done or presented for a degree in this or any other university. The results shown herein are a true reflection of what was obtained from the study.

HIRI MWAPE THOMAS

UNE 2012

ABSTRACT

Edible plants contain biologically active compounds that have antioxidant capacity; these biologically active compounds are distributed in leaves, roots stems and fruits. Among other uses of biologically active compounds, they have been used in broiler production for improving weight gain and for their medicinal properties. The experiment aimed at evaluating the effect of antioxidant properties of selected edible plants on performance of broilers. Seven edible plants (*Bidenspilosa, Ficussycomorus, Cleomegynandra, Solanumaethiopicum, Hibiscus meeiusei* , *Opuntia vulgaris* and *Piliostigmathonningii*) that are also used as medicinal plants were selected, of which three plants were used in broiler trials (Cobb 500) after screening for total antioxidant capacity. The experiment was conducted at the University of Zambia, School of Agricultural Sciences over a period of six weeks.

The experiment was done in a completely randomized block design with 7 treatments. Six treatments were *Ficussycomorus, Cleomegynandra andOpuntia vulgaris* plant parts preserved in each of alcohol and vinegar. The control had no plant extract. There were three replications of each treatment. Differential white blood cell count was done on blood collected at 43 days of age.

No significant differences ($p<0.05$) were found among the treatments for mean live weight, feed consumption and feed conversion ratio at 43 days of age. Significant differences ($p<0.05$) were found in heterophil levels but not basophils, eosinophils, monocytes and lymphocytes.

ACKNOWLEDGEMENTS

My appreciation goes out to my supervisor Ms. M.N Musukwa of the Animal Science Department, School of Agricultural Sciences for her supervision, guidance and immeasurable support towards the success of the project.

Many thanks go to the Head of Department of Animal Science Dr Drinah Banda Nyirenda for ensuring that all materials required at the field station were purchased on time for the smooth running of the project.

Special thanks also go to Mr. Mungili the supervisor for the field station, for the help offered on usage of necessary materials required from beginning to end of project.

Sincere gratitude also go to Mr. Banda for their help in analyzing the data.

Special thanks also go to the security guards for enabling the safety of the field station at night.

Humble gratitude to my friends Ashley chishiba, Bless Siwale, James Chishiba and Simfukwe Soft for their help during and after the project

Special thanks also go to my family (Mwapes) for their moral support during the project.

To God be the glory.

DEDICATION

This report is dedicated to my family and to God almighty for His unconditional love.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	ANALYSIS OF VARIANCE
KG	KILOGRAM
WBC	WHITE BLOOD CELL
IBDV	INFECTIOUS BURSAL DISEASE VIRUS
IBD	INFECTIOUS BURSAL DISEASE

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CHAPTER 1

1.0 INTRODUCTION

Broiler production is the rearing of chickens for their meat. Broilers are important worldwide as sources of food protein and cash income. When reared under good management, chickens grow very fast and are ready for sale at about five or six weeks of age. Broiler production has several benefits, it helps to improve food self-sufficiency and alleviate malnutrition as it provides an excellent protein source, minerals and vitamins which are essential for growth and maintenance of the body *(Farrell, 2000)* and it creates income generating activities *(Wethli, 1999)* as a lot of labour is employed. Poultry production has evolved gradually as a commercial venture since its introduction in the 1950s. *Oluyemi* and *Roberts (2000)* and *Isika et al. (2006)* postulated that poultry was strategic in addressing animal protein intake shortage in human nutrition because of its high fecundity, fast growth rate, short generation interval and unparalleled competence in nutrient transformation to high quality animal protein.

Antibiotic feed additives have been used for more than 50 years to enhance growth performance and prevent disease in livestock feeding environments (*Dibner and Richards, 2005*). Many antibiotics are used in animal production, especially in intensive husbandry. While some of the antibiotics are used therapeutically to improve the health and well-being of animals, a large portion are used for prophylactic purposes such as improvements in growth rate and feed conversion efficiency. The use of antibiotics for enhancing the performance of poultry production has been in use since their discovery. Antibiotics have undoubtedly had an effect on the improvement of animal performance and health status *(Thowke and Elwinger 1998).* It is common practice to add antibiotics to poultry diets to improve chicken health production and meat quality *(Schwarz et al 2001).*

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria and is administered at a low, sub therapeutic dose. The use of antibiotics for growth promotion has arisen with the intensification of livestock production. Infectious agents reduce the yield of farmed food animals and, to control these, the administration of antibiotics and

ntimicrobial agents at sub-therapeutic levels has been shown to be effective. *(Hughes et al '002)*

'he condition and function of the intestinal tract are key factors in health and performance of irds. In the past, poultry feed was supplemented with antibiotics to modulate the intestinal nicrobial ecology. However, the emergence of bacterial resistance to antibiotics in humans has aused an increase in public and governmental interest in eliminating sub-therapeutic use of ntibiotics in livestock production *(Hughes and Heritage, 2004).*

Vith increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage n Europe and the potential for a ban in US, there is an increasing interest in finding alternatives o antibiotics for poultry production globally. A public health concern associated with pathogenic acteria is the increased incidence of strains that are resistant to antimicrobial agents. Those esistant microorganisms can be disseminated via animal feces to other animals *(Ravikumar, 011).* Thus, current research involving feed additives for diets of young animals is focused on earching for alternatives to antibiotics that would have at least similar growth promoting effects f antibiotics without causing bacterial resistance. Today, the poultry industry has focused its ttention towards addressing public concern for environmental and food safety. As in many other ndustries, the global paradigm is shifting from an emphasis on efficiency to one of public ecurity *(Peter R. Ferket, 2002).* As a consequence, new commercial additives of plant origin onsidered to be natural products acceptable to the consumer have been proposed to livestock roducers (*Cabuk, 2006).*

.1 Background on use of Plant Extracts

lants have an almost limitless ability to synthesize aromatic substances, most of which are henols or their oxygen-substituted derivative. Most are secondary metabolites, of which at least 2,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, ese substances serve as plant defense mechanisms against predation by microorganisms, sects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and nnins) are responsible for plant pigment. Many compounds are responsible for plant flavor .g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by mans to season food yield useful medicinal compounds (*Schultes 1978).*

'lant extracts, more commonly known as essential oils, have been used for centuries in Chinese ierbal medicine. Plant extracts have a positive effect on feed intake (mainly due to their ppetizing flavour or aroma), endogenous enzyme production, the immune system as well as acteriostatic and fungistatic effects (*Kamel, 2000; Mellor, 2000*). The kind of bioactive ompounds and their concentration varies significantly with the source) and supports the fact that ixtures of different plant extracts are supposed to induce greater effects than single plant xtracts *(Kamel, 2000).*

.2 OBJECTIVES

.2.1 Overall Objectives

he main objective of the research was to determine the effect of selected antioxidant containing dible plant extracts on the performance of broilers.

.2.2 Specific Objectives

i. To find out the total antioxidant levels of selected edible plant species.
ii. To determine the weekly live weight, feed consumption and feed conversion ratio of broilers supplemented with antioxidant-containing plant extracts.
ii. To determine the effect of the extracts on Differential white cell count (Levels of Basophils, Monocytes, Heterophils, Eosinophils and Lymphocytes in broilers.

CHAPTER 2

2.0 LITERATURE REVIEW

The poultry industry has been quick to adapt to genetic progress and to incorporate improved husbandry practices that allow producers to realize a greater return from their investment. It must be acknowledged that even in state-of-the-art facilities, there are common sources of stress which exist as a major factor in reducing productivity. Stress is any biological response elicited when an animal perceives a threat to its homeostasis. When a stressor is actually causing a negative impact on the well-being of an animal, this can be defined as distress *(Moberg, 2000)*. Heat stress is of major concern for poultry industry, especially in the hot regions of the world because of the resulting poor growth performance, immunosuppression, and high mortality (*Bottje and Harrison, 1985; Young, 1990; Yahav et al., 1995*).

2.1 STRESS IN BROILER PRODUCTION

Continuous selection for fast growth has been associated with increased susceptibility of broilers to heat stress *(Geraert et al., 1993; Cahaner et al., 1995; Berong and Washburn, 1998)*. Exposure of chickens to heat stress causes significant behavioral and physiological responses *(Harrison and Biellier, 1969; Altan et al., 2003)*. Thermal stress exerts its deleterious effects on feed intake and body weight gain *(Geraert et al., 1996)* as well as on carcass yield and mortality rates *(Smith, 1993)*.

2.1.1 Types of Stress and their Effects on Broilers

Stress can be grouped under one or more of the following categories:

i. Climatic stress refers to the extreme changes in temperature, i.e. extreme heat and cold and high humidity in the environment.

ii. Environmental stress refers to factors relating to bright light, wet litter that can lead to accumulation of ammonia gas and poor ventilation which prevents proper circulation of air. Environmental stress also includes heat stresses, these are known to be one of the major

problems facing broiler rearing in tropical and subtropical areas, In broiler, the feed consumption and growth rate decrease as the ambient temperature rises *(Sabah et al; 2008).*

ii. Nutritional stress refers to all factors relating to shortages of nutrients, feed intake problems that can be as a result of environmental factors *(Brake, 1987).*

v. Physiological stress includes rapid growth of birds and process of maturing sexually.

v. Physical stress refers to stress that is due to catching, immobilization, injections, and transport of birds. This commonly happens during daily management routines in broiler production.

vi. Social stress includes overcrowding of the birds and poor body weight uniformity of the birds.

ii. Psychological stress includes all psychological factors that affect broiler production such as fear or a prolonged period of fear and harsh caretakers.

Birds raised under stressful conditions can be identified from flock records. Stocking density, mortality, body weight gains, and uniformity denote deviations from established standards.

Birds that are raised in such environmental conditions become highly susceptible to free radical formations in their body and thus susceptible to different diseases *(Gregorio, 1994).* For example, under normal physiological conditions about 3-5% of the oxygen taken up by the cell undergoes univalent reduction leading to the formation of free radicals *(Singal et al., 1998*).

Free radicals in broilers can be causal agents that can be a source of a number of diseases, Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin).Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress, that means a situation where the cell does not control the excessive presence of oxygenated toxic free radicals *(Favier, 2003).* Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease *(Atoui, 2005).*

'ree radicals are atoms, molecules or any compounds containing one or more unpaired electrons. Aost biologically-relevant free radicals are derived from oxygen and nitrogen. Both these lements are important for animal life, but in some circumstances they can be converted deliberately or by chance) into free radical molecules. Highly reactive free radicals and oxygen pecies are present in biological systems from a wide variety of sources, Free radicals (super ıxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, ıypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause ıxidative damage of amino acids, lipids, proteins and DNA because they are highly unstable *Gutteridge, 1995*). Free radicals are highly reactive molecules or chemical species capable of ndependent existence. Generation of highly Reactive Oxygen Species (ROS) is an integral 'eature of normal cellular function like mitochondrial respiratory chain, phagocytosis, Arachidonic acid metabolism, ovulation, and fertilization. Their production however, multiplies ;everal folds during pathological conditions (*Halliwell, 1989*).

2.1.2 Antioxidant Supplementation for Stress in Broilers

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) ıow appears the fundamental mechanism underlying a number of neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders *(Sinha, 2010)*. Oxidative stress caused by excessive levels of reactive oxygen species that are induced under stressful environments such as heat exposure and coccidiosis has been regarded as ɔne of the major factors negatively affecting performance of birds in the concentrated poultry industry *(Dalloul et al., 2006; Lin et al., 2006; Mujahid et al., 2007*) and as a main factor in the pathogenesis of several serious diseases (*Krichibbzs-Etherton et al., 2004*). Cells are under constant attack by free radicals, many of which are formed as a natural consequence of normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms. Therefore, supplementation of synthetic antioxidants (e.g., alpha-tocopherylacetate or butylated hydroxytoluene) to mitigate the oxidative stress has become a common practice in the poultry industry. Recently, use of plant extracts as natural antioxidants has gained increasing interest because of the global trend of restriction in use of synthetic substances *(Ahn et al., 2002). Naidoo et al. (2008)* demonstrated that antioxidant-rich plant

extracts have potential benefits in treating coccidial infections. Plant species from the families of Zingiberaceae (e.g., ginger and curcuma) and Umbelliferae (e.g., anise and coriander), as well as plants rich in flavonoids (e.g., green tea) and anthocyanins (e.g., many fruits), are described as exerting antioxidative properties. Pepper (*Piper nigrum*), red pepper (*Capsicum annuum L.*), and chili (*Capsicum frutescene*) contain antioxidative components *(Shibamoto, 2007).*

The most widely studied dietary antioxidants are vitamin C, vitamin E, and beta-carotene. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Beta-carotene and other Carotenoids also provide antioxidant protection to lipid rich tissues. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains, i.e., cereals and high quality vegetable oils are major sources of vitamin E *(Halliwell, 1989).*

2.1.3 Stress-Related Blood Parameters in Birds

On the basis of literature published in the last decade, researchers have concluded that stressors such as severe feed restriction high and low environmental temperatures fear and frustration, noise and road transportation cause different leucocytic responses. However, determining levels of corticosteroid levels or heterophil/lymphocyte ratios cannot always be accepted as an accurate measurement of stress in poultry. It appears that more reliable methods include measurements of the suppression of the immune system determined by counts and proportions of leukocytes with different immunological functions (*Gregorio, 1994*).

The normal packed cell volume ("PCV") for most psittacine species ranges from 37-50% with juvenile birds on the low end of the range. Values below 37% are considered anemic. Values below 15% might indicate the need for a blood transfusion. Generally, normal total leukocyte counts in chickens (*Gallus gallus domesticus*) are between 1.2- 3.0 × 104cell/μl (average 1.2) *(Jain, 1993).* A count that is greater than normal range is considered suggestive of leukocytosis. General causes of leukocytosis include infection, trauma, toxicities, hemorrhage into a body

vity, rapidly growing neoplasm and leukemias. The leukocyte count aids in the assessment of e leukocytosis, because a heterophilia is usually present in leukocytosis caused by flammation, *(Ritchie et al., 1994).* Typically, heterophil infiltration predominates in the first 6 12 hrs of the inflammatory response, but macrophages, lymphocytes, and even giant cells are esent at 48 h and there are numerous giant cells at 72 h. Low white blood cell counts are casionally observed with severe viral infections, particularly psittacine circovirus (beak and ather disease virus) infection in young birds, *(Harmon, 1998).*

2. CHEMICAL COMPOUNDS OF IMPORTANCE IN MEDICINAL PLANTS

2.1. Phenols

ienols are the major constituent of these plant species. Phenolic Compounds are composed of ie or more aromatic benzene rings with one or more hydroxyl groups (C-OH). This enormous ass includes numerous plant compounds that are chemically distinct from terpenes. Although e essential oils are often classified as terpenes, many of these volatile chemicals are actually enolic compounds, such as eucalyptol from (*Eucalytus globulus*), citronellal from (*E. riodora*) and clove oil from *Syzygium aromaticum* (Vivanco, *2003*).

2.2 Flavonoids

avonoids have been reported to exert multiple biological effects including antibacterial, tiviral, antitoxic and anti-inflammatory activities (*Cook and Samman, 1996*). Many of these eged effects of flavonoids have been linked to their known functions as strong antioxidants, e radical scavenger and metal chelators (*Torel et al., 1986; Nakayama et al., 1993*). The sitive effects of glycosides and cardiac glycosides are not common but their toxic effects clude decreased heart rate, decreased sympathetic activity and decreased systemic vascular istance (*Seigler, 1998*). Flavonols are colorless or yellow flavonoids found in leaves and many wers. The presence of some of these antinutrients could however be reduced by various ocessing techniques (*Elegbede, 1998*).

Like the terpenes, many Phenolic compounds are attached to sugar molecules and are called Glucosides or glycosides, depending on the type of sugar. Most vanilla flavorings sold in markets are synthetic vanillin containing artificial food coloring and preservatives. The double-ring phenolic compound called coumarin imparts the distinctive sweet smell to newly-mown hay. Coumarin is also an anticoagulant that represses the synthesis of prothrombin, a plasma protein produced in the liver in the presence of vitamin K. Prothrombin is the precursor of the enzyme thrombin which catalyzes the conversion of fibrinogen to fibrin in the clotting process. Threads of fibrin wind around blood platelets in the damaged area of a blood vessel and provide the framework of a blood clot (*vivanco, 2003*).

2.2.3. Saponins

Saponins are glycoside components often referred to as "natural detergent" because of their foamy nature (*Seigler, 1998*). Saponins in seeds have been known to posses both beneficial and deleterious properties depending on its concentration in the sample (*Seigler, 1998; Oakenful and Sidhu, 1989*). Seigler (1998) reported that saponins have anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cells and cholesterol lowering activity.

2.3 MEDICINAL PROPERTIES OF THE SELECTED PLANT EXTRACTS

2.3.1 Cat's Whiskers (*Cleome gynandra:* Family *Capperdiceae*)

Cleome gynandra is used as a medicinal plant and can be found in all over world .It grows as a weed in paddy fields and also in road sides and in open grass lands. *Cleome gynandra* is a common, widespread herb occurring in southern Africa extending from the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State, the Northern Cape and Namibia (*Mishra, 2011*).

The vegetable is important as a leafy vegetable in the following African countries: Nigeria, Zaire, Malawi, Zimbabwe, Cameroon, Botswana, Namibia, Swaziland, Tanzania, Zambia, South Africa, Ghana, Uganda and Kenya Indigenous knowledge possessed by rural women in Kenya indicates that *C. gynandra* has several nutritional uses. Leaves may be crushed to make a

concoction that is drunk to cure diseases such as scurvy. In many cultures, boiled leaves are regarded as a medicinal meal. In other communities, leaves are boiled and marinated in sour milk for 2-3 days and eaten as a nutritious meal, which is believed to improve eyesight, provide energy and cure Marasmus (*Opole et al. 1995*).

The leaves can also be used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash. Seeds have been reported to have anti helmintics properties, oil is used as fish poison and also used as alternative treatment of malaria, piles (haemorrhoids), and rheumatism (*Mule et al., 2008*). *Cleome gynandra* leaf extract is used to treat the arthritis disease through its stabilizing action on lysosomal membranes and thereby preventing the spread of inflammation. It is responsible for the anti-inflammatory and membrane stability modulating effect due to its biologically active ingredients (*Narendhirakanan et al., 2007*).

2.3.2 Prickly pear *(Opuntia vulgaris:* Family *Cactaceae)*

Rocky bluffs, sand dunes, dry rocky or sandy grasslands the edible parts of this plant include; leaves fruit and seeds. Fruit - raw, cooked or dried for later use. They are described as sweet gelatinous lean and insipid. The unripe fruits can be added to soups etc, imparting an okra-like mucilaginous quality. The fruit can hang on the plant all year round.

Prickly pear is widely cultivated and used in juices, jellies, candies, teas, and alcoholic drinks. The fruits and flowers of the plant are used as natural food colorants. Cactus gum is used to stiffen cloth. Essential oils from the flowers are used to make perfumes, and the seeds are a source of oil. Prickly pear has also been used as a source of animal feed and dye.(*Saleem et al 2006)*

There are numerous medicinal uses of the plant. American Indians used prickly pear juice to treat burns. Often a cone of plant material would be burned on the skin to treat irritation or infection, a process known as moxabustion in Chinese medicine. The Lakota tribe used prickly pear in a tea to assist mothers during childbirth.

2.3.2.1 Prickly pear uses and pharmacology

This plant species has been use in diabetis treatment. A mechanism of action remains to be discovered in diabetes; however, polysaccharides may be responsible for the plant's hypoglycemic activity (*Alarcon et al, 2003*).

In animals an extract of O. fulginosa prickly pear maintains normal blood glucose and glycated hemoglobin levels in streptozotocin-induced diabetic rats after insulin has been withdrawn. Similar results were obtained with O. megacantha in reducing blood glucose in normal and streptozocin-induced diabetic rats. However, O. megacantha was nephrotoxic as shown by elevated plasma urea and creatinine concentrations and reduced plasma sodium concentrations (*Trejo, et al 1996*).

2.3.2.2 Chemistry

The medicinal components of the plant are found in the flowers, leaves or pads, and fruit. Isorhamnetin-glucoside, kaempferol, luteolin, penduletin, piscidic acids, quercetrin, rutin, and β-sitosterol have been found in the flowers of prickly pear. The leaves or pads are rich in mucilage and consist primarily of polysaccharides that contain galactose, arabinose, xylose, and rhamnose (*Katsuhiro. 2006*).

Prickly pear fruit is high in nutritional value. Ethanol-soluble carbohydrates are the most abundant components of prickly pear fruit pulp and skin, making up 50% of the pulp and 30% of the skin. The betalain compounds are responsible for the various colors of the fruit. The skin contains calcium, iron, potassium, magnesium, manganese, sodium, and selenium. The edible pulp contains biothiols, taurine, flavonols, tocopherols, and carotenoids. However, industrial processing of juice components results in some loss of vitamins A, E, and C. The seeds are rich in phosphorus and zinc. The oils from the seeds and peel are a good source of polyunsaturated fatty acids (*Tesoriere, 2005*).

2.3.3 Bush Fig (*Ficus sycomorus:* Family *Moraceae)*

2.3.3.1. Chemical Properties

Ficus species are rich source of naturally occurring antioxidants of which phenolic compounds and flavonoids play a vital role in preventing innumerable health disorders related to oxidative stress including cardiovascular diseases, neurodegenerative diseases and cancer. Ficus species due to their strong antioxidant and biological properties are also known to diffuse the toxic free radical and can be used as a possible food additive or in nutraceutical and biopharmaceutical industries. The methanol extracts prepared from bark, fruits and leaves of F. microcarpa exhibited strong antioxidant activity assayed by the four different methods including DPPH and free radicals scavenging, PMS–NADH system superoxide radical scavenging and β-carotene–linoleic acid system. The antioxidant activity is mainly due to the presence of phenolic compounds and hence the bark contains high level of phenolic compounds.

2.3.3.2 Other Properties of the *Ficus* species

From previous research, the methanol extract of Ficus spp bark also exhibit anti bacterial activity against Gram positive and Gram negative bacteria. This antibacterial activity is mainly due to the presence high level phenolic compounds. Various researches carried out on F.microcarpa reported the presence of several triterpenoids such as oleanolic acid, ursolic acid, α-hydroxy ursolic acid, protocatechic acid and maclinic acid in the fruits, aerial roots and bark. It has been known that ursolic acid, oleanolic acid and other triterpenoids are efficient protectors against lipid peroxidation and hence these are potent antioxidants (*Changwei, 2008*)

2.4 PROPERTIES OF OTHER SCREENED PLANT EXTRACTS

2.4.1 *Piliostigma thonningii*

Piliostigma thonningii is a leguminous plant belonging to the family *Caesalpiniacea*, a family that comprises of trees, shrubs or very rarely scramblers. *Piliostigma thonningii* is a leguminous plant belonging to the family *Caesalpiniacea*, a family that comprises of trees, shrubs or very rarely scramblers (*Lock and Simpson, 1999*).

Piliostigma thonningii grows in open woodland and savannah regions that are moist and wooded grassland in low to medium altitudes. It is widely distributed in Africa and Asia (*Djuma, 2003*). *Piliostima thonningii* is highly valued in African traditional medicine, Lewis *et* al. ('1979), stated that apart from its use as an antiseptic chewing stick, *P. thonningii* roots are locally used in treatment of dysentery, fever leprosy, respiratory ailments snake bites and tooth ache. Locally an infusion of the bark of this plant is used as an astringent and haemostatic in the treatment of wounds and for diarrhea. The roots and the bark contain 20% of tannin (which probably account for the astringent action) and also contain traces of alkaloids.

The phytochemical screening of the seed shows the presence of saponins, flavonoids, Phenolic Glycosides, Anthraquinones as well as cardiac glycosides; while tannins, steroids, phylobatannins and triterpenes are known to be absent in the seeds. Some of these chemical compounds have been reported to have inhibitory effects on some gram-negative bacteria such as *Escherichia coli* and *Bacillus subtilis* amongst others (*Kamony*, 1995).

These chemical compounds also have prominent effects on animal systems and microbial cells (*Liu* et al., 1990; *Topcu* et al., 1993; *Oyagade* et al., 1999). The presence of these chemical compounds therefore suggests the pharmacological activities of *P. thonningii*.

CHAPTER 3

3.0 MATERIALS AND METHODS

The experiment was conducted at the University of Zambia, School of Agricultural Sciences Field Station. It was conducted using 459 Cobb 500 day old chicks, purchased from Hybrid Poultry in Lusaka. Upon arrival the birds were immediately distributed randomly to the treatments.

3.1. EXPERIMENTAL DESIGN

The completely randomized design (CRD) was used as the experimental design; this had six treatments and one control. The treatments were replicated three times. 21 experimental units were used throughout the experiment, of which 18 had 22 birds and 3 experimental units had 21 birds. The birds were placed at random into the experimental units. The treatments were as follows; cats whiskers leaves and stems (*Cleome gynandra*) in alcohol, Cats whiskers (*Cleome gynandra*) in vinegar, bush fig bark in alcohol bush fig in vinegar, prickly pear (*Opuntia vulgaris)* in alcohol prickly pear (*Opuntia vulgaris)* in vinegar.

3.2 BIRD MANAGEMENT

3.2.1 Housing Preparation and Equipment

The poultry house that was used for the trials had an east west orientation. It was disinfected using Virukill. A week before the arrival of birds the house was fumigated using formalin.

The 21 experimental unit pens each measuring 2x1m for 20 birds were covered with polyethene black plastic for heat conservation. The floor of the pens was covered with wood shavings. Infrared lamps placed in each pen and a heater which was moved around as required was used for brooding. Before the chicks arrived the place was warmed to raise the temperature for the drinking water which was left in the house overnight. A polyethene plastic was used to cover the sides of the units to prevent cold stress during brooding.

3.2.2 Feeding and Drinking Management

Vitamin and mineral supplements were given to the birds in water from the first day to the fifth veek. The birds were fed using the two phase feeding system. The feed given was compounded s starter and finisher feeds (Table3.1.). The starter feed was given up to four weeks and the inisher was fed in the last two weeks of the experiment. Throughout the experimental period all he birds were given the same feed *ad libitum*.

Table 3.1 Feed Ingredients used in the Starter and Finisher Diets

NGREDIENT	STARTER (KG)	FINISHER (KG)
Maize meal	51.38	68.45
oya bean meal	44.5	28
)CP	2.5	2
ime stone	0.8	0.7
Iethionine	0.12	0.2
ysine	0	0.05
alt	0.3	0.3
roiler premix	0.4	0.3

2.3. Preparation of the Plant Extracts used as Treatments

he treatments or plant extracts were prepared as follows: 400 g of fresh plant parts was weighed id soaked in 1.5 liters of either alcohol or vinegar. This was then stored for a minimum of 5 ys before use. During this period the material was stored under shade to prevent light reactions i the plant extracts. The material was subjected to hand shaking three times a day.

ie prepared plant extracts were given to the birds in drinking water at a dosage of two blespoons in 10 liters.

2.4. Vaccination Programme

ıe birds were vaccinated against New castle Disease and Infectious Bursal Disease. The birds the control treatment were vaccinated first against Gumboro (Infectious Bursal Disease) on ɛ 10th day of rearing. Then the second gumboro was vaccinated against on the 22nd day of ıring. All the birds were then challenged on the 14th day of rearing with the Lasota vaccine. All ɛ birds were given another Lasota vaccine on the 27th day of rearing.

2.5 Slaughter Procedure and Blood Sampling

ood samples were collected on the 43rd day from three birds that were selected at random from ch unit. Blood was collected from the sampled 63 birds. The blood was drained from the birds ing the jugular vein. This blood was immediately stored in the anticoagulant test tubes, these ere stored in the refrigerator in preparation for analysis.

3. DETERMINATION OF ANTIOXIDANT CAPACITY (TOTAL PHENOLICS) USING THE FOLIN CIOCALTEAU METHOD

3.1. Plant Extract Preparation

e Folin Ciocalteu (*Slinkard and Singleton*, 1977) method was used to determine the total tioxidant capacity in the plants to be used in the trials. In this method Gallic acid stock solution ıs used as a standard for determination of the antioxidant strength. Seven selected edible plants ere first air-dried for about two weeks. The dried plant parts were then pulverized using a ɔdak kitchen blender grinder. Approximately 1g of the finely ground sample was then ighed. A hot water infusion was used; 1 gram of the weighed plant sample was used for 100ml hot water. This was then filtered through a Whatman # 1 filter paper.

3.3.2 Gallic Acid Stock Solution.

'or the Gallic acid stock solution 0.5g of dry Gallic acid was dissolved in 10ml of ethanol and ıis was diluted to a volume of 100ml with water. This was then stored in a refrigerator for use ıe next day.

.3.3 Sodium Carbonate Solution

)g of anhydrous sodium carbonate was dissolved in 200 ml of water and was brought to boil, ter cooling few crystals of sodium carbonate were added. After 24 hours the mixture was tered and water was added to 250ml.

3.4. Gallic Acid Standard Curve

r the standard calibration curve 0, 1, 2 , 3, 5 and 10ml of the Gallic acid stock solution was ded to volumetric flasks and this was diluted with water to volume. The concentration in each sk was 0, 50, 100, 150, 250 and 500 mg/l Gallic acid, respectively as the effective range of the ay. 1ml of each of the stock solution was then pipette into 25 ml beakers to which 10 ml water l 1.5ml of Folin Ciocalteu reagent were added. After 3-5 minutes 4 ml of 20% sodium arbonate was added up to the mark with distilled water. The mixture was then allowed to ıd and after 30 minutes the absorbance was read at 765nm.

. of the plant extracts was also used for the determination of total antioxidant. Water, Folin calteu reagent and sodium bicarbonate amounts were added as for the stock solutions.

DATA COLLECTION

l Live weight

live weights of the birds were taken on a weekly basis until the last day of rearing.

. Feed Consumption

ımount of feed given to the birds was weighed from the first day of the experiment and from he feed consumption and feed conversion ratio were calculated.

3.4.3 Bird Mortalities

The mortalities that occurred during the experimental period were recorded.

3.4.4. Differential White Blood Cell Count

The whole blood samples taken from randomly selected birds in each pen were analyzed for differential white blood cell count. This was done at the Hematology Laboratory in the School of Veterinary Medicine of the University of Zambia.

3.4.5 Determination of Antioxidant Capacity

The antioxidant levels of the seven edible plant species were determined using the Folin Ciocalteu method.

3.5 STATISTICAL ANALYSIS

The data analysis was done using Genstat 3rd edition. Analysis of variance was done on live weight, feed consumption, feed conversion ratio, different types of white blood cells (Basophils, Eosinophils, Heterophils, Monocytes and Lymphocytes) and line graphs were drawn using Excel.

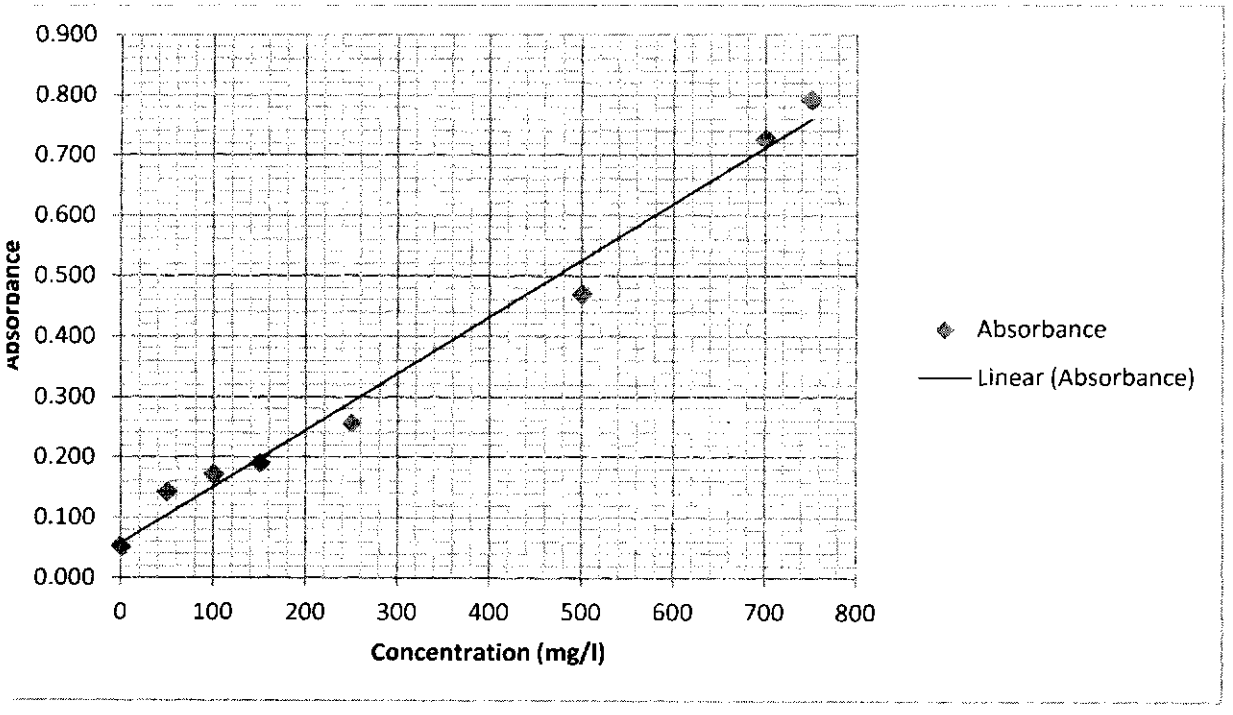
CHAPTER 4

) RESULTS AND DISCUSSION

. ANTIOXIDANT CAPACITY

standard curve was obtained using the Gallic acid as a standard, from the curve an ›roximation of the concentration the plant extracts had their Gallic acid concentration ıivalents approximated using a curve (figure 4.1.).

ure 4.1. Standard Curve for Gallic Acid Concentration



antioxidant activity in plants are contributed and related to the Phenolic acid content. The ›xidant activity is related to total Phenolic content. The higher the Phenolic acid contents in ılant the higher the antioxidant activity (*Juliani and Simon, 2002*).

Gallic acid equivalent content of the screened plant extracts as read at 765nm is in Table 4.1.

'able 4.1 Gallic acid equivalent content of the screened plant extracts as read at 765nm

cientific name (local name) common name	**Absorbance**	**Gallic acid equivalent mg/l**
'iliostigma thonningii (musekese) wild bauhinia	0.792	750
'icus syncomorus (mukuyu) bush fig	0.727	700
)puntia vulgaris (tolofiya)prickly pear	0.220	175
'ibiscus meeusei (lumanda) hibiscus fresh	0.320	150
'ibiscus meeusei (lumanda) hibiscus frozen	0.206	150
'leome gynandra (suntha) cats whiskers	0.125	50
)lanum aethiopicum (impwa) African eggplant	0.116	48
idens pilosa (kanunka) black jack	0.110	45

2. BIRD MORTALITIES

'om a total of 459 broilers at the beginning of the experiment, 16 birds died, giving a :rcentage mortality of 3.5%. The 2 mortalities experienced in the first few days after arrival ere attributed to unabsorbed yolk by post mortem results from the University of Zambia :hool of Veterinary Medicine Table 4.2 below shows the number of mortalities from each :atment.

able 4.2. Mortalities from each Treatment.

Treatment	**Mortality (Number of birds dead)**
Alcohol, Leaves and stem of cat's whiskers *(Cleome gynandra)*	2

Vinegar , Leaves and stem of cat's whiskers *(Cleome gynandra)*	2
Alcohol, Bark of bush fig (*Ficus sycomorus)*	2
Vinegar, Bark of bush fig (*Ficus sycomorus)*	2
Alcohol, Leaves of prickly pear *Opuntia vulgaris*	1
Vinegar, Leaves of prickly pear (*Opuntia vulgaris)*	1
Control (No plant extracts)	6
Total	16

ble 4.3 Mortality Means

analysis of variance was done on the mortalities. Mortalities showed no significant erences *($p<0.05$)* (Table 4.3).

atment	**Percent Mortality Means**
ohol ves and stem cats whiskers *(Cleome ndra)*	3.1[a]
egar es and stem cats whiskers *(Cleome*	3.0[a]

ynandra)	
Alcohol Bark bush fig (*Ficus sycomorus)*	3.0[a]
Vinegar Bark bush fig (*Ficus sycomorus)*	3.1[a]
Alcohol Leaves prickly pear *Opuntia vulgaris*	1.5[a]
Vinegar Leaves prickly pear (*Opuntia vulgaris)*	1.5[a]
Control (No plant extracts)	**9.1**[a]
COVARIANCE %	**139.8**
GRAND MEAN	**3.5**

Means in the same column followed by the same superscript are not significantly different (p≤0.05)

4 PERFORMANCE OF BIRDS

4.1 Mean Liveweights

Both the control and the treatments produced no differences ($p<0.05$) in mean live weight in the broiler trials. This means that the plant extracts enabled normal growth of the experimental birds despite not being vaccinated for Infectious Bursal Disease (Table 4. 3.).

4.2 Mean Feed Consumption

The mean feed consumption showed no significant differences *($p<0.05$)* (Table 4.3).

4.3 Feed Conversion Ratio

The feed conversion ratio showed no significant differences *($p<0.05$)* (Table 4. 3.)

ıle 4.4. Means of Live weight, Feed Consumption and Feed Conversion Ratio.

EATMENT	MEAN LIVE WEIGHT (Kg)	MEAN FEED CONSUMPTION (Kg)	FEED CONVERSION RATIO
ıhol *ıme gynandra*	2.007[a]	3.403[b]	1.696[c]
egar *ıme gynandra*	1.999[a]	3.416[b]	1.708[c]
ıhol ***Ficus morus***	2.047[a]	3.401[b]	1.662[c]
:gar ***Ficus morus***	1.998[a]	3.459[b]	1.733[c]
ıhol *ntia vulgaris*	2.035[a]	3.405[b]	1.674[c]
:gar *ıntia vulgaris*	2.048[a]	3.401[b]	1.664[c]
\TROL	2.173[a]	3.626[b]	1.668[c]
\ND MEAN	2.044	3.444	1.686
%	5	5.4	4

ıs in the same column followed by the same superscript are not significantly different 05).

5 Differential White Blood Cell Analysis

ıere were no significant differences *($p \leq 0.05$)* for Basophils, Monocytes, Eosinophils and ymphocytes . Significant differences were found for the Heterophils.

able 4.5. Means of Basophils, Eosinophils, Heterophils, Lymphocytes, and Monocytes

ɛans in the same column followed by the same superscript are not significantly different

ATMENT	HETEROPHILS	BASOPHILS	EOSINOPLILS	MONOCYTES	LYMPHOCYTES
ıol *ne ındra*	60 [a]	0[c]	0[d]	6[e]	34[f]
ar *ne ındra*	40 [a b]	0[c]	4[d]	5.3[e]	51[f]
ol *morus*	39.7 [a b]	0.7[c]	1.3[d]	5[e]	54[f]
ar *s morus*	37.3 [a b]	0[c]	3.3[d]	5.3[e]	54[f]
ıol *ia ·is*	31.3 [a b]	0.7[c]	4[d]	6.7[e]	58[f]
ar *ia ·is*	43.3 [a b]	0.7[c]	1.3[d]	2.7[e]	52[f]
ol	30 [b]	0[c]	2[d]	4.67[e]	63.3[f]

able 4.6 Table of Means for Heterophils to Lymphocyte Ratio

leans in the same column followed by the same superscript are not significantly different

reatment	**Heterophils/lymphocyte Means**
lcohol :aves and stem of cats whiskers *(Cleome nandra)*	1.765[a]
inegar :aves and stem of cats whiskers *(Cleome nandra)*	0.951[ab]
cohol ırk of bush fig (*Ficus sycomorus)*	0.761[ab]
negar ırk of bush fig (*Ficus sycomorus)*	0.716[ab]
cohol aves of prickly pear *Opuntia vulgaris*	0.612[ab]
negar aves of prickly pear (*Opuntia vulgaris)*	0.856[ab]
ntrol (No plant extracts)	0.509[b]
variance %	42.2
and mean	0.881

Immune Enhancing Effect of *Cleome Gynandra* Leaf Extract on Experimental Birds

: *Cleome gynandra* extract had an effect on levels of heterophils. It stimulated production of h levels of heterophils. Immune enhancement may have contributed to the normal formance of the birds despite not being vaccinated against Infectious Bursal Disease.

ıfectious bursal disease (IBD) also known as Gumboro disease, is an acute, highly contagious ral disease of young chickens. Live vaccines are used to produce an active immunity in young ıickens. A complementary approach to this is to provide chickens with passive protection by ıccinating the parents using a combination of live and killed vaccines. Effective vaccination of eeding stock is therefore of great importance. Severe acute disease of 3–6-week-old birds is sociated with high mortality, but a less acute or subclinical disease is common in 0–3-week-old rds. IBD virus (IBDV) causes lymphoid depletion of the bursa, and if this occurs in the first 2 :eks of life, significant depression of the humoral antibody response may result (Brown 1994). odulation of the immune response through and stimulation or suppression may help in ıintaining a disease-free state. Agents that activate host defense mechanisms in the presence of impaired immune responsiveness can provide supportive therapy to conventional emotherapy. The presence of immunostimulant compounds in higher plants has been tensively reviewed but only a limited amount of immune suppressive products have been ɔorted.

ıother indicator of stress in birds, Heterophil/lymphocyte ratios (H/L), (*Gross and Siegel, 83*), was calculated by dividing the number of heterophils in 1 ml of blood by the number of nphocytes. A relatively higher amount of heterophils was found in the 'cats whiskers in ohol' compared to other treatments. This also suggests that 'cat's whiskers preserved in ohol' is a good immunostimulant to assist in combating stress.

similar experiment was conducted in broiler chicks to determine the total antioxidant activity dried aerial part powder of *Echinacea purpurea (EP).* The antioxidant activity was higher in ilers fed the diet containing *Echinacea purpurea* (EP) diet than those on control and antibiotic ıtments significantly. The results of this trial showed that, use of Echinacea purpurea diet ɔroved total antioxidant activity in serum of broiler chicks (*Gholamreza, 2011*).

Chapter 5

CONCLUSION

tigma thonningii, followed by Ficus sycomorus had the highest antioxidant level with Black s the least in total antioxidant levels. There, however, does not appear to have been any nship between antioxidant activity and performance of the birds.

lant extracts for *Cleome gynandra, Ficus sycomorus* and *Opuntia vulgaris* resulted in l live weight, feed consumption and feed conversion ratio even though the birds were not ated against the notorious Infectious Bursal Disease which is endemic and normally causes ortalities in broilers which are not vaccinated.

leome gynandra preserved in alcohol promoted high Heterophil production and consequent Heterophil/lymphocyte ratio. The extracts showed no effect on levels of basophil, ytes, eosinophils and lymphocytes on the birds.

ECOMMENDATIONS

ure research in this area the experiment should be done with the following considerations

For the laboratory determination of total antioxidant capacity ethanol should be used as solvent as it extracts more from the plant than extraction using hot water infusion method.

Further analysis should be done on lymphoid organs to detect any effects of the extracts on their performance.

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APPENDICES

APPENDIX AI **Screened plant extracts and their Gallic Acid Equivalents in dry and in fresh sample.**

me	common name	scientific name	wavelength	GAE mg/l	mg/g sample	extracted weight g
	African eggplant	*Solanumaethiopicum*	0.116	48	48	1.009
	Black jack	*Bidenspilosa*	0.110	45	45	1.037
	Cat's whiskers	*Cleome gynandra*	0.125	50	50	1.025
	Lumanda	*Hibiscus meeusei*	0.320	280	280	1.010
(F)	Lumanda (Frozen)	*Hibiscus meeusei (F)*	0.206	150	150	1.025
	Bush fig	*Ficussycomorus*	0.727	700	700	1.014
e	Wild bauhinia	*Piliostigmathonningii*	0.792	750	750	1.056
	Prickly pear	*Opuntia vulgaris*	0.220	175	175	1.016

APPENDIX AII

scientific name	air dry weight	GAE in air dry amount	GAE in 500g	GAE/100g of fresh sample	GAE/100g dry weight sample
Solanumaethiopicum	32.4	1.54	1.54	0.31	4.76
Bidenspilosa	102.7	4.46	4.46	0.89	4.34
Cleome gynandra	43.0	2.10	2.10	0.42	4.88
Hibiscus meeuwsei	70.0	19.41	19.41	3.88	27.72
Hibiscus meeuwsei (F)	66.0	9.66	9.66	1.93	14.63
Ficussycomorus	260.5	179.83	179.83	35.97	69.03

70.0	19.41	19.41	3.88	27.72
66.0	9.66	9.66	1.93	14.63
260.5	179.83	179.83	35.97	69.03
500.0	355.11	355.11	71.02	71.02
35.3	6.08	6.08	1.22	17.22

APPENDIX B **Analysis of variance for Mean Feed Consumption**

Source of variation	**Degrees of freedom.**	**Sum of Squares**	**Mean Squares**	**variation**	**f probability**
treatment	6	0.12291	0.02049	0.6	0.727
Residual	14	0.47939	0.03424		
Total	20	0.60231			
Least significant differences of Means at 5% level				0.3241	

APPENDIX C Analysis of variance for Mean Live weight at 43 days

Source of variation	degrees of freedom	Sum of Squares	Mean Squares	variation	f probability
Treatment	6	0.0668	0.01113	1.05	0.437
Residual	14	0.14885	0.01063		
Total	20	0.21565			
Least significant differences of Means at 5% level					

APPENDIX D Analysis of Variance for Feed Conversion Ratio at 43 days

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	variation	f probabili
treatment	6	0.013096	0.002183	0.48	0.813
Residual	14	0.063847	0.00456		
Total	20	0.076943			
Least significant differences of Means at 5% level					

APPENDIX E analysis of variance for Percent Mortality at 43 days

Source of	Degrees of	Sum of	Mean Sum of	Variation	F pr.

Treatments	6	119.68	19.95	0.84	0.559
Residual	14	331.95	23.71		
Total	20	451.63			

APPENDIX F **DIFFERENTIAL WHITE BLOOD CELL ANALYSIS AT 43 DAYS**

APPENDIX FI Analysis of variance for Packed Cell Volume

'ce of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probability**
ıtment	6	39.209	6.535	0.8	0.59
dual	11	90	8.182		
ıl	17	128.5			
;t significant differences of Means at 5 % leave				15.14	

APPENDIX FII **Analysis of Variance for Basophil Cells**

Source of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probability**
Treatment	**6**	**2.2857**	**0.381**	**0.67**	**0.678**
Residual	**14**	**8**	**0.5714**		

Total	**20**	**10.2857**			
Least significant difference of Means at 5% level					1.324

APPENDIX FIII **Analysis of Variance for Monocytes.**

Source of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probability**
Treatment	6	28.476	4.746	0.76	0.612
Residual	14	87.333	6.238		
Total	20	115.81			
Least significant difference of Means at 5% level				4.374	

APPENDIX FIV **Analysis of Variance for Eosinophils**

Source of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probabili**
Treatment	6	42.286	7.048	2.47	0.077
Residual	14	40	2.857		
Total	20	82.286			
Least significant difference of Means at 5% level					2.96

APPENDIX FV Analysis of Variance for Lymphocytes

Source of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f proba**
Treatment	6	1487.2	247.9	2.06	0.125

Residual	14	1688	120.6		
Total	20	3175.2			
Least significant difference of Means at 5% level					19.23

APPENDIX FVI **Analysis of Variance for Heterophils**

ce of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probability**
ment	6	1779.14	296.52	3.18	0.035
dual	14	1304.67	93.19		
l	20	3083.81			
t significant difference of Means at 5% level					16.91

APPENDIX F VII. Analysis of Variance for Heterophil to Lymphocyte Ratio

Source of variation	**Degrees of freedom**	**Sum of Squares**	**Mean Squares**	**Variation**	**f probability**
Treatment	6	3.1185	0.5198	3.76	0.019
Residual	14	1.9338	0.1381		
Total	20	5.0523			
Least significant difference of Means at 5% level					0.6508