EFFECTS OF GENOTYPE AND GRADED LEVELS OF GARLIC (*Allium sativum*) AS FEED ADDITIVE ON SHORT TERM PRODUCTION OF LAYING PULLETS

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A Ph.D THESIS DEFENSE SEMINAR SUBMITTED TO THE FACULTY OF AGRICULTURE, DELTA STATE UNIVERSITY, ASABA CAMPUS, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN ANIMAL SCIENCE OF DELTA STATE UNIVERSITY, ABRAKA

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JULY, 2021

CERTIFICATION

We certify that this research work was duly carried out by Gbayisomore Oyewole Samuel in the Department of Animal Science, Delta State University, Asaba Campus, Asaba.

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Prof. S.I. Omeje **Date**
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Head of Department (Animal Science)

DECLARATION

I declare that this is an original work carried out by me, Gbayisomore, Oyewole Samuel (PG/17/18/245804).

_____

Gbayisomore, Oyewole Samuel

DEDICATION

This research work is dedicated to God Almighty for His mercies and enablement upon my life to carry out this study.

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ABSTRACT

A four months feed preliminary was examined on the effect of genotype and garlic powder supplementation on the performance characteristics, egg quality characteristics, hematological and serum biochemistry of Bovan Nera and ISA brown pullets. A two hundred and forty (240) of 20 weeks old pullets involving one hundred twenty each for Bovan Nera and ISA Brown individually. Each group were fed dietary treatments as T1 (control 0.00%), T2 (0.75%), T3 (1.5%), T4 (2.25%) and T5 (3.00%) garlic inclusion respectively. The treatments were replicated four times (4) with six (6) birds per replicate. 2 x 5 factorial in a Randomized Complete Block Design (RCBD) was used and the birds were fed *ad libitum*. Two eggs each were collected daily and analysed for the internal and external characteristics on the two pullet strains. The proximate and phytochemical compositions of garlic powder (*Allium sativum*) were investigated. The results on proximate composition showed significant ($P<0.05$) differences in crude protein (19.66% and 23.31%) ether extract (1.45% and 4.55%), dry matter (3.20% and 9.88%), ash content (2.10% and 4.59%), crude fiber (1.75% and 5.78%) nitrogen free concentrate (71.84% and 51.89%) of garlic powder and feed respectively. On the phytochemical composition of garlic powder showed higher flavonoids, tannin, saponing and steroids contents. The outcomes of performance characteristics showed significant ($P<0.05$) differences in feed and genotype interaction in the final body weight, weight gain, body weight at first egg, weight gain/bird/week and mortality rate. There were no significant ($P<0.05$) effect of feed and strain on weight of first egg and age of bird at first egg. Bovan Nera pullets on T1(0.00%), (control), T3 (1.5%) garlic powder, T4 (2.25% garlic powder) and T5 (3.0% garlic powder) had significantly ($P<0.05$) higher final body weight, body weight at first egg, feed intake, feed conversion ratio and percentage mortality than ISA Brown pullets which consumed the same dietary treatment except for T5. ISA Brown pullets on T2, T4 and T5 diets had significantly ($P<0.05$) higher body weight. The external and internal egg quality characteristics were significantly ($P<0.05$) influenced by dietary treatments and genotype. Bovan Nera pullets on dietary treatments T2 (0.75%) and T3 (1.5%) garlic inclusion had significantly ($P<0.05$) higher egg weight, egg volume, egg width, egg length, yolk, height, yolk diameter, yolk colour, Albumen weight and Albumen maximum diameter than ISA Brown pullets on same diets. The haematological parameters showed significant ($P<0.05$) difference in feed and genotype interaction of pullets in all parameters measured. The serum biochemical constituent was significantly ($P<0.05$) affected by feed x genotype interaction. Cholesterol level decreased with increased level of garlic powder in both strains. No significant effect of feed x strain interaction was obtained Albumin level. Bovan Nera pullets performed better than ISA Brown pullets in egg quality and production. Garlic supplementation for maximum productive yield should not exceed 1.5% for laying birds as indicated by the blood profile of both strains in this study.

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CHAPTER THREE

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Recently, concerns finding non-synthetic alternatives for antibiotics among the scientists. The use of antibiotics as growth promoters is facing serious criticism Tekeli et al., (2006).As stated by Ajayi et al., (2009) that there are some significant reasons that confine the utilization of antibiotics for example, bacterial resistance of synthetic drugs and its deposits in meat. In overcoming the poor performance and the increased susceptibility to diseases resulting from removal of antibiotics from the diet of poultry birds, attempts were made to investigate other alternatives. Natural, original growth promoters have been encouraging recently. Alodan et al., (1999) showed positive effects of herbal plants on broilers as studied by researchers. The microbial potential, hypo-cholesterolemic effects, growth-promoting effects, availability and other beneficial properties of herbs, have drawn the scientists' attention. Several years ago, the resistance bacterial and increased blood cholesterol in animals prompted the ban of these synthetic drugs. These probiotics which are of natural origin also has the propensity to increase the carcass quality, egg quality and obviously the organoleptic qualities of the animal because of its efficacy to increase taste, colouration and aroma respectively. According to recent concerns about cholesterol, there are a lot of attempts to produce foods with low or no cholesterol. There are reports that probiotics can decrease the cholesterol level of blood in poultry chickens, Lim et al., (2006).

Garlic has been discovered to have high medicinal and prophylactic benefits dating from ancient civilizations (Kendler, 1987). Garlic stimulatingly affects the gastric system (Dewit et al., 1979)

and bactericidial effects against gram-negative organisms Rode et al. (1989). Garlic oil has the anthelmintic effects on poultry worms, Ascaridia galli. (Kavinda et al., 2000). Also, garlic had positive effects such as antioxidant, antimicrobial in chickens and reduction rate of mortality (Koninfca et al (1997); Sivam, (2001); Stanader *et al.,* (2010). Hanafy *et al* (1994) observed decrease lead content in chickens muscles and liver tissues when fed garlic supplements.

Garlic fed to laying hens had significantly effects on the performance characteristics and enhanced the egg quality of the hens Lim *et al.,* (2006); Yalain *et al.,* (2006); Khan *et al.,* (2007). Additionally, garlic had some potentials in reducing the cholesterol in blood serum of chickens (Abdo et al., 1983 and El. Nawawi 1991).

The meat and eggs from chickens has boosted the supplies of animal protein to the emerging population (Leta and Bekana, 2010). Fertility and hatchability are significant indicators of reproduction that are correlated to the genetic makeup and environmental influence of an animal.

The nature of egg can be examined under two general classes, to be specific, external and internal qualities (Monira et al, 2003). The external features of the egg are; the egg size, structure, thickness and shell strength (Bain, 2005). The internal features is estimated based on the nature of the egg whites (albumen) as demonstrated by the Haugh Units (HU), the overall size of the different segments and the shell membrane. Several authors have examined these egg quality assessments in chicken (Tong *et al.,* 2002, De Ketelaere *et al.,* 2004, Bain, 2005) with it immediate environmental factors during storage and early brooding, what these mean for hatchability (Narushin and Romanov, 2002, Tona *et al.,* 2002; Reijrink *et al.,* 2008).

Protein deficiencies result in various clinical and sub clinical conditions such as reduced growth rates, poor physical and possibly mental development in children and adolescents. Improved health reduces resistance to diseases, and lowers working efficiency in adults (Ojewole, 1988), hence the expression of concerns at the rather low level of animal protein intake estimated to be

at 8gm per caput per day, about 27gm less than the minimum requirement recommended by Nation Research Council of the United States of America (Obioha, 1992). This has generally been attributed to the shortfall in animal production which encourages scarcity and high prices of animal products. Garlic is mainly the essential species of the onion genus, Allium, belonging to the family Alliaceae (Lonzotti, 2006: Eric, 2010). It is readily available and widely used around the world as it is grown year-round (Charlson and McFerreri, 2007). It is the second widely consumed species globally and its popularity has been boosted by the growing awareness of its health potential benefits (FAO, 1992). Garlic is well known for its dietary and medicinal implications (Lawson, 1998). Garlic bulbs contain alliin (S-allyl cysteine sulfoxide), the antecedent of allicin, which is hydrolyzed by catalyst allinase after pulverizing to its dynamic structure, allicin (S-allyl-2-propenthiosulphinate). It has been established that garlic enhance the growth performance characteristics parameters and decrease mortality when supplemented as additive in broiler chickens diets. Studies recently have indicated that allicin is the most potentially active component of garlic that is responsible for its characteristic odour, flavour as well as most of its biological properties (Chowdhury *et al*, 2002 and Heinrich *et al*, 2004). Sveral authors has observed the antifungal (Adetumbi et al, 1986; Durak et al., 2002) antiviral and against malignant growth (Weber et 01., 1992; Durak et al., 2002) and in-vitro antibacterial (Shalaby *et al.*, 2006) actions of extracts from freeze-dried garlic and fresh garlic has been recorded and researched globally. The fundamental oil of garlic has likewise been accounted for its cancer prevention agent (antioxidant) possibilities (Hui, 1996). Sovova and Sova (2004) have recommended conceivable potentials of garlic on cardiovascular system in forestalling and lessening hypertension, collection of cholesterol on the vascular walls and atherosclerosis. Grounded garlic into paste was accounted for its adequacy in decreasing cholesterol in serum of laying hens and egg yolk (Chowdhury). In spite of the fact that several authors have shown that garlic fed to animals as additive showed some vascular changes accordingly, a randomized

clinical preliminary revealed that the utilization of garlic in any composition had no effect on blood cholesterol levels in patients with moderate gauge cholesterol levels (Chan *et al.,* 2007). In addition to this, reports of Gardner *et al*,, (2007) and Ademola et al., (2009) about the effects of garlic supplementation on low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerols are conflicting. These contradicting reports on garlic as additive in broiler chickens on the performance, abdominal fat, plasma lipid profile, lipoproteins, and haematological indices of broilers as well as the dearth of information on the standardized dosage or level of garlic supplementation has called for further studies to obtain further results in order to explore the scientific advantages of garlic.

1.2 Statement of problem

The increasing prices faced by farmers on antibiotics usage as growth promoters have been facing serious criticism. There are some important reasons that restrict its usage, e.g drug resistance in bacteria and the drug residues in poultry products which causes health problem in man. To overcome the performance and the increased susceptibility to disease resulting from removal of antibiotics from bird's diets, high cost of antibiotics, their unavailability to rural farmers, residual effect of antibiotics on consumers ,health effect on birds , which could also have adverse effect in animal production attempts were made to find other alternatives, whose effects need to be ascertained. The tropical environment enhances microorganism multiplication and unfortunately disease incidence or outbreaks are not easily eliminated (Ziggers, 2012), which make it imperative for continuing investigations on finding alternative and effective approaches to the solving the problems. Therefore, this research is designed to ascertain, evaluate and explore the positive medicinal and antibiotic properties of garlic as an alternative source as a growth promoter at varied levels of inclusion especially as regard to egg production, egg quality and the growth performance of the layers birds. Also, the interactive effect of genotype on egg production is to be evaluated.

1.3 Objectives

1.3.1 General objectives

The general objective of the study is to determine the effects of genotype and varying levels of garlic as a feed additive on short-term production of layer pullets.

1.3.2 Specific objectives

The specific objectives of the study are to determine the effect of genotype and graded levels of dietary garlic on the growth, feed intake and weight variations of laying pullets. genotype and varying levels of the test ingredient on egg production and egg quality characteristics of laying pullets genotype and varied levels of garlic on haematology and serum biochemistry of laying pullets 1.4 Justification Poultry egg is perceived as the cheapest, most affordable and acceptable animal products. Egg are highly nutritious and contribute greatly to the animal protein needs of the populace (Oleforu-Okele *et al.*, 2016).Garlic has some chemical components like sulphur containing compounds (allin dialysulfides and allium) that have antibacterial, antifungal, and antiviral characteristics, it reduce serum and liver cholesterol and inhibit bacterial growth which eventually result into better egg quality production. It is therefore important to ascertain the effect of this additive as growth promoter on the health status of the different genotypes of the laying birds, as expressed in egg production and variations in haematology parameters compared with birds not treated with them especially in the tropical region. Also, because of the availability of this dietary spice in the tropical region and its beneficial properties to local poultry farmers as previously investigated, this now constitutes the motivation for the justification to further the research in this work thereby improving both the profits and quality of poultry products by our local poultry farmers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meeting the Human Nutritional Demands

Over the last two decades, there has been a radical rise in Nigerian population with an obvious corresponding rapid rise in demand for food. Availability of high quality food such as meat, eggs, milk has become limited because of the poor economic status of average Nigerians. The result as pointed out by Olayide and Olayemi (1998) showed that not only a high rate of demand for food, but also high cost, and so elusive to many.

Consequently, a frantic effort by the government and individuals to get involved in farming business and much more in animal production. In Nigeria, commercial poultry farming particularly came into being, soon after the Nigerian civil war in 1970 (Oruseibo, 2002). Poultry products also supply animal protein immensely to the the population.

According to Odunsi (2003), the rapid growth of human and livestock population, has created food and feed in the less developed countries, demand that alternative feed sources must be identified and evaluated.

There is need therefore, to explore non-conventional scientific methods to improve yield and at the same time reduce production cost.

Hence, any natural spices that could enhance growth with antimicrobial properties in the poultry industry with little or no cost attached would be a welcome substitute for the conventional, costly, synthetic antibiotics in the industry (Adewunmi *etal.*, 1998).

2.2 Poultry Layers

Poultry farmers' activity largely depended on various production purposes. These stages involves the birds age which is according to their development, necessary space and different feeds. Layers are generally meant for egg production and in Nigeria some of the popular strains include: Isa-Brown Bovan-nera Leghorn Ancona Star cross Minorca Funaab-Alpha There are other breeds used for

dual purpose of both meat and egg production. There are other breeds used for dual purpose of both meat and egg production. Examples are:

a. Plymouth rock

b. New Hampshire

c. Road Island Red.

2.3.0 **Performance and Characteristics of ISA-Brown and Bovan Nera Strains**

2.3.1 **Characteristics of Isa-Brown**

ISA-Brown exhibit the following characteristics; ISA-Brown a production laying performance of attractive brown eggs. It is suitable for a variety of management systems. Scott Sliversides and (2001) display recorded that eggs from ISA-Brown hens had a greater percentage of eggs shell thickness. Production performances and egg quality of ISA-Brown, increased egg weight, egg mass and feed efficiency from ISA-Brown (Grobas *et al.,* 2001). Tumova *et al.,* (2007), Zita *et al.,* (2009) and Ledvinka *et al.,* (2011) reported that the genotype has a major effect on productivity and egg quality characteristics. The shell breaking strength is over 35 Newtons, and optimum egg weight.

2.3.2 **Characteristics of Bovan Nera**

Bovan black has the highest body weight, lay heavier eggs and superior external qualities Singh *et al.* (2009). Olawumi (2007) reported that Bovan black layer strain had lower mortality rate, more adaptable to hot weather. It has better large egg size, heavier yolk weight, albumen weight, better percent albumen, better haugh unit and better albumen height. Bovan Nera display a productive laying performance of attractive brown and white eggs.

2.4.0 **Stages in Layer type of Poultry Production**

2.4.1 Pullet stage

The term pullet often refers to 12 – 20 weeks old hens. At this stage, the birds are fed with feeds suitable for their growth. During this developing period, the interest for feed, water, and floor space are essential as the birds developed.

The quality and quantity of feed with sufficient water decides the birds egg-laying abilities. Furthermore, congestion of birds ought to be tried not to by give sufficient floor space.

The bird's weight corresponds to its optimum egg productivity. The birds ought to achieve and keep up with its body weight for optimum egg productivity.

2.4.2 Grower Stage:

2.2.3 Laying Stage

At 20 weeks old, egg laying birds starts producing eggs. The utilization of "layer ration" starts at this age. Nonetheless, receptacles ought to be moved to their laying quarters when they are 18 weeks old. Sick and stunted birds in the pens should be culled to allow general performance of laying birds. The useful egg-laying time of layers is about two years.

In poultry farms, the steady production of eggs within a period of time, attention and measures should be created for possible replacement of older birds with another group. Keeping up with old birds can be expensive due to its feed cost and poor egg production.

2.5.0 Layer Requirements

i. laying birds feed on diets containing approximately 18% crude protein, good quality water ought to be made accessible consistently. Sudden changes in their feeds must be avoided. The feeding and water troughs should be placed outside the pen for their easy cleaning. This is common in battery cage system.

ii. About 14 hours daylight is essential for effective laying cycle. Contingent to the period of the year, an extra 2-4 hours of man-made lighting should be enhanced to guarantee 14 hours of sunlight. The utilization of 50-watt lights, one for each 100 birds, might be available. Also, a clock can be introduced for the lights to be switched on and off naturally. Lights are expected to invigorate egg production and permit more opportunity to feed of during the evening.

iii. It is suggested that the laying pen should be situated in a calm segment of the property. Endeavors ought to be made to keep away excessive noise which can alter their egg production.

iv. An nesting section should be available to the birds to lay their eggs when layers are placed on litter. A nesting section of about 2' x 6' for each 100 layers ought to be adequate. An egg carry out can likewise be appended to the pens so egg assortment should be possible outside the layer pen. Twice daily, eggs collection ought to be done in the morning and at the early evening. These eggs are stored after assortment in cool environment. Stains on eggs shell should be washed with water. It is suggested that cleaned eggs be refrigerated quickly to shield from any bacterial pollution like Salmonella.

v. it is recommended that the floor space of about 1.75 square feet per bird is essential. For example, 500 layers required about 875 square feet floor space. The walls should be made up of wire mesh for ventilation. An approximately 12" x 18" floor space should be used for cages with not less than three birds and five birds maximum. Its awareness has made this system to be popular since it curtail diseases and provide a better environment condition for optimum egg productivity. Layers spend majority of their activity in an free range system hence security must be provided against predators.

vi. During production, stunted, unproductive or sick layering birds could be culled. This could reduce the feed consumption. Inefficient hens ought to be separated (taken out) from the herd as they will ceaselessly devour feed while not producing eggs. Some visible signs includes pale and shrunken combs/wattles thin and emaciated bodies, inactive and dull appearance. Day by day checking for such birds ought to be regular. Note: increase in the numbers of culled birds indicates some managerial problems and must be addressed.

2.6.0 Potentially Active Chemical Constituents of Garlic

Garlic contains about 33 sulfur compounds, some enzymes and minerals such as iron, potassium, magnesium, selenium, germanium, calcium, copper, and zinc. Also, some vitamins such as A, B1 and C, and fiber and water. In addition, it contains about 17 amino acids (Josling, 2005). It concentration of sulfur compounds is higher than some other allium species which are capable both for garlic's sharp scent and a significant number of its therapeutic effects. Perhaps allicin organic compound mixtures in garlic is common while alliin (5-allyltysteine sulfoxide) is significantly higher, which is available at 10 and 30 mg/g in fresh and dried garlic, respectively (Lawson, 1998). The processing of garlic e.g, cleaving, mincing and pounding released S-allyl cysteine sulfoxide and presented it to allinase chemicals, rapidly changing it over to diallyl thiosulfinate, which gives garlic its trademark smell. The allinase catalyst is liable for diallyl thiosulfanate change and becomes inactivated under a pH of 3.5 or with warming (Pezzadra-Chaverri *et al.,* 2006). Despite the fact that allicin is viewed as scavenging compound and had significant antioxidant potentials, several authors has observed that different mixtures might assume more grounded parts; like polar mixtures of phenolic and steroidal source, which avail several therapeutic properties devoid of smell and are stable under heat (Lanzotti, 2006).

2.7 The effects of Garlic on health

Garlic can legitimately be nature's awesome plant with therapeutic potentials. It can restrain and kill microscopic organisms, parasites, reduces blood pressure, blood cholesterol and blood glucose, forestalls blood coagulating, anti-turmor properties and anti-immune (Abdultah *et al.*, 1988). It can animate the lymphatic system that facilitates the expulsion of waste. It is additionally viewed as a successful cancer prevention agent to secure cells against damages. It can assist with forestalling a few types of malignancy, coronary illness, strokes and viral diseases. Garlic can furnish us with more than 200 surprising synthetic substances with ability of shielding the human body from various sicknesses. The sulfur-compounds in garlic helps to protect the body by assurance by invigorating essential enzymes production that are beneficial (Mansell and Reckless, 1991).

2.7.1 Anticardiovascular

Diseases associated with the circulatory system have affected our affection ones. These are blockages in coronary veins which causes a bigger number of passings than some different components. The sections, which supply blood and oxygen to the heart, become dynamically more modest as plaque creates over a period. At the place of restricted blood supply, a particular section in the heart is denied oxygen and prompts coronary assault. Hypertension and high blood serum cholesterol levels prompts coronary illness which are influenced by the remedial movement of garlic. In coronary sickness, garlic could reestablish wellbeing to affected hare heart when taken care of with garlic (Bordia, 1981).

In India, an investigation on 432 coronary sickness patients were chosen arbitrarily into two gatherings where a big part of the populace were furnished with milk mixed with garlic juice, while the other patients did not consume garlic juice. In a time of three years of the test, about twice as various patients had passed on in the gathering not gave garlic juice (Yeh *et al.*, 2006). It recorded that it lessen platelet collection, vein plaque development, decrease homocysteine,

lower blood presurre, and miniature course increments. It may in like manner help with keeping mental rot by safeguarding neurons from neurotoxicity and apoptosis, thus hindering ischaemia or reperfusion-related neuronal passing and by additional creating learning and memory support (Borek, 2006).

2.7.2 Reduction of High Blood Pressure/Hypertension

Garlic has probably been most advanced as a necessary treatment for hypertension (Capraz *et al.,* 2006). An in-vitro research has asserted that, the vasoactive limit of garlic sulfur intensifies where red platelets convert garlic normal polysulfides into hydrogen sulfide, a known endogenous cardio-cautious vascular cell hailing molecule (Benavides *et al.,* 2007). Using 2400 mg garlic tablet containing 31.2 mg allicin has diminished diastolic squeezing factor by 16 mmHg after 5hours of organization (McMahon and Vargas, 1993). In a previous report, 415 patients uncovered a lessening of 7.7 mmHg diastolic pressing factor (Silagy and Neil. 1994).

2.7.3 As Natural Blood Thinner

The platelets and fibrin accept unimaginable part in blood coagulating and more elevated levels of fibrin in food may cause cardiovascular breakdown. Garlic constituents reduce fibrin and besides help with decreasing the fibrin present in the blood better contrasted with against inflamatory medication (ibuprofen) (Fukao *et al.,* 2007).One of the sulfur compound Ajoene present in garlic had been obligated for its enemy of coagulating impact; yet at room temperature or above, ajoene is feasible. It is absent in fresh or freeze dried garlic. Reports of garlic in man counts calories aided the breakdown of fibrin from up to 30% (Ernst, 1994).

2.7.4 Anti-immunity

Viral irresistible illnesses like HIV/AIDS, has lead to the insusceptible framework boosting. Since these sorts of disorders have no convincing fixes or treatments, supporting the body's

shielding-off infection in various researches. Garlic has monstrous sulfur containing amino acids and various combinations seem to begin extended development in the invulnerable system (Lau *et al.,* 1991). In any case, the titanic squeezing factors on the resistant framework, fundamental enhancements like garlic are clearly required (Salman *et al.,* 1999). Its eminent substance germanium alone offers astonishing safe movement. Regardless germanium, garlic additionally contains niacin, phosphorous, thiamine, sulfur and selenium (Morioka *et al.,* 1993).

Several investigations in man, using alum-standardized garlic powder (GP), have shown viable results on immunoreactions and phagocytosis. In developed people, administering 600 mg (GP) every day for 90 days provoked critical ($p<0.0l$) expansions in the degree of phagocytosing periphery granulocytes and monocytes when attempted ex vivo for their ability to overpower Escherichia coli microorganisms. In one more human examination on rough garlic separate (5 to 10 days) was managed to HIV/AIDS patients. For seven patients who completed the 12 weeks study showed a huge expansion in the regular executioner cells action from a low mean (Abdullah *et al.,* 1988). In USA, fundamentals concentrates on HIV/AIDS patients have displayed the improvement of regular executioner cells activity using garlic separates. Chinese examinations with viral illnesses in bone marrow relocate patients have shown a "amazing antiviral activity". A twofold outwardly hindered treatment controlled outline using 100% allicin yielding improvement has itemized that allicin can lessen the occasion of the typical cold and recovered from the symptoms (Josling, 2001).

2.7.5 Atherosclerosis and Hyperlipidaemia

Advancing garlic's ability in diminishing the cholesterol levels and lessen lipid peroxidation to upset plaque developments. An in vitro research, it was shown that it has an ability to subdue low thickness lipoprotein (LDL) and increment the obstruction of LDL to oxidation (Lau, 2006). The outcomes from the controlled human assessments were converge with different

examinations directed during the 1990s which uncovered fruitful results. Spin-off of a few examinations, on the concentrates of garlic, ongoing consequences of 15 hypercholesterolemia patients surveyed a material conveyed from garlic separates with shape Monascus pilosus. It was seen that a decline in the serum absolute cholesterol and low thickness lipoprotein cholesterol levels when seen at forward night and a month in the wake of beginning the treatment. The degrees of unsaturated fats had a tendency towards decline in hyper-triglycerdemic patients likewise, however high thickness lipoprotein cholesterol was unaltered (Sumioka et al., 2006). Following 60 days of organization, low-thickness lipoprotein, serum greasy oil and incredibly low thickness lipoprotein, were lessened by 21, 37, and 36.7%, separately (Jeyaraj et al., 2006).

2.7.6 Anti-diabetic Effect

several examinations on animals has support the practicality of garlic in diminishing blood glucose in streptozotoxin provoked similarly as alloxan-prompted diabetes mellitus in mice. An examination to survey oral association of garlic extract (GE) for 2weeks on blood glucose, blood cholesterol, greasy substances, urea and uric destructive, in customary and streptozotocin-induced diabetic mice. The result showed significant ($p<0.05$) effects in blood glucose cholesterol, ucea, uric destructive, aspartate amino transferase and alanine amino transferase levels, while hoists serum insulin in diabetic mice, anyway not in commonplace mice. From a connection study made between the action of GE and glibenclamide, also the antidiabetic effect of garlic is proficient compared to glibenclamide (Eidi *et al.,* 2006).

2.7.7 Anticancer Effect

The various activities of garlic, restraint of the improvement of harmful development is generally observable. A couple of synergistic impacts may fight disease development. The activities of garlic have been credited to strengthen resistant effector cells. Different epidemiological, clinical studies have revealed garlic exceptional influence in sickness expectation especially similar to

stomach related framework cancers. Human studies have typical affirmation of garlic decreases esophageal risk, intestinal and colon disease. This was accepted to be a direct result that supports allicin effects in diminishing the formation of cancer growth in the gastrointestinal tract (Galeone *et al.,* 2006).

In Netherlands, a decrease was seen in the progression of stomach disease development in people eating garlic straightforwardly or onions (Dorant *et al.,* 1996). Garlic diminishes patients with prostate cancer, particularly persons with restricted contamination. The large group had total allium vegetables (>10.0 g/day) an estimations colossal reduce danger of prostate disease development than those with most decreased arrangement (<2.2 g/day). Similar connections between's arrangements showed diminished risk in men compared to most confirmation classes for garlic expressly. The diminished prostate cancer risk relies on the body size, affirmation of various food sources and full scale calorie utilization and was expressed in men with confined prostate sickness than prostate disease (Hsing *et al.,* 2002). Prostate express antigen serum markers had critical impact during short ingestion period, yet returns to measure following a month (Mehraban *et al.,* 2006).

A fundamental epidemiological examination on Americans accounted for whereby 127 food assortments were burned-through (tallying 44 vegetables and organic) not set in stone in 41.387 women between 55 to 69years for a time of 5 years seeing of colon disease recurrence. The aftereffect of this "Iowa Women's Health Study" uncovered that garlic had quantifiably enormous relationship with reduced colon disease development peril. Cancer growths found in the colon when garlic was devoured, either new or in controlled structure every week achieved 35% lower danger, while a half lower peril was for distal colon sickness (Steinmetz *et al.,* 1994).

2.7.8 Dermatologic Applications

An examination reviewed 43 individuals for their skin usage of GE for mole and corn medicines. Of these individuals, 15 volunteers consumed water concentrate of garlic whereas 23 volunteers applied lipid separate on affected areas two times each day. All lipid extricate volunteers experienced none objective of mole and 80% of corn within one to around fourteen days. The water separate seemed, by all accounts, to be less extraordinary, with complete breaking down of more unobtrusive moles and corns, and simply fragmented deterioration of greater ones. The lipid separate caused some burning-through, redness, annoying and skin clouding which was settled after it usage (Dehghani *et al.,* 2005).

2.7.9 Antimicrobial Potential

The antimicrobial exercises of garlic was first reported by (Pasteur 1958), and thus, a few creators had shown its suitability and expansive range having a more extensive scope of antimicrobial activity against various sorts of microorganisms, contaminations parasites, protozoan and life forms (Jaber and Al-Mossawi, 2007). Garlic have mild incidental effects contrasted and engineered anti-toxins; subsequently, it treats infectious diseases (Tepe *et al.,* 2004). In many plants with remedial capacities, garlic has antimicrobial efficacy that safeguards the host against various microorganisms compared to ordinary antimicrobial medicines (Bajpai *et al.,* 2005; Wojdylo *et al.,* 2007).

Several authors has concurred that garlic isn't simply convincing against Gram +ve and Gram -ve microorganisms however also have antiviral and antifungal possibilities (Tsao and Yin, 2001).

2.7.10 Antiviral Potential

The sulfur constituents in garlic had antiviral potentials against some viral diseases. Several authors have reported the potentials of garlic in the successful treatment of seasonal influenza B contamination and herpes. In isolated separate researches in Japan and Romania revealed that the

plant can protect living creatures from influenza contamination (Tsai *et al.*, 1985). A few decades prior, a twofold multiplied visually impaired fake treatment controlled examination has uncovered huge guard from the ordinary cold infection. The Garlic Center, revealed the important significant work to have showed expectation, treatment and decline of re-contamination benefits if Allimax Powder capsule is administered one time each day (Josling, 2001).

2.7.11 Antibacterial Effect

Garlic eliminates and obstructs the improvement of Gram +ve and Gram -ve microorganisms (Tsao and Yin, 2001). Its antibacterial efficacy relies upon allicin presence caused by enzymatic development of allinase on allium. Allicin is seen as the most extreme antibacterial expert in crushed garlic extricates, yet it very well might be temperamental, isolating at 16 hour at 23°C (Hahn, 1996). Moreover, the usage of water-based concentrate of allicin balances out the allicin molecule due to the hydrogen bond in water with the responsive oxygen particle in allicin in crushed garlic that undermine the iota (Lawson, 1996). The shortcoming of this strategy revealed allicin reaction with H_2O to form diallyl disulphide compound, which doesn't show a comparative effect of antibacterial development (Lawson and Wang, 1996).

2.7.12 Antifungal Effect

Ajoene compound present in garlic which accepts a staggering part as compelling antifungal effects (Ledezma and Apitz-Castro, 2006). It been revealed to limit advancement of parasitic ailments as comparatively to ketoconazole drug, when vaccinated against fungi infections (Shams-Ghahfarokhi *et al.*, 2006). A Chinese clinical journal depicts intravenous garlic on parasitic sickness treatment of the frontal cortex called Cryptococcus meningitis. The outcome from the Chinese investigation differentiated the ampleness of the garlic and standard clinical treatment which incorporated an incredibly harmful antidote poison called Amphotericin-B. The

examination showed that, intravenous garlic was effective and less-harmful than commercial drug even at higher portion (Lemar *et al.,* 2007).

In a different report in Candida regions uncovered altogether diminish effects in mice fed with GE. The assessment also uncovered garlic stimulated phagocytic activity. This recommends that pollutions, for instance, Candida could be controlled since garlic protects the body. Garlic oil also treat ringworm, skin parasites and moles. Wounds that were achieved by skin parasites in rabbits and guinea pigs were additionally treated with outside usages of garlic extricate and inside seven days critical mending was noticed (Sabitha *et al.,* 2005)

2.7.13 Antiparasitic Role

Globally, several botanists in general attribute garlic to treat gastrointestinal parasites. Some kids affected with helminthes were treated using crushed garlic. In China, most clinical meds uses it for stomach related infections using alcoholic concentrate on crushed garlic. Allicin shows strong effects to parasitic activity mainly in human gastrointestinal parasites (Kalyesa *et al.,* 1975). Entamoeba histolytica a protozoan found in human gastrointestinal is outstandingly fragile to 30 pg/mI of allicin (Mirelman *et al.,* 1987). Several authors have reported lower doses (5 pg/mI), allicin stifled 90% the danger of trophozoites of E. histolytica (Ankri *et al.,* 1997).

2.8.0 Role of Garlic Against Multi-Drug Resistant Bacteria

The efficacy of garlic on microorganisms are impenetrable to hostile to microbials and its compounds affects microbials prompts fragmentary and outright synergism (Didry *et al.,* 1992). The improvement of multi-drug safe strains of Gram -ve and Gram +ve microorganisms are affects living thing. The improvement of disease methicillin safe (MRSA) impenetrable to mupirocin has driven numerous makers to attest mupirocin usage to be controlled even more thoroughly, during a shorter time. Thus, garlic treats MRSA (Sharma *et al.,* 2005).

2.8.1 Role of Garlic Against Multi-Drug Resistant Tuberculosis (MDR-TB)

Coherent confirmation from randomized clinical studies maintains garlic usage and overhauls access for MDR-TB polluted individuals. Its use can allow an effective MDR-TB the leaders, due to its sensibility and the setback of hurtful effects (Catia *et al.,* 2011). Considering the extended pace of MDR-TB, the new adversary of tubercular meds reliant upon moderate and all the more impressive meds has adequately begun. Studies on inventive elective plant concentrates of therapeutic characteristics ought to be anxious, as plants are a critical wellspring of new antimicrobial subject matter experts, with little hurtfulness, prepared to replace meds to which Mycobacteriurn resistance has occurred (Amin *et al.*, 2009).

A fascinating in vitro test about the counter tubercular movement of garlic was acted in Nigeria utilizing plate dispersion technique and contrasted and standard anti-toxins. The counter tubercular movement of garlic on different medication safe Mycobacterium was explored among Nigerian HIV-infectedpersons and it displayed maximaal against all separates even at diminished fixations. Just two of the standard enemy of tubercular anti-infection agents utilized, streptomycin and rifampicin, showed critical action against detaches tried (Dibua, 2010).

2.8.2 Antioxidant

Entire garlic and matured GE display direct cancer prevention agent impacts and upgrade the serum levels of two cell reinforcement chemicals (Prasad *et al.,* 1995). GE, allicin is productively rummaged exogenously created hydroxyl extremists in a portion subordinate style, yet their adequacy was decreases about 10% heating to 100°C in 20 min. Some garlic constituents, for example, S-allyl cysteine, likewise affirmed critical cell reinforcement impacts. The sulfur intensifies viewed in new garlic give off an impression of being almost multiple times more powerful as cancer prevention agents than rough, matured garlic separate.

2.8.3 Drug Toxicities and Pharmacokinetics

Glutathione compound is essential in the liver for the detoxification of substances. It has been attributed to garlic organo-sulfur mixtures that might have the option to forestall glutathione consumption. Patients experiencing expanding in receptive oxygen actuated weight on liver capacity might be protected when garlic is ingested (Sabayan *et al.,* 2006). In E. coli, matured GE S-allyl cysteine, diallyl sulfide and diallyl disulfide don't meddle with the anti-toxin movement of gentamycin however may improve gentamycin-incited nephrotoxicity (Maldonado *et al.,* 2005). Matured garlic has additionally been displayed to turn around oxidant effects of nicotine poisonousness in rodent examine. More explores are required later on garlic might be a one of a kind decision to assist with limiting the poisonous impacts of helpful medications (Sener *et al.,* 2005).

2.8.4 Reduces Stress

Garlic to several authors seems to be ensuring against the adverse consequences of stress that influences the autonomic anxious and neuroendocrine framework. Rodents that were prepared with perseverance activities to actual weakness appreciated further developed boundaries of oxygen consuming glucose digestion, weakened oxidative pressure, and vasodilations, administering 2.86 g/kg garlic for 30 mm before work out (Morihara et al., 2006). In rodents presented to mentally distressing circumstances, matured GE altogether forestalled the reductions in spleen weight found in charge creatures. Furthermore, the garlic fundamentally forestalled the decrease of hemolytic plaque shaping cells in spleen cells.

2.9.0 Adverse Effects of Garlic

The primary unfriendly impact generally connected with garlic admission is breath scent, particularly when crude types of the spice are utilized. Sickness and spewing are other major

antagonistic impacts and care ought to be taken in devouring high amounts. Albeit a whole bulb delivers little squeeze, it is intense and can go about as a solid emetic, even in little amounts. Despite the fact that garlic for the most part presents little danger as far as wellbeing issues, there are disengaged instances of effective garlic consumes (Friedman *et al.*, 2006) and hypersensitivity (Yinand Li, 2007). Uncommon garlic sensitivity has been credited to the protein allinase, which has actuated immunoglobulin E (IgE) interceded touchiness reactions from skin prick-testing (Kao *et al*, 2004). Accordingly, the writing has commonly forewarned against utilizing garlic while utilizing anticoagulant treatment. A detailed instance of unconstrained spinal or epidural hematoma in a 87 years of age man with related platelet brokenness identified with exorbitant garlic ingestion (Saw *et al.*, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of the Delta State University Asaba Campus, Asaba. Nigeria. The farm location is on longitude $6^{o}49^{l}$E of the Greenwich meridian and latitude $6^{o}14^{l}$N of the equator. It has an annual rainfall range of 1500mm to 3000mm, temperature range of 27.5^{o}C to 30.9^{o}C and relative humidity of 77.2% to 80% (Asaba Meteorological Service Centre, 2019).

3.2 Poultry House and Housing Preparation

One of the poultry houses assigned by the Department was used. The poultry house was fully covered with wire netting for good ventilation and it was built with dwarf walls. The pullets were managed on deep litter system. The poultry house was always kept in high hygienic and good sanitary conditions. Both the interior and environment (exterior surrounding) were swept, disinfected and washed three weeks before the birds arrived. The house was partitioned into 20 units (cells) (2m x 2m) of equal size. New wood shavings were spread on the pen floor to absorbed the faecal droppings, and were regularly replaced as required.

3.3 Preparation of Test Ingredients

Fresh garlic bulbs (*Allium sativum*) were procured from the local Ogbeogonogo local market in Asaba. They were air-dried under room temperature. The garlic bulbs were separated into cloves, peeled and sliced to form flakes of 3-4mm thick thereby increasing the surface area for faster drying up to 5-6% moisture content. This was then sun-dried, and milled using a micro-miller blender. The resultant dried garlic meal was stored in air-tight containers prior tothe commencement of the experiment.

3.4 **Proximate Nutrient Analysis of the Test Ingredient and Experimental Diet (MGM) and Experimental Diet**

The Proximate nutrient Analysis of theMilled Garlic Meal (MGM)samples was carried out using the AOAC (2015) methods as follows:

i. **Moisture**

The percentage moisture was determined by drying weighed samples of the experimental diet in a silica dish and placing in a thermostatic air oven set at 105° C for 24 hours, and then cooled in desiccators for 30 minutes and weighed. The percentage moisture content was calculated thus;

$$\% \text{ Moisture} = \frac{W_{OS} - W_{DS}}{W_{OS}1} \times \frac{100}{}$$

Where W_{OS} = Weight of Original Sample

W_{DS} = Weight of dried Sample

ii. **Ash**

The dish with the dry sample was reserved for the ash determination. The dry sample was charred on a heater at low heat with caution not to flame it. The heat was increased until a glow went all over the residue, which on cooling was transferred to a thermostatic muffle furnace at 600°C for 30min, until the ash became gray powder but not fused, was cooled in desiccators and weighed. The percentage total ash was calculated thus;

$$\% \text{ Total ash} = \frac{\text{Weight of ash alone}}{\text{Weight of sample used}} \times \frac{100}{1}$$

iii. **Ether Extract**

For the ether extraction, the sample was weighed into a fat extraction thimble which was placed in a soxhlet flask (previously dried in an oven, cooled and weighed) and a double surface condenser was fitted in turn into the extractor. Then the flask, 3/4 filled with petroleum ether was placed on a heater. The extractor process went on for about 7 hours, after which the ether will maximally recover, the flask was removed and oven dried overnight (to dry off the last traces of ether or water from the flask), then cooled and weighed.

$$\% \text{ ether extract} = \frac{\text{Weight of flask and oil - Weight of flask}}{\text{Weight of sample}} \times \frac{100}{1}$$

iv. **Crude Fibre**

For crude fibre (CF) determination, the content of the dry thimble (from extraction) was transferred into a conical flask, to which were added dilute sulphuric acid and sodium hydroxide solution, one after the other and brought to boil. After series of washing the reacting substances with methylated spirit and petroleum ether, the residue was placed in a silica dish and dried in an oven overnight, then cooled and weighed. The residue was charred and ignited in a muffle furnace to complete ash, cooled and weighed.

$$\% \text{ Crude fibre} = \frac{\text{Wt. of dish and fibre — wt. Of dish and silica dish}}{\text{Weight of sample}} \times \frac{100}{1}$$

v. **Crude Protein**

Crude protein (CP) was determined by the Kjeldahl method, where by the sample in a kjeldahl flask was digested with the following chemicals:- anhydrous sodium thiosulphate, copper sulphate, a trace of selenium powder, concentrated sulphuric acid, with the aid of glass beads. 1-leafing continued until cessation of frothing and resultant bright greenish blue solution was obtained. Distilled water was added and transferred to a 250m1 graduated flask, made up to the volume with distilled water and shaken vigorously to mix. Then using N4arkanì apparatus with subsequent titration, the percentage crude protein was calculated thus:

$$\% \text{ Crude protein} = \frac{4.375 \times \text{Titre}}{\text{Weigh of sample}} \times 100$$

vi. **Nitrogen Free Extract**

Percentage Nitrogen - Free Extracts (NFE) was calculated using the formula;

% NFE = 100 - (% CP + % CF + % EE + % Ash + % Moisture)

3.5 Phytochemical Analysis

3.5.1 Phytochemical screening

The Milled garlic meal (MGM) crude extract (200 ml) was filtered and concentrated by sequential extractions with ethylacetate at various partitions. The secondary metabolites were

examined using phytochemical qualitative reactions. The screening was performed for triterpenes/steroids, ‘alkaloids, flavonoids (anthocyanins) and saponins, tannins, (Schenkel *et al*., 2007; Santos *et al*., 2007; Farnsworth, 1966; Evans, 2009; Harborne, 1998; Pearson, 1976). The color intensity or the precipitate formation was used as analytical responses to these tests.

3.5.2 Determination of the total alkaloid content

The alkaloid content was achieved by liquid-liquid extraction and gravirnetric analysis. The chloroform fraction (20 mL) is marked at various stages with HCI, at 1, 0.5, and 0.25mo1/L respectively. The aqueous phase was collected, alkalinized with NH_4OH, 6mol/L, and partitioned with chloroform. The evaporated to dryness in this organic phase pass through a water bath (60°C). The alkalinization with NH4OI--I, hydroethanolic fraction (20 mL), 6mol/L, partition with chloroform, and evaporation to dryness were also determined. The chloroformand hydroethanolic fractions were accurately determined. The total alkaloid content was expressed in milligrams of alkaloids per l00g of the dry sample which is from free base and salt forms.

3.5.3 Determination of the anthocyanins content

Flavonoids were verified for the studied ethyl acetate, and residual ethanol fractions when treated with the Shinoda reagent, boric acidloxalic acid solution, and zinc/hydrochloric acid reaction. The flavonoid content has been widely investigated in *Ipomoea* species. Recently, anthocyanins, catechins, flavonols, and proanthocyanidins from SP leaves were identified and quantified using high-perform once liquid chromatography combined with a photodiode-array detector (Carvalho *et al.*, 2010)

3.6 Procurement and Management of The Experimental Layer Pullets

A total of two hundred and forty (240) 18 weeks old layer pullets were procured from Integrity Agricultural Venture Farms, Eku, Delta State for the experiment. These comprised one hundred and twenty (120) of Bovan-Nera one hundred and twenty (120) Isa Brown birds. The layers were acclimatized for a period of 2 weeks, during which they were fed commercial grower diets

(hybrid),The one hundred and twenty (120) birdsof each genotype were divided into 5 treatment groups of 24 birds per group. Each treatment groupwas further divided into four (4) replicate pensof 6 birds/replicate, (6x4x5). The body weights of the pullets were taken on arrival and subsequently on weekly basis

All the pullets were fed with grower's mash until they started laying and then with layers mash, thereafter clean drinking water were provided *ad libitum* daily for sixteen weeks (16). Weighed quantities of feed was given and left over was collected, air-dried and weighed in order to determine the feed intake.

The layers mash (Hybrid) contained 17% crude protein (CP) and average of 2,500kcal/kg metabolizable energy (ME). Multivitamins and anti-stress were given to the layers on arrival and all necessary vaccinations and medication were also administered accordingly.

Data on feed intake per replicate was taken daily. The first eggs laid by each replicate group were weighed and used to get the weight of first egg for each treatment group. Other data that were collected include: age at first egg, age at sexual maturity, (50%) hen-day production according to (Leeson and Summers, 1989), and feed conversion ratio (FCR) which is the body weight gain and kilogramme eggs produced.

3.6.1 Parameters Monitored

The following parameters were determined during the experimental period:

Growth Parameters

* Initial body weight of birds on arrival (kg)
* Weekly body weight of birds for another 16 weeks
* Weekly body weight gain of birds for 16 weeks (kg)
* Final body weight at the end of experiment (at 16 weeks) kg
* Weekly feed intake (kg)
* Body weight of birds at first egg (kg)

* Age of birds at first egg (days)
* FCR
* Percentage mortality (%)

Egg External Quality Characteristics:

* Egg Weight (g)
* Egg Volume (ml)
* Egg Length (mm)
* Egg Width (mm)
* Egg Shell weight (g)
* Egg shell thickness (cm)
* Haugh unit (cm)
* Egg length: width index

Egg Internal Quality Characteristics:

* Egg Yolk Height (cm)
* Egg Yolk Width (cm)
* Egg Yolk Weight (g)
* Egg Yolk Index
* Egg Albumen Weight (g)
* Egg Albumen Height (cm)
* Egg Albumen Maximum Diameter (cm)
* Egg Albumen Minimum Diameter (cm)
* Egg Yolk Colour Score

3.7 Experimental Diets

Experimental diets were formulated into five dietary treatments as follows: (Treatment 1 (T1) which served as the control contained 0.00%garlic meal (MGM), (Treatment 2) (T2) contained

(0.75%) milled garlic meal (MGM), (Treatment 3) (T3) had 1.5% MGM),(Treatment 4) (T4) contained2.25% MGM whilediets 5 (Treatment) (T5) had 3.00% MGM for the layer pullets. The percentage composition of the experimental diets are as shown in Table 3.1

Treatment 1 (T1) = Control(0.00%)

Treatment 2 (T2) = 0.75%

Treatment 3 (T3) = 1.5%

Treatment 4 (T4) = 2.25%

Treatment 5 (T5) = 3.00%

Table 3.1. Percentage Composition of Layers Experimental Diets: Dietary Treatment

Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**
Maize	57.00	56.90	56.20	56.25	54.5
Milled Garlic Meal	0.00	0.75	1.5	2.25	3.00
Soya Bean Meal	16.90	16.25	16.2	15.45	16.40
Fish Meal	4.00	4.00	4.00	4.00	4.00
Wheat Offal	10.50	10.50	10.50	10.50	10.50
Rice Offal	1.50	1.50	1.50	1.50	1.50
Oyster	6.50	6.50	6.50	6.50	6.50
Bone Meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Common Salt	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100
Calculated Crude Protein (%)					
ME(KCAL/KG)	2581.23	2545.09	2509.16	2503.65	2560.05

Metabolizable Energy (KCAL/Kg DM) was calculated according to NRC (1994)
CP, etc

3.8 **Experimental Design**

The experiment was a 2 x 5 factorial in a Randomized Complete Block Design (RCBD). The factors were the two genotypes (Bova Nera and ISA-brown) and dietary treatments (garlic inclusion) at five levels (0.00%, 0.75%, 1.50%, 2.25% and 3.00%)

The Statistical Model used is

$Xijk = \mu + \alpha i + g_j + \alpha sij + eijk$

Where X_{ijk} = An observation made on the K^{th} individual bird belonging to the i^{th} strain receiving the J^{th} dietary garlic level

μ = The overall population mean effect common to all observations.

ai: Effect of the I^{th} strain (i = 1,2)

g_j = effect of the J^{th} garlic dietary level (j = i …..5)

as_{ij} = Effect of the i and j interaction

e_{ijk} = random error made while taking measurement on the K^{th} individual bird belonging to the i^{th} strain and receiving the J^{th} garlic dietary level.

3.9 Data Collection

Data were collected and recorded on replicate basis for a period of sixteen (16) weeks of the experiment, the parameters determined include feed intake, initial weight, weight gain, feed conversion and percentage mortality were recorded weekly on replicate basis. Egg quality characteristics (external and internal) such as egg weight(g), length and width in (cm) diameter and height of the egg yolk (cm) albumen weight (g), and height (cm), egg shell weight (g), shell thickness (mm), egg-shape index(%) yolk index (%) and haugh unit were collected.

Haematological and serological parameters: haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular heamoglobin concentration (MCHC), plasma protein, total protein, albumin and cholesterol were also determined.

3.9.1 Feed Intake

Feed intake of the pullets were obtained by supplying a pre- weighed quality of feed daily according to replicate and the stale feed collected next day morning, weighed and subtracted from the pre-weighed feed provided previously while the mean daily feed obtained as well. Thus,

Feed intake (kg) = weight of allotted feed- weight of staled feed.

$$\text{Mean daily feed intake (kg)} = \frac{\text{daily feed intake}}{\text{Number of Birds in Replicate}}$$

3.9.2 Live Weight Gain

The initial weights of the pullets were obtained on arrival on replicate basis as well as the weight at weekly basis in intervals throughout the experiment period. The mean weight gain obtained by subtracting the live weight gain of first week (WK.1) from week two (WK.2) up to week (WK.4) simultaneously up to the end of the experiment and then result divided by the number of bird (N) in each replicate.

Weight gain (kg) = current weight — initial weight.

Mean weight gain (kg) = $\frac{\text{final weight— initial weight}}{\text{N}}$

3.9.3 Feed Conversion Ratio.

Feed conversion ratio was determined by dividing the total feed intake consumed by birds, by total weight gain per replicate outside the weight of during the period of study.

Feed conversion = $\frac{\text{Feed Consumed}}{\text{Current weight gain- initial weight of bird}}$

3.9.4 Percentage mortality.

The percentage mortality was determined per replicate as number of dead bird(s) in the replicate divided by initial number of birds in the replicate and multiplied by

Percentage Mortality = Number of dead birds in the replicate x 100

Initial number of birds in the replicate

3.9.5 Blood sample collection and analysis

At 16 weeks, blood samples were collected from one bird per replicate for haematological and biochemical analysis. The blood samples were collected from each bird from the under their wings vein using a sterilized disposable soil syringe and needle between 6.00 and 7.00 am. Prior to bleeding, a cotton swab soaked in 70% ethanol was used to dilate the vein and to prevent infection. An initial 2.0ml blood was collected into labeled sterile universal bottles containing Ethylene- Diamine-TetraAcetic acid (EDTA) acting as an anticoagulant. This was used to

determine the haematological components within an hour of sample collection. Another 3.0ml of blood was collected into labelled sterile sample bottles without anticoagulant and used to determine the biochemical components.

The serum obtained was analysed colorimetrically for total protein (TP) by the Biuret method with kits. Colorimetric determination of TP is based on, the principle of Biuret reaction (copper salts in alkaline medium) in which cupric ions form a blue complex, in alkaline solution, with NH2 of two or more peptide bonds. The intensity of the blue colour formed is proportional to the protein concentration in the plasma or serum. Albumin concentration was determined by the Bromocrcsol Green (BCG) method (Peters et al., 1982); albumins (Alb) bind with BCG to form a green compound. The concentration of Alb is directly proportional to the intensity of the green colour formed. Globulin (Gb) concentration was computed as the difference between total protein and albumin concentrations. Cholesterol was determined as described by Coles (1986).

The red blood cell (RBC) counts, total white blood cell (WBC) counts, haemoglobin (Hb) concentration and Packed cell volume (PCV) parameters were determined followingstandard procedures described by Davice and Lewis (1991). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobinconcentration (MCHC) was calculated as follows:

$$MCV = \frac{PCV}{RBC} \times 100$$
expressed in cubic microns

$$MCH = \frac{HB}{RBC} \times 100$$
expressed in microgram

$$MCHC = \frac{HB}{PCV} \times 100$$
expressed in percentage

Differential leukocytes count was determined using electronic microscope after staining in thin film.

3.9.6 Egg Production Determination

Internal and external egg qualities were determined. Eggs were collected and recorded on daily,

Parameters calculated were;

Total egg produced/bird

$$\frac{\text{Total no. of egg produced/replicate during the experiment}}{\text{Total number of birds in that replicate}}$$

$$\text{Total egg produced/bird/week} = \frac{\text{Total egg produced/bird}}{\text{Weeks of the experiment}}$$

$$\text{Total egg weight/bird (kg)} = \frac{\text{Average egg weight x Total egg produced/bird}}{1000}$$

Eggs were collected from each treatment and were randomly selected at the end of the experiment for egg quality parameters analysis. The eggs was properly cleaned and weighed with sensitive analytical balance to the nearest 0.01cm. The egg length and width were determined with Vernier Calliper while the egg volume was determined using a measuring cylinder. The egg shape index was calculated by dividing egg width by egg length.

Shell thickness was determined with Micrometer Screw Gauge, while shell samples from the broad, middle and narrow portions of the eggs were measured for shell thickness and the mean was calculated as,

$$\frac{\text{Apex + Middle + Base}}{3}$$

Shell weight was determined using electronic balance to the nearest 0.1g

$$\text{Shell weight expressed as percentage of egg weight (SEW)} = \frac{\text{Egg shell weight}}{\text{Egg weight}} \times \frac{100}{1}$$

The yolk height and diameter was measured using Vernier Calliper after breaking the egg and separation of yolk from the albumen while the yolk colour was determined using the (Roche, 1988) method in 1—15 scales. The color measurements were also conducted using L, a and b values using a Minolta chromometer (CR 300, Minolta-Japan. The yolk index was calculated by

dividing yolk height by yolk width. Haugh unit was determined according to (Kaugh, 1937; Stadelman and Cotterill, 1986).

Haugh unit was determined with the formula below

Haugh Unit (HU) = 100 x log (h-1 .7w $^{0.37}$ + 7.6)

Where, h = Observed height of the albumen (mm)

w = Weight of the egg (g)

Yolk weight expressed as percentage of egg weight (YEW) (%) = $\frac{\text{Yolk weight}}{\text{Egg weight}} \times \frac{100}{1}$

Albumen weight expressed as a percentage of egg weight (AEW) (%)

$$\frac{\text{Albumen weight}}{\text{Egg Weight}} \times \frac{100}{1}$$

3.9.7 Hen-day egg production and Hen-Housed egg production

Hen-day production was determined by dividing number of eggs produced per day by the [total number of birds in the replicate multiplied by 100 (expressed in percentage) and Hen-Housed egg production was obtained by dividing the total number of eggs laid during the period of the experiment by the total number of birds housed as at when the experiment began (expressed in numbers).

3.9.8 Egg Mass Production

This was calculated by multiplying the total egg production per replicate by the average weight of the eggs in the replicate.

Egg Mass Production = Total Egg Production x Average weight.

Egg cholesterol and triglyceride were analysed using enzymatic colorimetric method.

3.9.9 Content of IgG in Serum and IgY in Egg Yolk

Egg yolk IgY is contained in the water-soluble fraction. Egg yolk was separated from the white albumin using a yolk separator, gently rolled on a paper towel toremove the attached white, and then transferred to a graduated cylinder. The yolk volume was recorded and diluted

(1:6, vol vol-l) with acidified deionized water (pH 2.5), adjusted with 0.1 N HCI, mixed well and kept at 4°C for 6 h or overnight, egg IgY contents were quantified by. ELISA using rabbit anti-chicken lgG (Rockland Inc., Gilbertsville, PA) (Ulmer-Franco et al., 2012). The solution was centrifuged at 12 000 g at 4°C for 15 mm and the supernatant was collected. The contents of lgG in serum and egg yolk were determined by radial immune diffusion technique as described by Sunwoo *et al*., (1996).

3.10 **Statistical Analysis**

All data were subjected to Analysis of variance (ANOVA) and Randomized Completed Block Design (RCBD) carried out using GenStat (Release 4.24) statistical package (Genstat, 2014). Differences between means were separated by the Duncan's Multiple Range Test (DMRT).

Where the age of the birds was the block, the two strains of birds and the five (5) levels of Garlic meal inclusion makes it 2 x 5 factorial in a Randomized Complete Block Design (RCBD)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Results

4.1. Proximate Composition of the *Allium sativum*(Garlic) meal and the Feed (Layers mash).

The sample of ground *Allium sativum* and the feed layers mash were analysed for their different proximate composition including; moisture content, ash content, crude fibre content, crude fat, crude protein, carbohydrate as nitrogen free extract and dry matter, using standard methods of AOAC (1980) and the results are presented in Table 4.1.

Percentage of moisture content was 3.20% in garlic and 9.88% in layers mash. Percentage of Ash content was 2.10% in garlic to4.59% in the layers mash, crude fibre was 1.75% in garlic and (5.78%) in feed sample. Percentage of Ether extract varied from (1.45%) in garlic to (4.55%) in feed sample. Crude protein varied from (19.66%) in garlic to (23.31%) in feed sample. Carbohydrate varied from (71.84%) in garlic to (51.89%). The dry matter also shows $P<0.05$ with garlic (96.80%) and feed sample (90.12%).

Table 4.1 Proximate analysis of garlic meal and feed sample.

Parameters	Garlic sample	(Layers Mash) Feed Sample
% Moisture content	3.20	9.88
% Ash content	2.10	4.59
% Crude fibre	1.750	5.78
% Ether Extract	1.45	4.55
% Crude protein	19.66	23.31
% NFE or Carbohydrate	71.84	51.89

The values of the moisture content, ash, crude fibre, ether extract and crude protein were generally lower than those of layer mash except that of NFE which was higher. The crude protein content was considerably appreciable

4.2 Phytochemical constituents of Garlic (*Allium sativum*).

The phytochemical constituents present in the aqueous and ethanol extract of *Allium sativum* are shown in Table 4.2. Alkaloids, flavonoids and steroids were present in the ethanol extract while tanninsand saponins were not detected when screened. Alkaloids and flavonoids, tamins and steroids were detected under ethanol extract while Tannins, saponins and steroids were not detected in distilled water extracts.

Table 4.2.1 The phytochemical (quantitative) analysis of Garlic (*Allium sativum*)

Parameters (mg/100g)	**Ethanol Extract**	**Distilled H_2O Extracts**
Alkaloid (mg/l)	1.8	0.95
Flavanoid (mg/100g)	7.4	6.8
Tannins (mg/l)	0.24	ND
Saponins (mg/dl)	ND	ND
Steroid (mg/l)	0.21	ND

ND – not detected

Table 4.3 shows the quantitative phytochemical analysis of ethanol extract and aqueous distilled water extract of *Allium sativum*. The result revealed that there were significance difference (P>0.05) in alkaloid (1.8), flavonoid (7.4), Tannin (0.24) and steroid (0.21) under ethanol extract, compared to flovonoid (6.8) and Alkaloid (0.95) under aqueous extract. Although Tannin, saponin and steroid were not detected using aqueous extract. However, the result did not show the presence of saponin under aqueous and ethanol extract.

Table 4.2.2 The phytochemical (screening) analysis of Garlic (*Allium sativum*)

Parameters (mg/100g)	Ethanol Extract	Distilled H_2O Extracts
Alkaloid (mg/l)	+	+
Flavanoid (mg/100g)	+|	+
Tannins (mg/l)	ND	ND
Saponins (mg/dl)	ND	ND
Steroid (mg/l)	+	ND

Key:
+: Present in trace quantity
+|: Present in medium quantity
ND: Not Detected

4.3 Effects of Genotype on performance characteristics of pullets

The performance characteristics of Bovan Nera and ISA brown pullets laying are presented in Table 4.3

Table 4.3 Effect of genotype on performance characteristics of Laying pullets

Parameter	Bovan Nera	ISA brown
Initial Body Weight (g)	1229.00±19.89^a	1167.50±24.73^b
Final Body Weight (g)	1922.20±26.35^a	1880.70±23.41^b
Weight gain at 16 weeks (g)	693.20±16.95^b	716.70±29.99^a
Body weight at first egg (g)	1305.78±51.14^a	1297.86±4.15^b
Age at first egg (days)	142.80±1.14	143.50±1.17NS
Weight of first egg (g)	49.40±0.39	49.09±0.69NS
Average weekly feed intake/bird (g)	847.55±16.89^a	790.20±4.88^b
Weekly weight gain/bird/day	43.33±0.45^b	44.79±0.62^a
Feed conversion Ratio	2.12±0.05^a	1.82±0.03^b
% Mortality	0.94±0.44^a	0.05±0.03^b

For each parameter, results with different superscript letter, differs significantly (P<0.05)

Final body weight, weight gain at 16 weeks, body weight at first egg, average weekly feed intake, weekly weight gain, feed conversion ratio and mortality were significantly (P<0.05) affected by genotypes difference in mean age at first egg and weight of first egg were not significant (P<0.05).

Bovan Nera had significantly (P<0.05) higher final body weight, body weight at first egg, feed conversion ratio and percentage mortality compared to ISA brown pullets. ISA brown pullets however, had significantly (P<0.05) higher body weight gain at 16 weeks (112 days), weekly weight gain and lower mortality compared to Bovan Nera pullets.

Table 4.4: Effect of Garlic Dietary Treatment on Performance Characteristics of Laying pullets

Parameters	T1(0.0%)	T2(0.75%)	T3(1.5%)	T4 (2.25%)	T5(3.00%)
Initial Body Weight	1286.75±27.64^{a}	1175.00±28.06^{b}	11.67.50 ± 7.50^{b}	1200.75 ± 48.29^{b}	116125 ± 40.33^{b}
Final Body weight	1966.00±38.5^{a}	1852.50±64.36^{b}	1848.25±25.36^{b}	1946.25±26.56^{a}	1901.75±33.67^{b}
Weight Gain at 16 weeks	679.25±33.11^{b}	677.50±59.56^{bc}	680.75±31.59^{b}	745.50 ±42.31^{a}	741.75±9.93^{ab}
Body weight at first egg(g)	1287.28 ±8.60^{b}	1298.08±7.28^{ab}	1308.88±6.42^{a}	1311.38±8.51^{a}	1303.50±6.36^{ab}
Age at first egg (days)	145.25±1.75^{a}	145.25 ±1.75^{a}	140.00±0.00^{b}	140.00±0.00^{b}	145.2±.73^{a}
Weight of first egg (g)	48.68±1.28	50.58±0.82	49.75±0.39	49.65±0.22	47.58±0.67^{NS}
Average weekly feed intake b/d	841.94±34.79^{ab}	782.44±15.21^{b}	858.90±20.22^{a}	813.43±7.02^{ab}	799.60±4.69^{ab}
Weekly gain/bird/day	42.45±0.64^{b}	42.34±0.92^{b}	42.55±0.72^{b}	46.59±0.95^{a}	46.36±0.87+
Feed conversion ratio	2.14±1.05^{a}	1.81±0.05^{b}	2.12±0.06^{a}	1.90±0.05^{b}	1.88±0.06^{b}
% Mortality	0.40±0.40^{b}	0.40±0.40^{b}	0.78±0.78^{a}	0.78±0.55^{a}	0.13±0.72^{c}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05

All the performance parameters as affected by garlic dietary treatments were significantly (P<0.05) different as presented in Table 4.4 except weight of first egg. The initial body weight was higher (P<0.05) in pullet fed control diet T1(0.0% garlic inclusion) than birds fed with dietry T2 (0.75%), T3 (1.5%), T4 (2.25%) and T5 (3.0%) respectively which had similar (P>0.05) body weight. Final body weight was higher (P<0.05) in birds fed dietary T4 (2.25%) and control T1(0.0%) than those fed dietary T2(0.75%), T3 (1.5%) and T5 (3.0%) respectively.

Mean weight gain at 16 weeks of birds fed T4 (2.25%) and T5 (3.0%) were significantly (P<0.05) different followed by T1(0.00%) and T3 (1.5%). T2 (0.7%) had the least weight gain at 16 weeks. Laying pullets fed T3 (1.5%) and T4 (2.25%) had the highest (P<0.05) mean body weight (1308.88 ± 6.42) and (1311.38 ± 8.51) than birds fed T2 (0.75%) and T5 (3.0%) respectively. Mean age at first egg were significantly higher (P<0.05) in pullets fed dietary

treatment 4, T2 and T5 (145.25 ± 1.75), (145.25 ± 1.75) and (145.25 ± 1.73) days than pullets fed T3 (140.00 ± 0.00) and T4 (140.00 ± 0,00) days.

The highest ($P<0.05$) average weekly feed consumption was recorded for pullets fed T3 (858.90±20.22) and the least average weekly feed consumption was weekly for pullets fed T2 (782.44 ± 15.21). Weekly gain per bird was significantly ($P<0.05$) different in pullet which consumed dietary T4(2.25%) and T5 (3.0%) than pullet fed T1(0.0%), T2 (0.75%) and T3 (1.5%) garlic inclusion. Mean Feed conversion of pullet fed dietary treatment T1(0.0%, 2.14 ± 1.05), T3 (2.12 ± 0.06) were significantly different ($P<0.05$) compared to birds which consumed dietary treatment T2 (1.81 ± 0.05), T4 (1.90 ± 0.05) and T5 (1.88 ± 0,06). Mean percentage mortality was significantly ($P<0.05$) higher in pullet which consumed dietary treatment T3 and T4 while pullets fed T5 had the least percentage mortality.

Effect of Garlic Dietary Treatments X genotype on performance characteristics of laying pullets

Table 4.5 shows Effect of Garlic Dietary Treatments X Genotype on performance characteristics of laying pullets

Parameters	Strain Type	T_1 (0.00g)	T_2 (0.75g)	T_3 (1.5g)	T_4 (2.25g)	T_5 (3.00g)
Initial Body Weight (g)	BN	1286.00±26.00^{a}	1212.50±22.50^{a}	1160.00±15.00^{b}	1276.50±43.50^{ab}	1210.00±60.00^{ab}
	IB	1287.50±62.50^{a}	1137.50±37.50^{b}	1175.00±0.00^{b}	1125.00±25.00^{b}	1112.50±37.50^{b}
Final Body Weight (g)	BN	2020.00±20.00^{a}	1829.00±6.00^{b}	1871.50±36.50^{b}	1951.00±64.00^{a}	1939.50±58.50^{a}
	IB	1912.00±50.00^{b}	1876.00±154.00^{b}	1825.00±38.00^{b}	1941.50±9.50^{a}	1864.00±23.00^{b}
Weight gain at 16 weeks (g)	BN	734.00±6.00^{c}	616.50±16.50^{c}	711.50±51.50^{b}	674.50±20.50^{c}	729.50±1.50^{b}
	IB	624.50±12.50^{b}	738.50±116.50^{ab}	650.00±38.00^{c}	816.50±15.5^{a}	754.00±17.00^{ab}
Body weight at first egg (g)	BN	1280.25±14.75^{b}	1308.60±8.40^{a}	1301.50±3.50^{a}	1324.75±8.75^{a}	1313.80±3.80^{a}
	IB	1294.30±11.30^{b}	1287.55±5.05^{b}	1316.25±11.20^{a}	1298.00±0.50^{ab}	1298.20±4.00^{ab}
Age at first egg (days)	BN	147.00±0.00^{a}	143.50±3.50^{ab}	140.00±0.00^{b}	140.00±0.00^{b}	143.50±3.50^{ab}
	IB	143.50±3.50^{bb}	147.00±0.00^{a}	140.00±0.00^{b}	140.00±0.00^{a}	147.05±1.45^{a}
Weight of first egg (g)	BN	49.25±0.75^{a}	50.60±1.40^{a}	49.50±0.90^{a}	49.55±0.45^{a}	48.10±0.10^{bc}
	IB	48.10±2.90^{bc}	50.55±1.45^{a}	50.00±0.00^{a}	49.75±0.25^{a}	47.05±1.45^{c}
Average weekly feed intake/bird (g)	BN	899.89±68.07^{ab}	783.88±24.61^{c}	928.23±35.71^{a}	827.09±15.69^{bc}	798.99±6.14a^{b}
	IB	783.99±8.27^{c}	781.00±18.28^{c}	789.56±8.75^{bc}	799.77±8.53^{b}	796.79±7.17^{b}
Weekly weight gain/bird/wk (g)	BN	45.88±0.67^{b}	38.53±0.44^{c}	44.47±0.86^{b}	42.16±1.08^{b}	45.59±1.26^{b}
	IB	39.03±0.66^{c}	46.16±1.52^{ab}	40.63±1.07^{bc}	51.03±1.15^{a}	47.13±1.20^{ab}
Feed conversion Ratio	BN	2.31±0.19^{a}	1.77±0.08^{c}	2.29±0.10^{a}	2.13±0.08^{a}	2.10±0.06^{a}
	IB	1.97±0.87^{ab}	1.85±0.05^{bc}	1.95±0.05^{ab}	1.65±0.03^{c}	1.67±0.0^{c}
% Mortality	BN	0.79±0.78^{b}	0.78±0.78^{b}	1.56±1.56^{a}	1.56±1.09^{a}	0.00±0.00^{c}
	IB	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.25±0.14^{c}

For each parameter, results with different superscript letter, differs statistically (P<0.05)
BN: Bovan Nera, IB: Isa Brown

From the Table 4.5 which showed the interaction between the Garlic dietary treatments X genotype, we deduced significant (P<0.05) interactions between treatments and genotype in the

final body weight, weight gain at 16 weeks (112 days), body weight at first egg, age at fist egg, weekly feed intake, weekly weight gain, feed conversion ratio and percentage mortality. There were no significant ($P>0.05$) interaction between treatment and genotype in weight of first egg. Initial body weight was significantly ($P<0.05$) higher for Bovan Nera fed T_4 that ISA Brown fed same diet.

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Table 4.6: Effect of Garlic Dietary Treatments on External Egg Characteristics of Laying Pullets

Parameters	T1(0.0%)	T2(0.75%)	T3(1.5%)	T4 (2.25%)	T5(3.00%)
Egg weight (g)	54.70±0.53^{b}	57.82±0.62^{a}	55.89±0.51^{b}	54.91±0.53^{b}	54.35±0.47^{b}
Egg Length (cm)	4.32±0.09	4.41±0.09	4.36±0.84	4.29±0.08	4.33±0.09^{NS}
Egg width(cm)	3.10 ±0.09	3.16 ±0.09	3.08 ±0.08	3.07±0.09	3.08±0.09^{NS}
Egg Vol (ml)	50.06 ±0.05^{ab}	51.96 ±0.59^{a}	50.91±0.52^{ab}	49.92±0.56^{b}	49.72±0.50^{b}
Shell Weight (g)	5.20 ±0.07^{b}	5.54 ±0.07^{a}	5.42±0.08^{ab}	5.34±0.07^{ab}	4.95±0.08^{bc}
Shell Thickness (mm)	0.54±0.01	0.56±0.01	0.55±0.01	0.56 ±0.01	0.54±0.01^{NS}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05

The effect of garlic dietary treatment on the external egg quality of laying pullets is presented in Table 4.6. The test ingredient had no significant (P>0.05) effects on egg length, egg width, and shell thickness. Egg weight, egg volume and shell weight were significantly (P<0.05) influence by dietary treatment. Birds fed Dietary treatment (T2) (0.75%) significantly increase in all the external egg quality followed by pullets fed dietary treatment T3 (1.5%) and pullets which consumed dietary treatment is (3.0%) had the least (P<0.05) external egg quality characteristics.

Table 4.7: Effects of Genotype on External Egg Characteristics of Laying Pullets

Parameters	Bovan Nera	Isa-Brown
Egg weight (g)	56.3±0.35^{a}	54.76±0.34^{b}
Egg Length (cm)	4.41±0.55^{a}	4.27±0.53^{b}
Egg width(cm)	3.10 ±0.53	3.10 ±0.56^{NS}
Egg Vol (ml)	51.31±0.35^{a}	49.73±0.33^{b}
Shell Weight (g)	5.15±0.05^{b}	5.44±0.47^{a}
Shell Thickness (mm)	0.54±0.01^{b}	0.57±0.00^{a}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05

Mean effect of genotype on external egg quality parameter of laying pullets fed (GP) are presented in Table 4.7. The result shows that Bovan Nera pullets significantly increase in egg weight, egg length and egg volume than ISA Brown pullets. ISA brown on the order, has significantly (P<0.05) higher shell weight and shell thickness than Bovan Nera. There was no significant different in mean egg width of both strains of pullets.

Table 4.8: Effect of Garlic treatments X Genotype on the External Egg Characteristics of Laying Pullets.

Parameters	Strain Type	T_1 (0.00g)	T_2 (0.75g)	T_3 (1.5g)	T_4 (2.25g)	T_5 (3.00g)
Egg Weight (g)	BN	53.88±0.59	60.43±0.79[a]	56.72±0.59	54.96±0.83	55.54±0.60[a]
	IB	55.51±0.87	55.21±0.70[b]	55.08±0.82	54.85±0.69	53.17±0.65[b]
Egg Length (cm)	BN	4.34±0.14[a]	4.53±0.13[a]	4.47±0.11[a]	4.29±0.13[a]	4.43±0.13[a]
	IB	4.30±0.12[a]	4.29±0.12[a]	4.26±0.13[a]	4.29±0.11[a]	4.22±0.13[a]
Egg Width (cm)	BN	3.03±0.12	3.21±0.12	3.09±0.11	3.07±0.12	3.10±0.12[a]
	IB	3.18±0.13	3.12±0.12	3.08±0.13	3.06±0.12	3.07±0.13
Egg Volume (ml)	BN	49.50±0.52	52.69±0.76[a]	51.69±0.66	50.03±0.87	50.66±0.72[a]
	IB	50.63±0.86	49.28±0.62[b]	50.13±0.82	49.81±0.71	48.78±0.63
Shell Weight (g)	BN	4.98±0.09[b]	5.44±0.08[b]	5.28±0.13[b]	5.21±0.09	4.82±0.09
	IB	5.43±0.09[a]	5.63±0.11[a]	5.59±0.08[a]	5.48±0.11	5.09±0.11
Shell Thickness (mm)	BN	0.52±0.01	0.55±0.01	0.54±0.01	0.56±0.01	0.53±0.01[a]
	IB	0.57±0.01	0.57±0.01	0.57±0.08	0.57±0.01	0.55±0.01

For each parameter, results with different superscript letter, differs statistically (P<0.05)
BN: Bovan Nera, IB: Isa Brown

Result showing effect of garlic treatment and genotype on external egg parameter of pullet layers are presented in table 4.8.Bovan Nera fed diet T_2 had significantly (P<0.05) higher egg weight and egg volume than ISA brown pullet which consu (56.72±0.54) and T_5 (55.54±0.60) of Bovan Nera were also hig(P<0.05) than egg weight of ISA Brown pullet fed same diet T_3 (55.08±0.82) and T_5 (53.17±0.65) respectively.

ISA Brown pullet fed T_2 (5.63±0.11) and T_3 (5.59±0.08) had significantly (P<0.05) higher shell weight than Bovan Nera pullet fed diets T_2 (5.44±0.08) and T_3 (5.28±0.13).

The result on ISA brown fed control diet T_1 (0.0g) had significantly (P<0.05) higher egg weight, egg volume, shell weight and shell thickness than Bovan Nera fed same control diet..

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Table 4.9: Effect of Dietary Garlic Treatments on Internal Egg Characteristics

Parameters	T1(0.0%)	T2(0.75%)	T3(1.5%)	T4 (2.25%)	T5(3.00%)
Yolk Height(cm)	1.5±0.03	1.56±0.03	1.51±0.03	1.52±0.03	$1.53±0.03^{NS}$
Yolk Dia(cm)	4.08±0.03	4.09±0.03	4.10±0.03	4.15 ±0.03	$4.12±0.03^{NS}$
Yolk Colour	$6.79±0.31^{a}$	$6.85±0.27^{a}$	$6.95±0.32^{a}$	$6.44±0.30^{b}$	$6.38±0.28^{b}$
Yolk Weight(g)	14.17±0.26	14.52±0.21	14.60±0.28	14.57±0.22	$14.60±0.22^{NS}$
Albumen weight(g)	$33.69±0.37^{b}$	$36.3±0.54^{a}$	$33.73±0.69^{b}$	$33.46±0.45^{b}$	$33.25±0.38^{b}$
Albumen max dia(cm)	7.70±0.47	7.71±0.04	7.62±0.13	7.69±0.06	$7.58±0.05^{NS}$
Albumen min dia (cm)	$6.48±0.07^{a}$	$6.46±0.06^{a}$	$6.49±0.06^{a}$	$6.28±0.08^{ab}$	$6.14±0.07^{b}$
Albumen height (cm)	0.886±0.01	0.91±0.01	0.89±0.01	0.89±0.01	$0.88±0.11^{NS}$
Haugh Unit	94.74±0.48	95.66±0.47	94.81±0.52	9496±0.51	$94.87±0.50^{NS}$
Yolk Index (%)	37.05±0.65	38.06±0.56	37.06±0.68	36.71±0.62	$37.27±0.76^{NS}$

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

Table 4.9 presents the effect of garlic treatment on the internal egg parameter of laying pullet. The result shows no variation (P>0.05) in Yolk height, yolk weight, yolk diameter, Albumen maximum diameter, Albumen height, haugh unit and yolk index. Significant different (P<0.05) existed in yolk colour Albumen weight and Albumen minimum diameter.

Table 4.10: Effects of Genotype on Internal Egg Characteristics of Laying Pullets

Parameters	Bovan Nera	Isa-Brown
Yolk Height(cm)	1.54±0.02	$1.52±0.02^{NS}$
Yolk Dia(cm)	$4.20±0.02^{a}$	$4.04±0.02^{b}$
Yolk Colour	6.83±0.18	$6.52±0.19^{NS}$
Yolk Weight(g)	$15.11±0.14^{a}$	$13.87±0.15^{b}$
Albumen weight(g)	34.12±0.36	$34.06±0.30^{NS}$
Albumen max dia(cm)	$7.76±0.56^{a}$	$7.60±0.33^{b}$
Albumen min dia (cm)	$6.52±0.04^{a}$	$6.21±0.05^{b}$
Albumen height (cm)	0.88±0.01	$0.90±0.01^{NS}$
Haugh Unit	$94.50±0.33^{b}$	$95.52±0.29^{a}$
Yolk Index (%)	$36.74±0.37^{b}$	$37.72±0.45^{a}$

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

Bovan Nera pullets significantly had higher (P<0.05) Yolk diameter, Yolk weight, Albumen maximum diameter and Albumen minimum diameter than it counterpart ISA Brown and presented in Table 4.8. The haugh unit and Yolk Index were significantly (P<0.05) higher in ISA Brown Pullets than Bovan Nera pullet fed same diets. Significant variation was not observed

($P<0.05$) in Yolk colour Albumen weight, Albumen height in both Bovan Nera and ISA Brown pullets.

Table 4.11: Effect of Garlic treatments X Genotype on the Internal Egg Characteristics of Laying Pullets

Parameters	Strain Type	T_1 (0.00g)	T_2 (0.75g)	T_3 (1.5g)	T_4 (2.25g)	T_5 (3.00g)
Yolk Height (cm)	BN	1.49±0.04^{b}	1.60±0.04^{a}	1.54±0.03^{ab}	1.51±0.04^{ab}	1.54±0.04^{ab}
	IB	1.52±0.03^{ab}	1.52±0.03^{ab}	1.49±0.04^{b}	1.54±0.04^{ab}	1.52±0.04^{ab}
Yolk diameter (cm)	BN	4.09±0.05^{ab}	4.22±0.04^{a}	4.19±0.04^{a}	4.24±0.04^{a}	4.21±0.04^{a}
	IB	4.07±0.05^{ab}	3.98±0.05^{b}	4.01±0.44^{ab}	4.07±0.04^{ab}	4.06±0.04^{ab}
Yolk Colour	BN	6.64±0.45^{b}	6.98±0.38^{ab}	7.09±0.45^{a}	7.22±0.32^{a}	6.19±0.38^{bc}
	IB	6.94±0.42^{ab}	6.72±0.39^{b}	6.79±0.34^{b}	5.66±0.42^{c}	6.56±0.41^{b}
Yolk Weight (g)	BN	14.29±0.28^{b}	15.21±0.28^{a}	15.52±0.39^{a}	15.08±0.32^{a}	15.45±0.22^{a}
	IB	14.05±0.45^{b}	13.82±0.63^{c}	13.68±0.34^{c}	14.06±0.29^{b}	13.74±0.32^{c}
Albumen Weight (g)	BN	32.70±0.44^{ab}	38.17±0.76^{a}	33.30±1.07^{c}	32.93±0.63^{c}	33.48±0.51^{bc}
	IB	34.68±0.55^{a}	34.45±0.63^{b}	34.17±0.87^{b}	33.98±0.65^{bc}	33.02±0.57^{c}
Albumen Maximum diameter (cm)	BN	7.78±0.05^{a}	7.84±0.04^{a}	7.68±0.25^{a}	7.93±0.06^{a}	7.56±0.08^{a}
	IB	7.63±0.08^{a}	7.58±0.07^{a}	7.56±0.08^{a}	7.45±0.08^{a}	7.61±0.06^{a}
Albumen Minimum Diameter (cm)	BN	6.56±0.09^{a}	6.59±0.05^{a}	6.61±0.08^{a}	6.67±0.07=	6.24±0.05^{ab}
	IB	6.39±0.09^{a}	6.33±0.10^{ab}	6.38±0.09^{ab}	5.88±0.09^{b}	6.05±0.10^{b}
Albumen Height (cm)	BN	0.87±0.01^{a}	0.92±0.02^{a}	0.87±0.01^{a}	0.87±0.02^{a}	0.88±0.02^{a}
	IB	0.89±0.02^{a}	0.90±0.01^{a}	0.91±0.02^{a}	0.90±0.02^{a}	0.88±0.01^{a}
Haugh unit	BN	94.65±0.70^{a}	95.56±0.68^{a}	93.64±0.65^{a}	94.22±0.75^{b}	94.40±0.84^{a}
	IB	94.81±0.68^{a}	95.77±0.64^{a}	95.98±0.01^{a}	95.70±0.66^{ab}	95.33±0.50^{a}
Yolk index (%)	BN	36.75±1.01^{ab}	37.98±0.77^{a}	36..87±0.71^{b}	35.54±0.71^{a}	36.57±0.79^{b}
	IB	37.36±0.82^{a}	38.15±0.82^{a}	37.26±0.76^{ab}	37.88±0.94^{b}	37.96±1.31^{a}

For each parameter, results with different superscript letter, differs statistically (P<0.05)
BN: Bovan Nera, IB: Isa Brown

Effect of dietary treatments X genotype on the Internal Egg quality parameters of Bovan Nera and ISA Brown pullet layers

Table 4.11shows the effect of graded level of garlic treatments and strain on internal egg quality characteristics.

There was no significant (P>0.0) interaction effect between treatments and strain in yolk height, yolk diameter, yolk colour, albumen maximum diameter, albumen minimum diameter and haugh unit respectively, while yolk weight, albumen weight and yolk index were significantly (P<0.05) influence by treatment and strain.

Bovan Nera pullets fed dietary treatment T_2, T_3, T_4 and T_5 had higher yolk weight than ISA Brown fed same dietary treatments. Albumen weight of Bovan Nera fed diet T_2 (38.17±0.76) was significantly (P<0.05) higher than albumen weight of ISA Brown pullet fed same diet T_2 (34.45±0.63).

Table 4.12: Effect of Garlic Dietary Treatments on Haematological Parameters of Laying Pullets

Parameters	T1(0.0%)	T2(0.75%)	T3(1.5%)	T4 (2.25%)	T5(3.00%)
PCV(%)	24.00±1.47^{b}	26.88±1.39^{a}	25.63 ±0.69^{a}	23.88±0.31^{b}	25.88±2.63^{a}
HB(g/dl)	8.25±0.60^{cd}	9.12±0.42^{a}	8.50±0.46b^{c}	7.92±0.22^{d}	8.79±0.91^{ab}
WBC x $10^3/mm^3$	77.00±15.30^{a}	5312.50±267.99^{b}	5275.00±449.77^{b}	5012.50±65.75^{c}	5075.00±340.04^{c}
RBC x $10^6/mm^3$	2.63±0.24^{a}	2.48±0.17^{ab}	2.38±0.63^{b}	2.38±0.17^{b}	2.25 ±0.32^{c}
Plasma Protein (g/dl)	6.25±0.59^{ab}	6.10±0.23^{b}	6.50±0.29^{a}	6.33±0.34^{ab}	5.25±0.29^{c}
Netrophils (%)	50.50±0.50^{c}	50.75 ±0.48^{c}	53.25±0.75^{b}	53.50±1.19	57.75±1.03^{a}
Lymphocytes(%)	40.75 ± 0.48^{b}	43.00±1.29^{a}	42.75±1.60^{a}	39.00±1.29^{b}	39.00±0.48^{b}
Monocytes (%)	2.00±0.00^{a}	2.00±0.00^{a}	0.50±0.29^{c}	2.00±0.00^{a}	1.00±0.58^{b}
Eosinophils (%)	5.75±0.48^{a}	3.00±0.58^{c}	3.00±0.58^{c}	4.75±0.48^{b}	2.0±0.00d
Basophils (%)	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00^{NS}
MCV (fl)	110.00±12.08^{bc}	106.20±13.15^{c}	121.37±8.20^{a}	108.99±9.07^{c}	115.45±10.84^{b}
MCH (pg)	36.15±3.32^{bc}	35.77±4.36^{c}	40.16±2.52^{a}	36.19±2.85^{bc}	38.63±3.59^{b}
MCHC(g/dl)	33.33±0.00^{a}	33.33±0.01^{a}	33.33±0.00^{a}	32.66±0.38^{b}	33.33±0.00^{a}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

The result of haematological parameter of laying pullets as affected by garlic treatment is presented in Table 4.12 above. Significant (P<0.05) difference existed in all the haematological parameters of the pullets except in Basophils of the layers (P>0.05)

The mean PCV (Packed Cell Volume) (26.88±1.39) and Haemoglobin concentration was significantly (P<0.05) different from other dietary. Birds fed dietary treatment T4 (2.25% garlic inclusion) had the least mean PCV (23.88±0.31) and Hb (7.92±0.22). White Blood Cell Count (7700.00± 153.25) and Red Blood Cell Count 92.63 ±0.59) birds fed T1 (0.00%) was observed to be higher (P<0.050 than the dietary treatment T2, T3, T4 and T5 respectively. The value decreases with increased level of garlic diets. Percentage Neutrophil value increased with

increased level of garlic powder in the diet of the pullets with dietary treatmentT5 having the highest (P<0.05) mean Neutrophil (57.75± 1.03) T1, T2, T3 and T4 respectively.

Pullet fed dietary treatment T2 and T3 were significantly (P<0.05) higher in mean Lymphocyte T2 (43.00 ± 1.29) and T3 (42.75 ± 1.60) than other treatments T1, (40.75 ± 0.48), T4 (39.00 ± 1.29) and T5 (39.00 ± 0.58) with similar (P>0.05) percentage of lymphocytes.

Mean percentage monocyte was observed to be least in birds fed dietary treatment T3 (0.50 ± 0.29) than other treatment T1 (62.00±0.00), T2 (2.00±0.00), T4 (2.00±0.00) and T5 (2.00±0.00) with similar (P<0.05) percentage of monocytes.

Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly different (P<0.05) in layers fed T3 (1.5%) than those fed T1(0.00%) control, T2 (0.75%), T4 (2.25%) and T5 (3.0%) respectively. Dietary treatment T2 (0.75%) had the least (P<0.05) MCV and MCH.

Mean corpuscular haemoglobin concentration was observed to be least in birds fed dietary treatment T4 compared to Treatments T1, T2, T3 and T5 respectively.

Table 4.13 Effects of Genotype on Haematological Parameters of Laying Pullets

Parameters	Bovan Nera	ISA Brown
PCV(%)	23.700±0.68^{b}	26.80±0.92^{a}
HB(g/dl)	8.08±0.26^{b}	8.95±0.38^{a}
WBC x 10^3/mm^3	5825.00±756.76^{b}	77.00±1530.25^{a}
RBC x 10^6/mm^3	2.22±0.12^{b}	2.63±0.24^{a}
Plasma Protein (g/dl)	5.91±0.27^{b}	6.25±0.59^{a}
Netrophils (%)	52.80±0.83^{a}	50.50 ±0.50^{b}
Lymphocytes(%)	41.20±0.74^{a}	40.60±0.97^{b}
Monocytes (%)	1.80±0.13	2.00±0.00^{NS}
Eosinophils (%)	3.40±0.40^{b}	5.75±0.48^{a}
Basophils (%)	0.20±0.13^{b}	1.00±0.00^{a}
MCV (fl)	114.10±5.34^{a}	110.70±7.45^{b}
MCH (pg)	39.01±1.75^{a}	36.75±2.27^{b}
MCHC(g/dl)	33.33±0.04	33.3±0.00^{NS}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

Table 4.13 presents the result showing the effect of genotype on the haematological parameters of laying pullets. Significant difference (P<0.05) was observed in the haematological parameters

of the two strains except in the monocytes, and mean corpuscular haemoglobin concentration (P>0.05). ISA Brown pullets were significantly (P<0.05) higher in PCV, Hb, WBC, RBC, Monocytes and Basophils than it Bovan Nera counterparts fed same diets. However, Lymphocytes, Neutrophils, MCV, and MCH were significantly higher (P<0.05) in Bovan Nera than ISA Brown pullets fed same diets

Table 4.14 Effect of Graded levels of Garlic dietary treatments X genotype on haematological parameters of laying pullets

Parameters	Strain Type	T_1 (0.00g)	T_2 (0.75g)	T_3 (1.5g)	T_4 (2.25g)	T_5 (3.00g)
PCV (%)	BN	21.50±0.50^{c}	24.50±0.50^{b}	26.75±0.25^{b}	24.25±0.25^{b}	21.50±0.50^{c}
	IB	26.50±0.50^{b}	29.50±0.25^{a}	24.50±0.50^{b}	23.50±0.50^{bc}	30.25±1.75^{a}
Hb (gldl)	BN	7.25±0.25^{a}	8.42±0.09^{ab}	9.25±0.25^{a}	8.25±0.25^{ab}	7.25±0.25^{b}
	IB	9.25±0.25^{a}	9.83±0.17^{a}	7.75±0.25^{b}	7.58±0.08^{b}	10.33±0.33^{a}
WBC X10^{3}/mm^{3}	BN	10,350.00±50.00^{a}	4,850.00±50.00^{cd}	4,500.00±100.00^{d}	4,925.00±25.00^{c}	4,500.00±100.00^{d}
	IB	5,050.00±50.00^{b}	5,775.00±25.00^{bc}	6,050.00±50.00^{b}	5,100.00±100.00^{c}	5,650.00±150.00^{bc}
RBC X10^{6}/mm^{3}	BN	2.25±0.25^{bc}	2.70±0.10^{a}	2.30±0.10^{b}	2.10±0.10^{c}	1.75±0.25^{c}
	IB	3.00±0.00^{a}	2.25±0.25^{bc}	2.45±0.05^{b}	2.65±0.05^{ab}	2.75±2.50^{a}
Plasma Protein (gldl)	BN	7.25±0.25^{a}	5.75±0.25^{b}	6.00±0.00^{b}	5.75±0.05^{b}	4.80±0.00^{c}
	IB	5.25±0.05^{b}	6.45±0.05^{ab}	7.00±0.00^{a}	6.90±0.10^{a}	5.70±0.30^{b}
Neutrophils (%)	BN	51.00±1.00^{b}	50.50±0.50^{b}	54.50±0.50^{b}	51.50±0.50^{b}	56.50±1.50^{ab}
	IB	50.00±0.00^{b}	51.00±0.00^{a}	52.00±0.00^{b}	55.50±0.50^{ab}	59.00±1.00^{a}
Lymphocytes (%)	BN	41.00±1.00^{ab}	45.00±1.00^{a}	40.00±0.00^{ab}	41.00±1.00^{ab}	39.00±1.00^{b}
	IB	40.50±0.50^{ab}	41.00±1.00^{ab}	45.00±0.50^{a}	37.00±1.00^{b}	39.00±1.00^{b}
Monocytes (%)	BN	2.00±0.00^{a}	2.00±0.00^{a}	1.00±0.00^{b}	2.00±0.00^{a}	2.00±0.00^{a}
	IB	2.00±0.00^{a}	2.00±0.00^{a}	0.00±0.00^{c}	2.00±0.00+	2.00±0.00^{a}
Eosinophils (%)	BN	5.00±0.00^{b}	2.00±0.00^{c}	4.00±0.00^{b}	4.00±0.00^{b}	2.00±0.00^{c}
	IB	6.50±0.00^{a}	4.00±0.00^{b}	2.00±0.00^{c}	5.50±0.50^{ab}	0.00±0.00^{d}
Basophils (%)	BN	1.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	IB	1.00±0.00^{a}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
MCV (fl)	BN	102.50±2.50^{b}	89.89±0.61^{a}	122.73±0.00^{a}	122.90±2.90^{aa}	132.41±8.26^{a}
	IB	117.50±27.50^{ab}	122.50±22.50^{c}	120.00±20.00^{a}	95.09±9.90^{bc}	98.41±8.26^{bc}
MCH (pg)	BN	34.50±0.50^{b}	29.88±0.12^{c}	40.91±0.00^{a}	40.43±0.43^{a}	44.33±2.33^{a}
	IB	37.79±7.79^{b}	41.67±6.67^{a}	39.42±6.08^{ab}	31.96±3.57^{bc}	32.93±2.63^{bc}
MCHC (gldl)	BN	33.33±0.00^{a}	33.34±0.02^{a}	33.33±0.00^{a}	33.32±0.02^{a}	33.33±0.00^{a}
	IB	33.33±0.00$_{a}$	33.32±0.01^{a}	33.33±0.00^{a}	32.01±0.09^{a}	33.33±0.00^{a}

a, b, cFor each parameter, results with different superscript letter, differs statistically (P<0.05)

The interaction effect between graded level of garlic and strain of pullets on PCV, RBC, WBC, Plasma protein, Neutrophils, Lymphocytes, Eosinophils, MCV and MCHC were significant

(P<0.05) while there was no significant (P>0.05) effect of dietary treatment and strain on Monocytes, Basophils and MCHC.

ISA Brown pullets fed diet T_5 had significantly (P<0.05) higher PCV (30.25±1.75) and Hb (10.33±0.00) than Bovan Nera fed the same diet T_5 (21.50±0.50) PCV and Hb (7.25±0.25). ISA Brown pullets fed dietary T_1 and T_2 follow the same pattern while Bovan Nera fed same diet T_1 and T_2 were lower (P<0.05).

Table 4.15: Effect of Garlic Dietary Treatments on Serological parameters of Laying Pullets

Parameters	**T1(0.0%)**	**T2(0.75%)**	**T3(1.5%)**	**T4 (2.25%)**	**T5(3.00%)**
Cholesterol (mg/dl)	179.68±2.24^{a}	175.95±0.90^{ab}	173.13±0.43^{bc}	170.85±1.44^{cd}	166.75±2.31^{d}
Total Protein (g/dl)	4.06±0.01^{d}	4.25±0.07^{c}	4.34±0.13^{b}	4.40±0.10^{b}	4.57±0.09^{a}
Albumin (g/dl)	1.96±0.01^{c}	1.96±0.01^{b}	1.99±0.01a^{b}	2.01±0.01^{a}	2.04±0.23^{a}
Globulin (g/dl)	2.20±0.04^{d}	2.29±0.06^{c}	2.39±0.12b^{c}	2.39±0.10^{b}	2.54±0.09^{a}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

The effect of dietary treatments on the biochemistry parameter of laying pullet is presented in table 4.15. The result obtained revealed that increase in garlic treatment cause progressive increase in the biochemical parameters, Total of Protein, Albumin of the bird and globulin cholesterol decreases with increase level of garlic powder from T1(179.68±2.24), T2 (175.95±0.90), T3 (173.13 ± 0.43) T4 (170.75 ± 1.44), T5 (166.75 ± 2.31) respectively. Birds fed T1 (control) consistent had significant lower (P<0.05), total protein, Albumin and globulin than the dietary treatment T2, T3, T4 and T5 respectively.

Table 4.16: Effects of Genotype on Serological parameters of laying pullets

Parameters	Bovan Nera	ISA Brown
Cholesterol (mg/dl)	173.29±0.82	173.21±2.33^{NS}
Total Protein (g/dl)	4.19±0.45^{b}	4.45±0.08^{a}
Albumin (g/dl)	1.98±0.18	1.98±0.02^{b}
Globulin (g/dl)	2.21±0.03^{b}	2.49±0.05^{c}

abc means on the same row having different superscripts are significantly different (P<0.05
NS means not significantly different (P>0.05)

Table 4.16 shows that cholesterol and Albumin were not significantly (P>0.05) influenced by genotype. Total protein and globulin of ISA Brown users observed to be significantly (P<0.05) higher than Bovan Nera counterpart.

Table 4.17 Effect of Garlic Dietary Treatments X Genotype on Serological parameters of laying pullets.

Parameters	Strain Type	T_1 (0.00g)	T_2 (0.75g)	T_3 (1.5g)	T_4 (2.25g)	T_5 (3.00g)
Cholesterol (mgldl)	BN	176.45±0.45^{b}	174.50±0.50^{b}	173.25±0.25^{b}	173.00±0.00^{b}	169.25±1.25^{bc}
	IB	182.90±3.00^{a}	177.40±0.60^{b}	173.00±1.00^{b}	169.00±1.50^{bc}	164.25±4.25^{c}
Total protein (gldl)	BN	4.06±0.01^{a}	4.13±0.02^{a}	4.12±0.01^{a}	4.23±0.03^{a}	4.43±0.08^{a}
	IB	4.05±0.03^{a}	4.37±0.04^{a}	4.56±0.04^{a}	4.58±0.03^{a}	4.72±0.04^{a}
Albumin (gldl)	BN	1.91±0.00^{a}	1.95±0.00^{a}	1.98±0.02^{a}	2.01±0.02a	2.05±0.05^{a}
	IB	1.89±0.01^{a}	1.97±0.02^{a}	2.02±0.02^{a}	2.02±0.02^{a}	2.02±0.02^{a}
Globulin (gldl)	BN	2.15±0.02^{c}	2.19±0.02^{c}	2.14±0.02^{c}	2.22±0.01^{c}	2.38±0.03^{bc}
	IB	2.25±0.07^{c}	2.40±0.02^{bc}	2.54±0.03^{b}	2.56±0.01^{b}	2.69±0.03^{a}

a, b, c; a, b, cFor each parameter, results with different superscript letter, differs statistically (P<0.05)

Table 4.17 showed the effect of genotypen and garlic treatment on Serum biochemical parameters of pullet birds.

The effect of interaction between garlic treatment and genotype of pullets on cholesterol and globulin were significantly (P<0.05) different while no significant (P>0.05) effect between garlic treatment and genotype on total protein and Albumin. Bovan which consumed diet T_4 (173.00±0.00) had higher (P<0.05) cholesterol than ISA brown fed same diet T_4 (169.00±1.50). ISA Brown fed control diet T_1 had significantly (P<0.05) higher cholesterol than Bovan Nera fed control diet.

ISA Brown pullet which consumed diet T_2 (38.15±0.82) and T_4 (37.96±1.31) had significantly (P<0.05) higher yolk index than Bovan Nera fed same diet T_2 (37.98±0.77) and T_4 (35.54±0.71).

Effect of graded levels of garlic on haematological parameters of Bovan Nera pullets.

Table 4.14 showed the effect of graded level of garlic on haematological parameters of Bovan Nera pullets. There were significant (P<0.05) effect of treatment on PCV, HB, WBC, RBC, Plasma Protein, Neutrophils, Lymphocytes, Monocytes, Eosinophils, MCV and MCHC while no significant (P>0.05) effect of treatment means on Basophil and MCHC. Birds fed diet (T_3) had significantly (P<0.05) higher packed cell volume and Haemoglobin concentration than pullets fed T_1, T_2, T_4 and T_5. WBC of birds fed T_1 control (10,350.00±50.00) had significantly (P<0.05) higher value than birds fed dietary treatment T_2, T_3, T_4 and T_5 which had similar WBC value (P>0.05) (4,850.00±50.00, 4,500.00±100.00, 4,925.00±25.00 and 4,500.00±100.00) respectively. RBC of pullets fed diet T_2 (2.70±0.10) was not significantly (P>0.05) different from RBC of pullets fed dietary treatments control T_1 (2.25±0.25) and T_3 (2.30±0.10) but had significantly (P<0.05) higher Red Blood Cell counts (RBC) than birds fed dietary treatments T_4 (2.10±0.10) and T_5 (1.75±0.25) respectively which had similar value (P>0.05).

Bovan Nera pullets fed control diet (T_1) (7.25±0.25) has significantly (P<0.05) higher plasma protein than pullets fed T_2 (5.75±0.25), T_3 (6.00±0.50) and T_4 (5.75±0.05) respectively. Birds fed diet T_5 (4.80±0.00) had significantly (P<0.05) the least plasma protein. The neutrophils of birds fed T_3 (54.50±0.00) and T_5 (56.50±1.50) were significantly (P<0.05) higher than birds fed control (T_1) diet, (51.00±1.00), T_2 (50.50±0.50) and T_4 (51.50±0.50) which had similar (P>0.05) values.

Bovan Nera birds fed diet T_2 (45.00±1.00) had significantly (P<0.05) higher lymphocytes than birds fed dietary treatment T_1 (41.00±1.00), T_3 (40.00±0.00), T_4 (41.00±0.00) and T_5 (39.00±1.00) had the least significant (P<0.05) lymphocyte value.

Monocytes of birds fed diet T_1, T_2, T_4 and T_5 was no significantly (P>0.05) different while birds fed diet T_3 had the least (P<0.05) monocyte value compared to birds fed T_1, T_2, T_4 and T_5 respectively.

Birds fed control T_1 diet had significantly ($P<0.05$) higher Eosinophils than birds fed T_2, T_3, T_4 and T_5 respectively. Birds fed diets T_2 and T_5 had the least ($P<0.05$) Eosinophils value.

MCV and MCH of Bovan Nera birds fed diet T_5 (132.50±7.50, 44.33±2.33) had significantly ($P<0.05$) higher MCV and MCH than Bovan Nera pullets fed diet T_1 (102.50±2.50), T_2 (89.89±0.61), T_3 (122.73±0.00) and T_4 (122.90±2.90) respectively. Bovan Nera pullet fed diet T_2 had significantly ($P<0.05$) the least MCV (89.89±0.61) and MCH (29.88±0.12) than birds fed diet T_3 and T_4 which had similar ($P>0.05$) values.

Effects of Garlic treatment on PCV of Bovan Nera and ISA Brown pullets

Bovan Nera pullets which consumed control T_1 diet had significantly ($P<0.05$) higher WBC counts than ISA Brown that consumed the same dietary treatment. However, ISA Brown pullet fed dietary treatments T_2, T_3, T_4 and T_5 were significantly ($P<0.05$) higher than Bovan Nera that consumed the same diet T_2, T_3, T_4 and T_5 respectively. The RBC of ISA Brown pullet fed control diet was significantly ($P<0.05$) higher than Bovan Nera fed same control diet.

4.2 DISCUSSION

4.2.1 Proximate Composition

The present results of proximate composition of garlic and the feed presented in Table 4.1. revealed that the moisture content (3.20%) was lower than the value (3.41%) reported by Omer *et al*., (2019), (5.52%) by (Muhammed and Idris (2019), and Yusuf *et al.,* (2018) report moisture content of (4.55%). The moisture content of protein concentrate (9.88%) was higher than (3.05%) reported by Omer *et al.,* (2019), Odebunmi *et al.,* (2009), Otunola *et al.,* (2010) and Sajid *et al* (2014) who reported moisture range of 3.26 - 3.55%.

Ash content and crude fibre were (2.10% Ash, 1.75% crude fibre) and protein concentrate. The ash (4.59%) and crude fibre (5.78%) results recorded were higher than ($P<0.05$) the Ash

(05.85%) and crude fibre (03.96%) reported by (Muhammed and Idris 2019), 0.75% crude fibre reported by Yusuf *et al*., (2018). However, the crude fibre range 2.13 – 2.64 reported by Lawal *et al.,* (2018) was higher than the value recorded in this study.

The low moisture content observed would help to prolong the shelf-life of *garlic* powder and discourage the growth of microorganisms. This agrees with the findings of Adeyeye and Ayejuyo (1994) and Lawal *et al.* (2018) that high moisture content of garlic powder is susceptible to microorganism and reduction in shelf-life.

The Ether extract (1.45%) and crude protein (17.66) result obtained were higher than the Ether extract (0.85%) and crude protein (8.37%) reported by Omer *et al.,* (2019), (16.23%) crude protein reported by (Muhammed and Idris 2019) and (17.08%) crude protein reported by Fajemiletin and Alamuoye (2019). The crude protein of the feed (23.31%) was lower compared to 45.00% value reported by Omer *et al.,* (2019).

Nitrogen free extract value (71.84) recorded was higher than the value 66.00% reported by (Muhammed and Idris 2019) but lower compared to the report of Yusuf *et al.,* 2018 and Omer *et al.,* 2019 (73.22% and 82.55%) respectively. The Nitrogen free extract value of protein concentrate (51.89%) obtained in this study were higher than (43.27%) value reported by Omer *et al.,* (2019). Dry matter (96.80%) of garlic was also higher than (33.43%) and (96.45) reported by Odebunmi *et al.,* 2009 and Omer *et al.,* 2019 while the value (90.12%) recorded in this study was lower compared to (92.72%) reported by Omer *et al.,* 2019.

The presence of moisture content, ash, ether extract and crude protein is an evidence that garlic may be useful for body building and prevention of infection. While high crude fibre content will help in bowel movement (Muhammed and Idris, 2019). The presence of ash content in both *Allium sativum* and feed concentrate is an indication of the minerals element preserved in any food materials (Iniaghe *et al.,* 2009). The high crude protein recorded in this present study is an

indication that garlic powder is a rich spice source needed by farm animal for growth and development of tissues.

4.2.2 Phytochemical Composition

Phytochemicals give plants their colour, flavor, smell and are part of a plant natural defense system against herbivorous insect and invertebrates, fungi, pathogens and parasite (Ibrahim *et al.,* 2010). The result of the phytochemical screening of aqueous extract and ethanlonic extracts on garlic are presented in Table 4.2.1 and 4.2.2

It was observed that flavonoids, Tannins, Alkaloids are steroids are present while saponin was absent. This agrees with Gazuwa *et al.,* (2013) findings that reported absence of saponin in garlic. This however, disagreed with the finding of Akeem *et al.,* (2016), Yusuf *et al.,* (2018), Muhammed and Idris (2019) who observed the presence of saponin in garlic bulb and powder.

The absence of saponin in this present study could be attributed to genetic and climatic factors rather than storage time, processing and extraction method. Akeem *et al.,* (2013).

The presence of these essential phytochemical substances support the observation of Pandey (1980) that plants contain vital chemical substance (Alkaloid, tannin, glycoside and other quantitative phytochemical analysis of aquaeous and ethanolic extract of garlic powder Table 4.2.2.

Flavonoids was the most abundant phytochemical value (7.40), followed by alkaloid (1.80), tannin (0.24) and steroid (0.21). The abundant of flavonoids recorded in this present study conformed with the findings of (Prohp and Onoagbe, 2012) who reported high level of flavonoid. According to Okwu (2001), flavonoids prevent oxidative cell damage and also have strong anticancer activity. It also helps in managing diabetes induced oxidative stress. The value of alkaloid (1.8, 0.95), flavonoid (7.4, 6.8), Tannin (0.24, 0.00) and steroid (0.21, 0.00) of garlic with ethanoic and aqueous extract observed in this present study were found to be higher

compared to the value reported by Akeem *et al.,* (2018), Alkaloid (0.84, 0.55), flavonoid (1.63, 1.13), Tannin (0.82, 0.08) and steroid (0.69, 0.03).

The important of tannins in promoting wound healing has been documented by (Okwu and Josiah, 2016) (Iwu, 1983) also reported that tannin possess anti-diabetic properties. Steroids are important compound that possess sex homones and are used to produce drug such as female contraceptives pills (Okwu, 2001).

The present results on phytochemical component of garlic supports the study conducted by Akeem *et al* (2018) who found that garlic and selected spices contained natural antioxidants which may be accountable for their use in treating infection, curing certain diseases and ingredients in traditional medicine.

4.4 Effect of Garlic Dietary Treatments on Performance Characteristics of Laying Pullets

The result showed positive effect of garlic powder on the performance characteristics of laying pullets except the weight of first egg which was not affected by dietary garlic inclusion. The result of final body weight and weight gain values observed agreed with Ozturk *et al*., (2021), Milosevic *et al.,* (2013) also reported positive effect of garlic on body weight and body mass of broiler fed 1.5kg up to 3% garlic powder. The weight gain of pullet layer fed diet T4 (2.25% garlic inclusion) was higher than the control (T1, 0.0%) and other dietary treatment. This finding is in agreement with that of Ramial *et al.,* (2014) who recorded higher (P>0.05) weight gain in dietary treatment than the control of broiler chicken fed basal diet of 0.5% garlic powder. Khan *et al* (2007) also reported higher weight gain for birds fed garlic powder than the control. Feed intake increased with garlic powder from 0.0% to T3 (1.5%)) but decreases with further addition of garlic powder. This result contradicts the findings of Khan *et al.,* (2007) who reported increase in feed consumption from 0% to 8%, it was observed that weekly weight gain and feed consumption were influenced by garlic inclusion. Gureshi *et al.,* (1983), Chowdhury *et al.,* 2002)

reported that feed consumption, feed efficiency and egg production of Deri laying hens were not influenced by garlic powder. The variation showed could be due to the difference in garlic proportion as in fed supplement.

The result on pullet fed dietary treatment T3 (1.5% garlic) had significantly ($P<0.05$) higher intake than control (T1 0.0%). This is similar to the report of Asirat *et al.,* (2018) that 1%, 2% and 3% garlic powder inclusion increased the fed consumption at higher levels of dietary treatment in laying hens. This could be attributed to the rich aromatic oil content of garlic which enhance digestion Asirat *et al.,* (2018) Adibmoradi *et al.,* (2006) also reported that garlic administration enhanced villus height and crypt death and decrease epithelial thickness, global cell numbers in duodenum jejunum and ileum of birds and body weight at first egg (1527.71±59.60) and (1489.02 ± 25.05) recorded by (Jesuyon, 2011) were higher that the result of the present study (1287.28±8.60) and (40-145.25±) – (1311.38±8.51). The mean age of first egg recorded in this experiment is lower in all the treatments than the mean age at first egg of Bhiyan *et al.,* ((2005) for white leghorn who reported a higher days (175.00 days) Deshi breed were reported to have 0 ± 8 days a lower age at first egg (120±9-124.0 ± 8 days) at a treatment 19^{o}C (Jesuyon, 2011). Also Farooq *et al.,* (2015) recorded a lower age at first egg (126.0 ± 1.02 days).

The variations in age at first age could be attributed to geographical location, environmental factors, (temperature relative humidity) effect of diets and breeds of layers used. Result on FCR of laying pullet Table 4.5 contrast the report of Chuowdhury *et al.,* (2002), Asirat *et al.,* (2018) who observed no significant effect on growth, feed intake, FCR and feed efficiency of laying birds. The birds on control T1 and those fed T2 (1.5%) garlic powder were significantly ($P<0.05$) higher than those fed T2, T4 and T5 respectively.

The result of percentage mortality among control and birds fed dietary garlic powder were higher in control T1(0.0%), T2 (1.0%), T3 (1.25%) and T4 (2.25) but lower in T5 (3%). This results is

similar to the report of Asrat *et al.,* (2018). In control Yalcin *et al.,* (2006) and Adebiyi *et al.,* (2018) reported that garlic powder supplementation has no effect on mortality in laying hens. The decrease in mortality of laying pullet fed garlic powder containing diet T5 (3.0%) in the present study could be attributed to the antiphrastic action of garlic (Fenwick and Hanley, 1985).

4.5 **Effect of garlic dietary treatments X Genotype on Performance Characteristics Of Laying pullets**

The result showed that Bovan-nera gained more weight after 16 weeks of experiment than ISA Brown pullets at T1 (control), while ISA Brown progressively gained more weight as the garlic inclusion increased especially at T4 (2.25%). The result also showed great significant ($P<0.05$) effect in the weight gain between the two strains at T4 (2.25%) garlic inclusion. This agrees with the report of Okwu *et al.,* (2001). This may be due to strain factor between the two layer pullets.

The age at first egg between the two pullets showed that ISA Brown started laying earlier than Bovan Nera at T1 (0.0%) garlic inclusion and Bovan nera's age at first egg reduced as the garlic inclusion increased except at T5 (3.00%) where both Bovan nera and ISA Brown age at first egg increased.

The inclusion of garlic reduced mortality rate in both strains of pullets but ISA-Brown performed better than Bovan nera at all levels of inclusion

4.6 Effect of Garlic Dietary Treatments on External Egg Characteristics of Laying Pullets

Eggs are an inexpensive source of high-quality protein, essential vitamins and minerals that are needed for a healthy diet and healthy life (Khalid, 2015). Thus improvement in egg quality is of paramount important in the field of production and management (Mahmoud *et al./,* 2010) Yalcin *et al.,* (2006) noted that egg weight increase when laying hens were fed 5g/kg and 10g/kg garlic powder. Carogulloris *et al.,* (2010) also reported that egg production increased significantly ($P<0.05$) by adding 1% garlic powder in the feed of laying hens. This study showed an increase

in egg weight of laying pullet. Feed garlic dietary treatment T2 (0.75) but decreases with increase levels of garlic T3 (1.5%) T4 (2.25) and T5 (3.0%) respectively. This findings supported the report of Omer *et al.*,(2019). Sakine and Onbasitar (2006) observed increase egg weight with dietary garlic powder supplementation. In contract Safaa (2007), Khan *et al.,* (2008) and Chowdhury *et al.,* (2002) reported that egg weight was not affected by 0, 2, 6 or 8% garlic powder.

The results on egg length, egg width and shell thickness agree with the report of Canogullari *et al.,* (2010) and Omer *et al.,* (2019). In contrast, the egg volume and shell weight values Table 4.6 does not support Adebiyi *et al.* (2018) findings who reported that the egg weight, yolk index, shell weight shell thickness and yolk weight were not affected by 1% garlic powder fed to laying hens. These differences in result could be from the use of different garlic source, method of garlic powder preparation, and age of layers used in the experiment..

4.7 Effect of Genotype on External Egg Characteristics of Laying Pullets

The present result shows that Bovan Nera had significantly ($P<0.05$) higher mean egg weight (56.31 ± 0.35), egg length (4.41 ± 0.55) than ISA Brown pullets fed the same diet. This is in constrast with Jesuyon (2010) that reported higher egg weight (59.99g) in ISA Brown compared to Bovan Nera (56.20gm). Fajemilehin *et al.,* (2019) also report a higher egg weight for ISA Brown (62.50 ± 0.05) at 1% garlic powder inclusion. The variation in these results might be the differences in strain, age and or environmental condition.

4.8 Effect of Graded Level of Garlic treatment X Genotype on the External Egg quality Characteristic of Laying pullets

Table 4.6 present the effect of garlic treatment and strain type. Bovan Nera and ISA Brown fed dietary T_2 and T_3 were higher in egg weight, egg length and volume than it ISA Brown counterpart. The present result obtained in this experiment is similar to the report of Ibrahim *et al.,* (2020) who worked on three strains of laying birds. The observation in this present study for

Bovan Nera over ISA Brown pullet fed same varying level of garlic powder could be due to difference in the genetic attributes of the two strains. These observations are in agreement with the report of Curtis *et al.,* (1985) and (Oyeagu 2014) who worked on two strains of layers Nera Black and Shaver Brown Hens.

ISA Brown on the other hand fed dietary T_2 and T_3 were higher in shell thickness and shell weight than Bovan Nera fed the same garlic level. There is dearth literature concerning the interaction effect of garlic and strain type of Bovan Nera and ISA Brown on egg quality characteristics. The observed variation in egg shell weight according to Pelicia *et al.,* (2009) could be due to availability of calcium in their feed and possibly bioavailability of calcium and phosphorus.

4.9 **Effect of Dietary Garlic Treatments on Internal Egg Characteristics of Laying Pullets**

The result presented in Table 4.8 for internal egg quality characteristics agree with those reported by Yalcin *et al.,* (2006) that supplementation of garlic did not affects the egg albumen index, egg shell index and haugh unit values of laying hens fed diets containing up to 10g/kg garlic powder for 22 weeks. The non effect ($P>0.05$) of garlic on yolk weight, yolk height, yolk diameter, Albumen maximum diameter, Albumen height, Haugh unit and Yolk index observed in this present study could be that garlic powder does not have any negative effect on the laying pullet. Similar result was reported by Chowdhury *et al.,* (2002), Canogullari *et al.,* (2010) and Omer *et al.,* (2019) who noted that the supplementation of garlic powder did not affect egg production.

However, Yolk colour, Albumen weight and Albumen minimum diameter were significantly ($P<0.05$) influence by garlic powder inclusion with pullet fed T3 (1.25%) having the highest value Table 4.8. This could be the phytochemical constituent of garlic and it colour.

The mean result of yolk colour obtain in this study range from (6.38±0.28 – 6.95 ± 0.32). The colour table (4.8) decreases with increase level of garlic powder. The present yolk colour falls below the normal range according to (Lesson and Summers 1997) that the acceptable colour of yolk by consumer range from 7-8 (deeper yolk colour) in most area. Ao *et al.,* (2010) recorded increased yolk colour in laying hen fed 2%, 3% fermented garlic powder (10.55 0 10.65)/ However, the present result on yolk colour was higher than the value recorded by Asrat *et al.,* (2018) and Omer *et al.,* (2019) (2.5-3.38) and (4.5-5.0) respectively.

In contrast, Birrenkot *et al.* (2000) reported no differences in colour and flavours of laying hens which consume up to 5% garlic powder. The difference in value or colour of yolk could be attributed to the method of preparation of garlic, the breeds of laying hen use, the age of birds among others.

Mean yolk index value in this study ranges from 36.71±0.62-38.06±0.56 with birds fed diet T2 (0.75%) garlic powder having better yolk index. This result does not agree with Fajemilehin *et al.,* (2019) who recorded no change in yolk index of ISA Brown fed garlic 1% - 2%.

4.10 Effect of Genotype on Internal Egg Quality Characteristics of Laying Pullets

The present study table 4.9 revealed that Bovan Nera had significantly ($P<0.05$) higher yolk diameter, Yolk weight, Albumen maximum and minimum diameter than it ISA Brown Counterpart. These findings are not in agreement with Oyeagu (2014) who reported no changes between strain and feed in all the internal egg parameter of Nera Block and Shaven Brown hens fed five different commercial feeds. This could be the effect of garlic in Bovan Nera and ISA brown compared to the five commercial feed used by the Oyeagu (2014). However, no significant ($P<0.05$) differences observed in yolk height, yolk colour, albumen weight and Albumen height between the two strains of similarity observed in the internal egg parasitic of Bovan Nera and Isa Brown could be that garlic powder is efficient and suggested stress was no

presence of toxic factor (such as haemoglobin) which has adverse effect on blood formation (Bello and Indo 2014, Oyawoya and Ogunkunle 2004).

In contrast to the present study, Oladele *et al.,* (2018) reported that garlic inclusion has no influence on PCV of laying hens. Etiagbo *et al.,* (2003) reported a lowder PCV value than those obtained in this experiment. DoMorunmtle and Best 2013) reported that haemocrit 9PCV) decreases in a variety of conversion conditions including lives, kidney, diseases, malnutrition, is tamin, B12, folic acid deficiency, from deficiency, pregnancy among others. The lower PCV obtained in birds fed T4 (2.25%) could be likened to the report by DeMoranvile and Best (2013). White Blood Cell counts obtained range from (5,075.00±3.00 – 7,700.00 ± 1520.25). Birds fed control had the highest (WBC) than those fed dietary garlic powder. The WBC decreases with increase level garlic (T2-T5) Tables 4.10. In contrast, Akanbi *et al.,* (2020) reported an increase in WBC count of laying birds fed garlic level 1% - 3% and ginger. The WBC value ranges from (3.85mg/dl – 7.58mg/dl). The RBC value recorded in this study decreases with increase garlic inclusion T1 (2.63) – T5 (2.25) x 10^6/mm^3 and are lower than the values recorded by Akanbi *et al.,* (2020) T1 (2.27) – T4 (3.48) mg/dl. The Haemoglobin concentration obtained for pullet fed diet T2 (0.75% garlic powder level were within the normal range 9-13g/dl reported by (Mitruka and Rawnsley 1997) attributed to the fact that those exotic birds are commercially hybrids that have been selected over many generation from interbreeding between specialized breeds, strains and lines. Duduyemi (2005) and Oyeagu (2014), the mean Haugh Unit (95.52±0.29) recorded in the study for ISA Brown is higher than Bovan Nera and the Haugh unit (76.12 ± 0,07) and ISA Brown layering hens reported by Fajemitehin *et al.,* (2019). In line with the finding observed on higher Haugh Unit recorded for ISA Brown in this study, Ahmad *et al.,* (2019) reported that higher haugh unit indicates that ISA Brown had better Albumen quality compared to Bovan Nera. This result is also in agreement with the findings of Dunga (2013) who found variation in

Haugh unit scores between Naked Neck and Arseel Chickens However, Rajkumar *et al.,* (2009) did not report any significant Haugh unit differences among chicken genotype.

4.11 Effect of Dietary Treatments X Genotype on the Internal Egg Quality Parameters of Laying Pullets.

The result of the present study on dietary treatment and strain type on the internal egg quality characteristics is shown in Table 4.7. There was no significant interaction on strain and dietary treatment in Yolk diameter, albumen maximum diameter, Albumen minimum diameter, Albumen height and Haugh unit of Bovan Nera and ISA Brown.

This result is similar to the earlier report of Yalcin *et al.,* (2006). That supplementation of garlic had no effect on egg albumen, index, egg haugh unit when laying hens fed diet containing 5% and 10%g/kg garlic powder for 22 weeks. The similarity observed in the internal egg parameter of Bovan Nera and ISA Brown pullet in this study could be attributed to the fact that these exotic birds are commercial hybrid that have been selected over generation for interbreeding between specialized breeds, strains and lines Oyeagu (2014)

Shell thickness is an important bioeconomic trait in commercial egg production as it may help to reduce the percentage of cracked eggs. It is a trait of commercial importance as thick eggs and strong shell are usually the most marketable, Abera *et al.,* (2010). The study revealed that, ISA Brown fed dietary T_1, T_2, T_3, T_4 and T_5 had thicker shell than it Bovan Nera counterpart fed same dietary treatment.

The interaction effects of garlic powder and strains type are presented in Table 4.7. The results this study showed that Bovan Nera fed dietary treatment T_2, T_3, T_4 and T_5 had significant higher yolk height, yolk diameter, yolk colour, yolk weight, Albumen maximum and minimum diameter, than it ISA Brown counterpart fed same dietary treatments.

The variation in yolk height, yolk colour, yolk weight and yolk index with effect of treatments and strain in this study is in agreement with the report of Kumar *et al.,* (1971) that variation due to genotype do occur with respect to yolk parameters.

Bovan nera pullet fed with T_2, T_3 and T_4 recorded higher yolk colour than ISA Brown pullets fed same diet. This is in line with the report of Safara (2007) and Asrat *et al.,* (2018) who reported increase in yolk colour, yolk weight and haugh unit with 20% garlic inclusion. In contrast, Birrenkot *et al.,* (200) reported no difference in yolk colour and flavor of egg hens consuming up to 3% dietary garlic powder.

4.12Effect of Garlic Dietary Treatments on Haematological Parameters of Laying Pullets

The results of mean PCV (Packed Cell Volume) obtained in this study table (4.10) which ranged from (24.00 ± 1.47 – 26.88 ± 1.39) are within the normal range for birds as reported by (Mitruka and Rawsley 1981). Birds fed dietary T2 (0.75%) garlic powder had the highest PCV. The normal values obtained in this experiment is an indication of normal range reported by (Mitruka and Rawsley 1981) (102-129), MCV, MCH (31.9 – 40.7) and MCHC (25.9-33.9) respectively.

The value of PCV, Hb (Haemoglobin concentration) RBC and EBC had significant influences across all treatments, which implies that the experiment diets, at the levels of inclusion could not be tolerated without compromising the welfare an immunity of the birds. Akanbi *et al.,* (2020). However, Akinola and Egwanumku (2017) reported that Red pepper had no significance effect on Hb, PCV and RBC of laying hens. These differences could be as a result of different species of birds, addictive and or environment conditions.

The differential leucocytes count were significantly ($P<0.05$) influenced by garlic inclusion except basophil. This is not in agreement with other researcher (Elagib *et al.,* 2015), Xeryehun *et al.,* (2017), Yang, *et al.,* 2007) who reported that garlic powder has no effect on differential Leucocytes (Neutroph. Monocytes, Lymphocytes) in white Leghorn fed garlic powder. The decrease in the Hb, PCV, RBC and WBC could be attribute of stress condition one of which is

temperature Ukanu and Pantaya (2018). Chineke *et al.*, (2006) reported that apart from genotype, age, sex differences in haematological indices may be could nutritional, environmental and hormonal factor.

The mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and MCHC (Mean Corpuscular Haemaglobin Concentration) falls within the normal range reported by (Mitruka and Rawsley, 1981) (102- 129), MCV, MCH (31.9 – 40.7) and MCHC (25.9 - 33.9) respectively. The slightly variation among treatments groups in blood parameters obtained in this present study affirms the fact that diets affect blood profile of poultry birds . (Adeyemo and Sanni.

4.13.Effect of Genotype on Haematological Parameters of Laying Pullets

Haematological examination is among the methods which may contribute to the detection of some changes im health and physiological conditions which may not be apparent during physical examination but it affects the animal fitness. Bamishaye et al.,(2009).

The result on genotype on haematological parameter in the present experiment showed that ISA Brown had significantly better ($p<0.05$) PCV, Hb, RBC,WBC, Eosinophils and Basophils than Bovan Nera fed same diets. On the other hand, Bovan Nera had higher MCV and MCH than ISA Brown counterpart.

The differences in the haematological parameters observed between the two genotype could be attributed to differences genetic content, responsiveness to dietary meal, climatic factors such as temperature, relative humidity, among other things. This is in agreement with the report of Esonu et al.,(2009) and Iheukwuemene et al., (2008) that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environment which include feeding and drugs administered.

Variations in blood parameters have been reported in animals due to several factors : age, attitude, management, feeding levels, sex, health status, method of blood of collection,

haematological techniques, diurnal and seasonal variations, ambient temperature and physiological status of the animal (Sherman and Many 1994), Forhead *et al.,* (2002).

The result of haemaological parameters obtained for both Bovan Nera and ISA Brown are within the record range (Mistruka and Rawnsley 1997) indicating that there was no adverse immune response due to the test ingredient. This findings is in line with Ebogbulem (2018)

The mean corpuscular Volume (MCV) Mean Corpuscular Haemglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) obtain for both strains in the present work, are within the normal range (Mitruka and Rawnsley 1981). Etim (2014) noted that normal range of value indicate that the vital physiological process has been maintained and were normal. Although Bovan Nera had significantly (P,0.05) higher MCV and MCH value than ISA Brown pullet No Significance difference (P<0.05) was obtained in Mean Corpuscular Haemoglobin concentration MCHC. The significance variation observed could be a reflection of inherent genetic differences amongst the two strain of laying chicken (Agail and Uko, 1998) Invboje et al., (2020) while the similarities observed may be a result of the traits that are highly conserved in both genotype.

Also (Egena and Aloa, 2012) reported that the variation in the harmatological indicator could be a way of delineating genetic variation in animals.

4.14 Interaction Effect of Garlic Dietary treatments X Genotype on Haematological Parameters of laying Pullets

Table 4.14 presents the interaction effect of garlic and genotype on haematological parameters of the experiment birds.

Significant differences (P<0.05) were obtained between treatment and strain in packed cell volume, haemoglobin concentration (Hb), White blood cell count, Red Blood Cell Counts, Plasma protein, Neutrophils, Lymphocytes, Mean corpuscular, volume and mean corpuscular

haemoglobin. No significance effect of dietary treatment and strain on monocytes, Basophils and mean corpuscular haemoglobin concentration in the present study.

ISA Brown pullet fed T_1, T_2 and T_5 had higher PCV, Haemglobin and RBC than Bovan Nera fed some diet. Packed Cell Volume, haemoglobin and Nuan corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes and are significant in the diagnosis of aenemia Peter *et al.,* (2011). They also serves as useful indices of the born marrow capacity to produce red blood cells in mammals Chineke *et al.,* (2006).

The White blood cells count of ISA brown pullet fed T_2, T_3, T_4 and T_5 were also higher than that of Bovan Nera except the control T_1 where Bovan Nera was higher table (4.17). The numerical increase observed in Hb, PCV, WBC and RBC of ISA brown birds fed dietary T_2 and T_5 garlic inclusion suggest that the diets were better utilized and assimilated into the blood streams for use by the birds. This observation is in agreement with the report of Zeryehun *et al.,* (2017) for White leghorn fed vary level of garlic.

The significant difference ($P<0.05$) observed in Neutrophils, lymphocytes and Eosinophils among the strain and dietary treatments in this study were not in agreement with the findings of Zeryhun *et al.,* (2017) who reported no significant difference in lymphocyte, monocyte and neutrophils of birds fed with garlic supplement. The present study revealed slightly increase in lymphocyte in Bovan Nera over ISA Brown and increase in Neutrophils of both Bovan Nera and ISA brown pullet fed vary level of garlic supplement agreed with the findings of Prasad *et al.,* (2009) who noted slight rise in lymphocyte and heterophil count observed in garlic supplement groups may be due to immune-stimulatory effect of garlic. Bovan Nera fed T_3, T_4 and T_5 had significant higher MCV and MCH than ISA Brown pullets fed same diets. The significant variation observed among the strains with varying level of garlic supplement could be a reflection of inherent genetic difference among two strains of laying chicken (Agaire and Uko 1998) while the observed similarity may be a result of the traits that are highly conserved in both

genotype. The observed variation in the haematological indicators could also be a way of delineating genetic variation in animals (Egena and Aloa, 2002).

4.15Effect of Garlic Dietary Treatments on Serological parameters of Laying Pullets

The result of serum biochemical influence of garlic is presented in Table 4.15. Total cholesterol decreases with increase level of garlic. This agrees with the report of El-Katcha *et al.,* (2006) who observed that serum total cholesterol concentration was significantly reduced by about 12.9%, 11.9%, 7.9% and 14.2% when garlic extract was supplemented at 0.1, 0.2, 0.3 or 0.4mg/kg diet respectively.

Khan *et al.,* (2007) also reported reduction in blood cholesterol in laying hens. This could be the present of Allicin in garlic as reported by Lawson (1998) that Allicine compound in garlic is responsible to health promotion and typocholesteronic benefit. Adebiyi *et al.,* (2018) reported reduction in serum cholesterol which agrees with the present study. The result of Total protein, Albumin and Globulin obtained increases with increase garlic powder inclusion from this finding is in line with other researcher El-Katcha *et al.,* 2016, Oladele *et al.,* (2018), Lee *et al.,* (2016) who reported increase in total protein and Albumin of laying hens fed garlic dietary treatment in contract, Zeryehen *et al.,* (2017) reported a decrease in total protein of laying hens with increase garlic powder from 0% - 30% with T1(0.0%) having the highest value (9.47 g/dl (T1) – 6.16g/dl (T4). Resrearchers have reported that the mechanism by which garlic reduces plasma cholesterol concentration is not fully understood. Some thought that garlic depressed lipogenic and choesterolgenic activities of the liver enzymes, fatty acids, synthase, methyl-glutanyl CoA (HMG- CoA).(Chowdhury et al.,2002, Canogullari et al., 2010, Mahmoud et al., 2010, Khan et al., 2012). The total protein (4.06 – 4.57) obtained in this study are within the normal ranges (2.5 – 4.5g/dl reported by (Mitruka and Rawnsley 1981). This indicates that garlic inclusion in diets can improve the protein level in laying birds.

The increased concentration of total protein may be explained by the very quick somatic plasma protein in bird, age of birds seems to be the most important factor. Higher value are generally found in adult birds compared to young birds. Silva et al., (2007). The findings of this work for increased Albumin and Globulin is in consonance with the report of Aikpitanyi and Egweh (2020).

4.16 Effect of Genotype on Serological Parameters of Laying Pullets

The serum biochemical profile of Bovan Nera and ISA Brown is presented in table 4.16. the mean values of Cholesterol and Albumin were not significantly (P>0.05) influenced by genetic grouping, however, Total protein and Globulin were higher (P<0,05) in ISA Brown pullets fed same diets. This is similar to the report of Ladokun et al., who reported no significant effect in cholesterol and albumin of Naked Neckand normally feathered. The present finding is in harmony with the report of earlier researchers, (Mitruka and Rawsley; El-Safty et al., 2006).

The significant difference in Total protein and Globulin between the two genotypes observed in this study could be as a result of fluctuations in environmental temperature (Donkoh, 1989). The value of Total protein obtained in this study (4.19- 4.45gl/dl) were higher than the values (3.4 - 3.8 gl/dl), reported by Albokhadaim *et al.,* (2012) but lower than the values (6.33 - 7.25gl/dl) reported by Nosike *et al.,* (2017).

The higher Globulin level in ISA Brown pullets aids in better cell-mediated immune response. This is in line with Ladokun *et al.,* (2008).

Serological parameters are important in the proper maintenance of the osmotic pressure between the circulating fluid and the fluid in the tissue spaces so that exchange of minerals between the blood and tissue cells could be facilitated. They also contribute to the viscousity and maintenance of normal blood pressure and Ph. Ladokun et al., (2008).

4.17 **Effect of Graded Level of Garlic treatments X Genotype on Serological Parameters of Laying Pullets**

Result of cholesterol, Total protein and Globulin in Bovan Nera and ISA Brown pullets Table (4.17) were significantly ($p<0.05$) influenced by dietary treatments. Increases level of garlic powder decreases the cholesterol level in both strains. This is in agreement with previous reports where dietary supplementation of garlic powder in broilers was found to cause significant ($P<0.05$) decreases in mean value of total cholesterol as compared to control bird. (Onyimonyi *et al.,* (2012); Abd El-Lafit *et al.,* (2013); Galib *et al.,* (2013); Hossain *et al.,* 2017). Higher polyunsaturated fatty acid like arachichonate and eicosapentenate in garlic could be responsible for preventing aheroscierosis (Issa and Omer 2012). This may be due to the possible mechanism of hypocholesterolemic and hypolipdemic action of garlic products which depresses the hepatic activities of lysogenenic and cholesterogenic enzymes such as malic enzymes, fatty acid synthase, glucose-6-phosphatase dehydrogenase (Chi *et al.,* 1982, Queshi *et al.,* (1983). Allicin also inhibit the action of HMG. COA (β-Hydroxyl, β-methyglutaryl-CoA) reductase, which is the most important enzyme that participate in the synthesis of cholesterol (Lawson and Bauwe, 1998). Oladele *et al.,* (2018) also reported decrease in cholestorel level in commercial layers chickens. Significant differences was not ($p>0.05$) observed Albumin among strain with effect of garlic treatments. ISA Brown pullet fed dietary T_2, T_3, T_4, and T_5, had significantly ($P<0.05$) higher total protein and Globulin Anan Bovan Nera fed same diets. There is increase in total protein and globulin with increase garlic in both Bovan Nera and ISA Brown. The result of this present study agreed with the findings of Oladele *et al.,* (2018) who reported higher total protein albumin, and globulin in commercial layers fed graded garlic inclusion than the control birds.

According to Doneley (2011) the liver is the site of protein synthesis including albumin and macroglobulins. Thus the higher level recorded in garlic group could indicate enhanced hepatic function Oladele *et al.,* (2018).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

In this study conducted to determine the response of Bovan Nera and ISA brown laying pullets with layers diets (control), (T1, 0.0%) and four other graded dietary treatments T2(0.75%), T3 (1.50%), T4 (2.25) and T5 (3.0%) garlic powder respectively. Seven experiments were conducted in this study.

i. The Proximate analysis of feed and garlic powder (Table 4.1)
ii. Phytochemical analysis (Table 4.2) with the presence of Flavonoid, tannins, Alkaloid and Steriods.
iii. Growth performance of two genotypes of laying pullet fed garlic powder.
iv. External egg quality characteristics of laying pullet fed garlic powder.
v. Internal egg quality characteristics of laying pullets fed garlic powder
vi. Haematological parameters of two genotypes of pullets fed garlic dietary treatment.
vii. Serum biochemical analysis of two genotypes of pullets fed garlic powder.

The results showed significance ($P<0.05$) differences were observed across the treatments and genotypes. Among the dietary treatments, laying pullets fed dietary T3 (1.5% garlic powder) and T4 (2.25% garlic powder) had significant ($P<0.05$) better growth performance in the final body weight gain, weight gain at 16 weeks, body weight at first egg, week feed intake, weekly weight gain per bird per day and fed conversion ratio. Although, mortality rate was higher in pullets fed T3 and T4 this can be attributed to the management practices or environmental factor.

The effect of garlic powder on external and internal egg quality parameters showed that laying pullets fed dietary garlic powder T2(0.75%) has the highest external egg quality; egg weight, egg

length, egg volume, shell weight, and internal egg quality characteristics, albumen weight, albumen diameter and haugh unit.

The Haugh Unit values obtained in this study ranges from (94.74-95.66) were within the normal range showing that, the albumen are in excellent condition and fresh.

Effect of genotype of the pullets on internal egg quality characteristics showed that Bovan Nera had better egg weight, egg length, egg volume than ISA Brown except; shell weight and shell thickness which was higher in ISA Brown than Bovan Nera.

Internal egg parameters of strain type also follow the same trend with Bovan Nera except for Haugh unit and Yolk index which was higher ($P<0.05$) in ISA Brown. The Haugh unit values obtain for the two strains were also within the normal range for albumen freshness, indicating that garlic has no negative influence on egg quality.

Results of garlic powder on hamatological parameters of the laying pullet were within the normal ranges by Mitruka and Rawnsley 1981 and other researchers. This shows that no negative influence of garlic on the Blood parameters of the laying pullets reared.

The haematological parameters of the experimental bird fed dietary T2 (0.75% garlic powder) and T3 (1.5% garlic powdr) had high packed cell volume (PCV), Haemoglobin concentration (Hb), plasma protein (PP), lymphocyte than other birds fed dietary treatment T1 (0,00%) and T2 (2.25%). For strain type, ISA Brown has higher PCV, Hb, WBC, (white blood cell), RBC (red blood cells, plasma protein, Eosinophil and Basophils than it counterpart Bovan Nera which consumed the same diet. This could be the genetic make-up of the strains, environmental factors and response of strain to stress among other factors.

Serum biochemical parameters also follow the same trend for treatments and breed type. The effect of garlic dietary treatment and strain interaction table results showed that Bovan Nera pullets which consumed control T1 diet (0.00%), T3 (1.5%), T4 (2.25%) and T5 (3.0%) garlic powder inclusion had significantly ($P<0.05$) higher final body weight gain, body weight at first

egg, feed intake, FCR and percentage mortality rate than ISA Brown pullet when consumed same diets T1, T3, T4 and T5 respectively.

Higher mortality could be variations in temperature, the external and internal egg quality characteristics were significantly ($P<0.05$) influenced by dietary garlic treatment and genotype. Bovan Nera pullets which consumed treatment T_2 (0.75g of garlic) and T_3 (1.5g garlic powder had significantly ($P<0.05$) higher egg weight, egg volume, egg width, egg length, among others than ISA brown pullets fed same diets. Shell weight, shell thickness and Albumen height were significantly ($P<0.05$) higher in ISA brown pullet fed dietary treatment T_2 and T_3 than Bovan Nera fed same diets. No significant ($P>0.05$) feed and strain type interaction in Albumen minimum and Haugh unit. Haematological content showed significantly ($P<0.05$) feed and strain interaction in PCV, Hb, WBC, RBC, plasma protein, Neutrophils, Eosinophils MCV (Mean Corpuscular Volume) and MCHC (mean corpuscular haemoglobin concentration) ISA Brown fed dietary treatment T_1 (control) T_2 and T_5 were significantly ($P<0.05$) higher in PCV, Hb, RBC and Eosionphils than Bovan Nera pullets fed same diets. WBC, MCV and MCH were significantly ($P<0.05$) higher in Bovan Nera fed T_1, T_3 and T_5 respectively. No significant ($P<0.05$) feed and strain interaction in monocytes, Basophils and MCHC.

The serum biochemical indices also showed significant ($P<0.05$) feed and genotype interaction in cholesterol, total protein and Globulin. No effect of feed and genotype interaction in Albumin. Cholesterol decreased with increased garlic addition while total protein and globulin for both genotypes ; Bovan Nera and ISA Brown increased. This could be the allicin compound present in the garlic.

5.2 Conclusion

The effect of garlic supplementation on egg quality and production performance of laying chickens have been widely studied but the information available on the basis of genotype and the issue of garlic level inclusions is an on going study. The results obtained from this study, under suitable conditions can be reported that the incorporation of garlic powder up to 1.5% can be safely utilised which improves the health, performance characteristics of egg quality traits and decreases blood cholesterol in laying pullets (Bovan Nera and ISA Brown).

i. It is therefore, concluded that Bovan Nera pullets fed garlic treatment performed better than ISA brown pullets fed same diets in this study in terms of growth performance, and egg quality production.

ii. Bovan Nera pullets were at their best with garlic level of T2 (0.75%) in egg quality characteristics (both external and internal egg quality) than its ISA Brown counterpart.

iii. ISA Brown had higher haematological and serum Biochemical parameters than Bovan Nera. Indicating that ISA Brown had higher immune system than Bovan Nera.

iv. This study has proven that the growth performance, egg quality, haematological and serum biochemistry of laying pullets can be improved with garlic inclusion without any adverse effect.

v. In order to have better quality egg products, I will suggest that Bovan Nera and ISA Brown should be utilized in cross breeding programmes.

5.3 Recommendations

In view of the genetic variability recorded on the response to garlic powder inclusions in diets of experimental pullets and its influence on growth performance, egg production and quality characteristics, haematological and serum biochemical constituent, it is recommended that:

i. that farmers should incorporate and adopt up to 1.5% garlic in compounding layers mash in feeding their poultry birds since its has positive health benefits and positive influence on production.

ii. Bovan Nera pullets should be used in poultry egg production because it has proven from this study to have higher genetic trait for fecundity, bigger egg size and can tolerate better, the climatic condition in the tropical region.

iii. Garlic powder has shown from this experiment that cholesterol content can be reduced with garlic inclusion up to 3.0% while increasing the protein content of the egg. It is therefore important that consumer with high cholesterol level can be advised to consume such eggs with garlic content.

iv. For maximum yield and performance in egg production in ISA brown pullets, garlic inclusion should not exceed 0.75%.

5.4 Contribution to Knowledge

This study has proven that:

i. 1.5% garlic powder diet inclusion resulted in optimal growth performance and egg production in Laying Pullets. Bovan Nera performed better than ISA Brown Pullets in terms of egg production.

ii. Haematological and serological parameters showed that the health status of the pullets were not adversely influenced.

iii. Garlic (*Allium sativum)* inclusion between 0.75% to 1.50% levels enhanced the internal egg quality characteristics of both strains of laying pullets.

iv. The study also showed that inclusion of garlic in the diet of the laying pullets improved the haugh unit values, reduced the cholesterol level in the serology and also reduced the percentage mortality of both strains of pullets used for the short-term production period. Hence, this is good news for our local poultry farmers and

consumers in terms of quality of products especially people with arteriosclerosis conditions.

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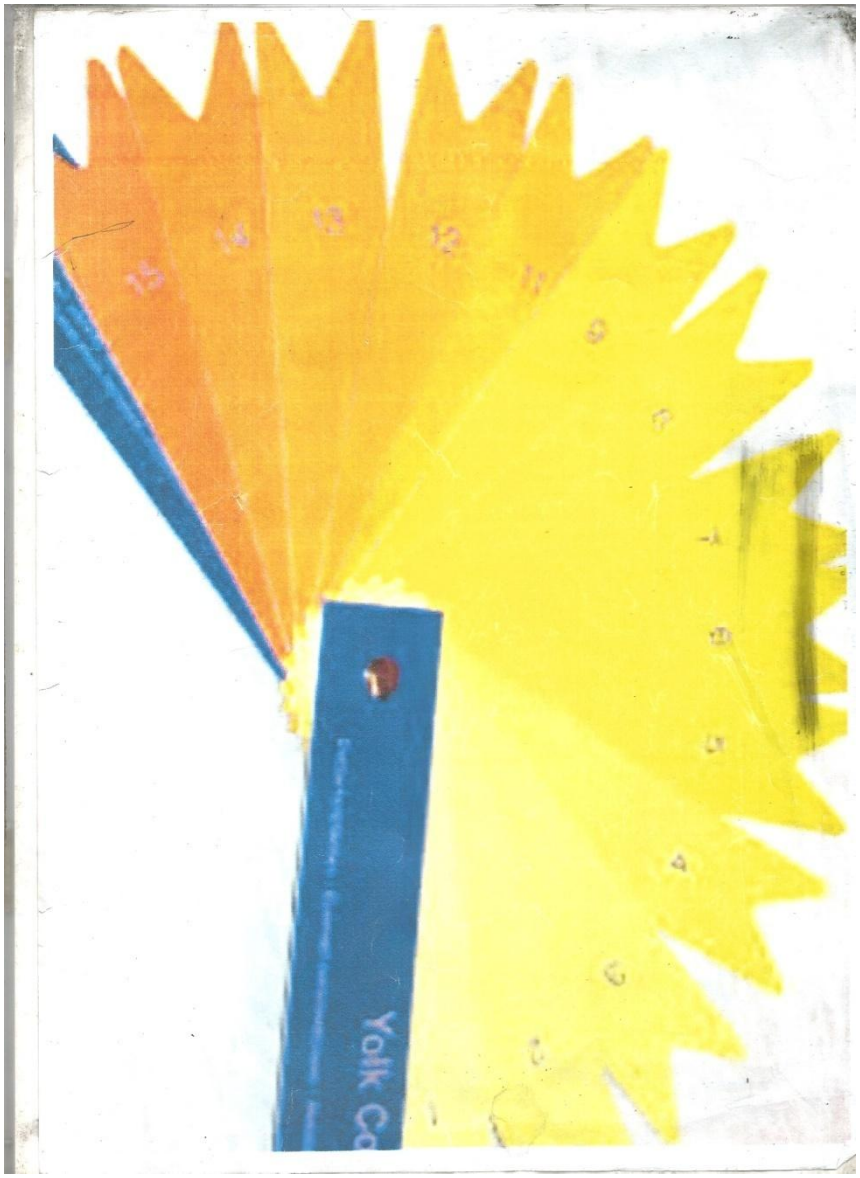
APPENDIX

Plate 1: Collection of Blood Samples for Haematology and Serology Screening

Plate 2: Stabilizing the birds on arrival in the pen at the experimental site

Plate 3: Vaccination of Birds

Plate 4: Weighing of Experimental Pullets



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