

**EFFECT ON NUTRIENT DIGESTIBILITY AND SERUM BIOCHEMICAL
PARAMETERS OF CHICKEN BIRDS FED DIETS CONTAINING BLACK PEPPER
(*Piper nigrum*) AND ZINC BACITRACIN**

A RESEARCH PROJECT

BY

BASSEY, PEACE EDEM

AK15/AGR/ANS/005

SUBMITTED TO

DEPARTMENT OF ANIMAL SCIENCE

AKWA IBOM STATE UNIVERSITY

OBIO AKPA CAMPUS

JULY, 2022

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**IN PARTIAL FULFILLMENT OF REQUIREMENT FOR THE AWARD OF
BACHELOR OF AGRICULTURE (B.AGRIC) IN ANIMAL SCIENCE**

JULY, 2022

DECLARATION

This project is authentic and original work carried out by me; **Bassey, Peace Edem** with the Registration Number **AK15/AGR/ANS/005** in partial fulfillment of the requirement for the award of Bachelor of Agriculture (B. Agric.) in Animal Science.

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CERTIFICATION

This is to certify that this research work "**Effect of Diets Containing Zinc Bacitracin and Black Pepper on Nutrient Digestibility and Serum Biochemistry of Broiler Birds**" is the original copy of research carried out by; **Bassey, Peace Edem** with the registration number; **Ak15/AGR/ANS/005**, in the Department of Animal Science, Faculty of Agriculture, Akwa Ibom State University.

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DEDICATION

I dedicate this work to God Almighty, my parents Mr./Mrs. Edem Bassey Edem and my siblings; Faith, Johnpaul, Happiness and Blessing.

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ABSTRACT

The study was carried out to evaluate the effect on nutrients digestibility and serum biochemical parameters of Broiler chickens fed diets containing Zinc Bacitracin and Black Pepper (Piper nigrum) at varying levels of inclusion. Completely randomized Deign was used for this experiment. 110 birds was divided into five (5) treatment groups which was further replicated twice with about 11 birds per replicate. The birds were raised intensively using deep litter system for 49 days. 15 birds (3 birds each from treatment) were randomly selected for serum biochemical parameters analysis. For the nutrient digestibility analysis, two birds from each groups were selected randomly and transferred to a metabolism cage, feaces collected separately and the Chemical composition of the feed and feces samples were determined according to the method described by AOAC (2000) and the metabolizable energy of feed and feces were also calculated. Two birds from different groups were selected randomly and transferred to a metabolism cage. The bids were acclimatized for four days in the cages with their irrespsective diets. Their feaces was collected separately and dried at the temperature of about 60oc to constant weight. The Chemical composition of the feed and feces samples were determined according to the method described in AOAC (2000) and the metabolizable energy of feed and feces were also calculated. The data obtained was subjected to one-way Analysis of Variance. For the nutrient digestibility; Crude protein, Crude fat, Crude fibre and Ash showed significant difference ($P<0.05$) across the treatments. For the serum parameters; Total protein, Globulin, Urea, ALT and AST showed significant difference ($P<0.05$) across the treatments. The study concluded that Black pepper (Piper nigrum) can be a safer and effective replacement for Zinc Bacitracin at an inclusion rate of 0.5%/10kg for broiler chickens.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Poultry is a term used to describe domesticated birds (*Gallus galus domestica*) with economic value that are primarily raised for either meat or eggs and are used as source of protein to human animals (Anosike *et al.*, 2018). Poultry is derived from the French word 'Poule' which is coined from Latin word '*Pollus*' meaning small animals. There are varieties of poultry species including turkey, chicken, fowl, duck, guinea fowl, goose and pigeon (FOASTAT 2018).

Among all domesticated poultry species, chickens are the most abundant and consumed globally. According to the Poultry Dictionary (2014), chicken production is among the most lucrative business in the livestock sector of the agricultural industry. They may be attributed to its minimum maturity period, low cost of management and its nutritional value (Adeyemo and Onkoyi, 2012).

Poultry production benefits the national economy, the populace and the producer. It is a major source of animal protein (food) to the humans. Keeping of poultry provides job creation opportunities and is a source of income to the producer (Nwafor, 2008). Poultry product serves as raw materials for several industries. The poultry sub-sector in the livestock industry has contributed to the Gross Domestic Product (GDP) of the Nigerian economy. It is estimated to account for approximately 25% to the agricultural GDP which itself contributes 25-35% to the Nigerian economy (NABC, 2020).

Due to the active contribution of poultry industry to the national economy, there has been a deliberate national drive of the Nigerian government and its agency to promote agriculture as a business (NABC, 2020). According to FAO, 2019 in the publication 'the future of livestock in

Nigeria', it was reported that Nigeria has the largest annual egg production and the largest chicken production in Africa, which implies that Nigeria is the highest producer of poultry products in West Africa.

Despite the numerous reported challenges facing the poultry industry, high cost of feed is significantly responsible for the reduction in production rate of the industry (Gadde *et al.*, 2017). The high cost of feed ingredient is the determining factor of the high prices of feed which affect farmers profit and production rate of the poultry industry (Sonaiya and Swan, 2004). The high cost of feed ingredients is attributed to the increased competition by humans for these feed ingredients for consumption and production purpose especially maize and soya bean (Al-Harthi *et al.*, 2006). To mitigate the challenges of the high cost of feed which affect production cost and rate of growth of the poultry industry, researches has been done in order to maximize general production cost through strategies like east cost feed formulation, used of growth promoting alternative feed stuffs (Dhama *et al.*, 2013, 2014, 2015). The use of agro-industrial product such as wheat offal, palm kernel cake (PKC) etc. to supplement major ingredient sources though these ingredients were found to contain some anti-nutritional factors and devoid of essential nutrients (Reigh, 2008) but through proper processing they can be reduced.

Furthermore, the utilization of feed additives was incorporated into the diet of poultry birds as growth promoters, health enhancers and appetite boosters. The use of zoo-technical antibiotics such as enzymes, hormones were recommended for utilization in poultry diet to boost feed efficiency (Kataria *et al.*, 2005; Angelakis *et al.*, 2019; Aghdam *et al.*, 2018).

Antibiotic growth promoters were mostly employed and these antibiotics were used in poultry feed continuously at a lower level of improve growth and feed conversion and not for the

purpose of any therapeutic reasons (Dhama *et al.*, 2007). Antibiotics compounds commonly used as growth promoters include Bacitracin, Penicillin, Tiamulin, Enrofloxacin etc (Chowdhury *et al.*, 2009). Overtime, the use of antibiotics in chicken birds diets became disadvantageous as it led to the development of antibiotics resistant strains of pathogenic micro-organisms like staphylococcus aureus and streptococcus SPP of bacteria in the gut of the birds (Dhama *et al.*, 2014). These pathogenic micro-organisms later deposit in the muscle of the animal causing health threatening situation in the human consumers (Alagawany *et al.*, 2019).

To combat the adverse effect of antibiotic growth promoters, researchers led to the advent of probiotics, prebiotics, phytobiotics (Angelakis *et al.*, 2013; Kuldeep *et al.*, 2014; Juse *et al.*, 2008). Recently, there has been several studies and investigation in the efficiency of the use of phytobiotics (plant extracts) and spices in the boiler diets (El-Deek *et al.*, 2003; Hassan *et al.*, 2004). Black pepper (*Piper nigrum*) is a common spice, mostly used in the diet of humans and traditional medicine for its varied potentials (Alkassie *et al.*, 2011). Black pepper (*Piper nigrum*) has numerous therapeutic potentials as it is used in the treatment of asthma, cold, cough and other general health disorders (Rakesh and Susphil, 2003). Black pepper (*Piper nigrum*) is reported to have antioxidant potential and anti-carcinogenic effect (Nalini *et al.*, 2006). Black pepper (*Piper nigrum*) outer fruit layer contains odour-contributing turpenes including pinene, sabinene and limonene which gives a tasty property of the spice (Al-Kassie *et al.*, 2011).

Black pepper (*Piper nigrum*) is found to be rich in glutathionine peroxidase, glucose-6-phosphate dehydrogenase and *piperine* (Al-Kassie *et al.*, 2011). Piperine is the bioactive compound in black pepper and it has been shown to increase the absorption of selenium vitamin B-complex, beta carotene, curcumin as well as other nutrients (Khalaf, 2008). Piperine also enhances the thermogenesis of lipid and accelerates energy metabolism in the body and increases

the serofonin and beta-endorphine production in the brain (Khalaf, 2008; Al-Kassie *et al.*, 2011). Black pepper (*Piper nigrum*) in the diet of broiler birds has been reported to enhance growth by improving digestion and absorption rate and intestinal properties (Mohan *et al.*, 1996; Hassan *et al.*, 2004).

Exploring the benefits of black pepper (*Piper nigrum*) on human diet, health and its reported action in chicken birds which is attributed to its chemical composition, the inclusion of this spice in chicken diet could help minimize health constraints posed by antibiotic growth promoter, reduce cost of production, increase profitability of the farmers and increase poultry production generally.

1.2 Statement of the Problem

In broiler production, aside from high mortality rate, high capital investment, disease outbreak and pest infestation (Anosike Enploring *et al.*, 2015). High cost of feeding is exclusively a major challenge faced in the poultry industry. The cost of feeding encompasses at least 60 – 70% of the total cost of production with cast of medication and operational cost exclusive (Hassan *et al.*, 2010). The high cost of feeding is related to the competitive use of feed ingredients by humans for consumption and production. In order to maximize profit and reduce cost, feed additives like spices, antibiotics enzymes and hormones are used to increase nutrients digestibility rate, boost growth and increase production but the tragedy staged by antibiotics has led to it restriction in broiler production as it causes resistance of drugs in human and animal (Dhama *et al.*, 2011). Therefore, there is need to source for alternatives measures to increase production and reduce cost.

1.3 Justification of the Study

Exploring the economic importance of broiler birds to the national economy and humans as it is a major source of animal protein, source of income generation to producers, source meat and other poultry produce and medium of foreign exchange and create job opportunities, there is need to boost the supply of poultry product (broiler produce) to meet the increasing demand of the economy (Alcicek *et al.*, 2010).

To curb this health threatening situation by antibiotics growth promoters, the use of spices have been exploited in other livestock nutrition which was found to increase production (Barreto *et al.*, (2008). Hence, there is need to steer research to explore the use of spice, such as; Black pepper, to increase production in broiler production. Black pepper (*Piper nigrum*) has been proven to contain certain important enzymes and nutrients which have been used in human medicine (Al-Kassie *et al.*, 2011; Rakesh and Sushil, 2003). Black pepper (*Piper nigrum*) in other livestock nutrition have been reported to increase feed efficiency promote health status, increase nutrient digestibility, boost growth and produce safe meat for human. Hence, there is need to explore black pepper used in the diet of broiler birds (Barreto *et al.*, 2008).

1.4 Objectives of the Study

- i. To evaluate the effect on Nutrients digestibility of Broiler chickens fed diets containing Zinc Bacitracin and Black Pepper (*Piper nigrum*).
- ii. To determine the effect on serum biochemistry of Broiler chickens fed diets containing Zinc Bacitracin and Black Pepper (*Piper nigrum*).

CHAPTER TWO

LITERATURE REVIEW

2.1 Poultry Industry in Nigeria

There are diverse definition regarding the term 'poultry' but does not deviate from the domestication of economic birds (Banerjee, 2007). These domesticated birds include: chicken, pigeons, turkey, goose and duck. Globally, chicken birds are the most domesticated class amongst the numerous classes of birds. Chicken birds are categoried into: broilers (meat producers) and the layers (egg producers) birds. (Marlo, 2008). The Poultry industry is one of the sub-sector of agriculture that have emerged drastically over the decades with over 100million birds in Nigeria and it has grown steadily despite the challenges faces (NABC, 2020). According to (Rabo Bank, 2017) report 'A time for Africa', Ivory Coast and Benin shows the most potential for poultry sector development in terms of increasing local demand and the incentives available, both fiscal and non-fiscal. Since 2008, the Nigerian Government have invested in the poultry sector by launching the commercial Agriculture credit scheme (CACS) to encourage farmers focus more on commercial farming than subsistence farming in order to produce more and boost the economic growth: noting that the poultry sub-sector contribute to the Gross Domestic Product (GDP), hence the poultry industry is significant for economic growth and development (NABC, 2020).

2.1.1 Economic Importance of Poultry in Nigeria

Poultry production plays significant role in human economy through the provision of food while also creating wealth job provision for the teeming population (Alders, et al., 2019). The poultry sector provides raw materials to industries as well as serve as a take-up industry for other industries (Omiti and Okuthe, 2015). Poultry production provides economic support and

development effect on the tourism sector as well as the fashion industries (Heinke *et al.*, 2015). Poultry production also provides nutrients in form of the supply of protein, vitamin, mineral and oil. It provides organic fertilizers in form of animal droppings. It provides affordable meat for the populace especially in urban areas (Wahyono and Utami, 2018). Poultry production industry serves as a means of foreign exchange as it has contributed about 25% of the total agricultural in the nations GDP (Atefeh et al., 2010).

2.2 Challenges of Poultry Production

Poultry production industry is faced with numerous challenges which cannot be over-looked. The challenges encountered in the poultry production industry includes;

- i. High cost of feeding (Agro-Ind, 2002; Adeyeme *et al.*, 2012; Sonaija and Swan, 2004)
- ii. Inadequate poultry extension services (Adeyoma and Onikoy, 2012)
- iii. Lack of technical knowledge in the management and production of poultry as majority ventures into the sector because of the huge profit regardless of the ethics (Aromalaran *et al.*, 2013)
- iv. High rate of disease and pest attack (Ajala *et al.*, 2007)
- v. Lack of access to loans and credits procurement (Aromalaran *et al.*, 2013)
- vi. High rate of mortality which is due to supply and use of poor quality materials (Anosike *et al.*, 2015: Ajala *et al.*, 2007)

Amongst the endless list of challenges faced by poultry production industry, high cost of feeding is the major challenge that impedes the growth of the production which has been reported by Agro-Ind (2002) to be anchored by the competitive nature of the feed ingredients with man and unavailability. Where the total cost of feeding covers about 60-70% of the production cost thereby endangering profit maximization by the producer (Anosike et al., 2015).

2.3 Nutrients Requirement of Broiler Chickens

2.3.1 Protein Requirement

Proteins are polymers that are composed of α -amino acids, which are linked together by peptide bonds. They are broken down and hydrolyzed in the digestive system into amino acids. Following absorption, the amino acids will be assembled and metabolized to form proteins that are used in the building of different body tissues (Aviagen, 2009). They also serve vital metabolic roles as blood plasma proteins, enzymes, hormones, and antibodies, each of which has a specific role in the body (Pond *et al.*, 1995). When formulating broiler diets, main emphasis is placed on the crude protein, because protein is the critical constituent of poultry diets, and together with the other main nutrients such as carbohydrates, fat, water, vitamins, and minerals, is essential for life (Cardoso, 2012).

Broiler requires about 19-24% of protein for both starter and finisher phase respectively (Adedeji *et al.*, 2009). The protein requirement of broilers is mostly obtained from animal and plant source (Adeyemo and Longe, 2007). Plant sources of protein include; Soybean, legumes, cereal by-products, etc, while animal protein sources is obtained from; fish meal, blood meal, feather meal, etc. Plant proteins are usually cheaper than animal proteins; however, there is a limitation to their use because of their content of anti-nutritional factors. Most of these anti-nutritional factors can be destroyed by thermal processing that causes an increase in the nutritional value sometimes and protein level of plant proteins due to the elimination of anti-nutritional factors and freeing the protein in the plant protein products (Adeyemo and Longe, 2007). The anti-nutritional factors such as; phytate, lectins, polyphenolic compounds, glucosinolates, saponin etc. and the processing methods of these protein sources drastically limits the digestibility rate of protein generally.

2.3.2 Energy Requirement

Simple carbohydrates, some complex carbohydrates, protein and fat in the feed are the main energy supplying summation of the energy requirement for maintenance, nutrients. The importance of energy feedstuff in poultry feeding cannot be over-emphasized because increasing or decreasing the dietary energy has been reported to affect feed intake in addition to promoting or undermining efficient feed utilization and growth rate (Moritz and Latshaw, 2001). Dietary energy is measured by metabolizable energy, which is the amount of energy used for growth maintenance and other activities in the animal (Gheisar *et al.*, 2015; Singh *et al.*, 2017). From researches, it is deduced that birds usually eat with the aim of satisfying their energy requirement, and once this aim is achieved, the birds will stop eating irrespective of the fact that other key nutrient requirements such as protein, minerals, and vitamins have not been met (Zamani *et al.*, 2017). Energy feed stuff are feedstuff that is high in energy, low in fibre and protein content less than 18-20%. The use of cereals (especially maize) as an important energy feedstuff in broiler diets has been drawing much attention recently, because these ingredients contain considerable amounts of non-starch polysaccharides substances that may impair nutrient utilization (Ndams *et al.*, 2009). Broiler chickens requires about 3000-3200kcal/kg for growth and body maintenance (Ndams *et al.*, 2009).

2.3.3 Amino Acid Requirement

Poultry, like all animals, synthesize proteins that contain 20 L-amino acids. Birds are unable to synthesize 9 of these amino acids because of the lack of specific enzymes: arginine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Ravindran, 2013). Histidine, glycine, and proline can be synthesized by birds, but the rate is

usually insufficient to meet metabolic needs and a dietary source is required. These 12 amino acids are referred to as the essential amino acids (Okamoto *et al.*, 2009). Tyrosine and cysteine can be synthesized from phenylalanine and methionine, respectively, and are referred to as conditionally essential because they must be in the diet if phenylalanine or methionine levels are inadequate (Khawaja *et al.*, 2007). The diet must also supply sufficient amounts of nitrogen to allow the synthesis of nonessential amino acids. Essential amino acids are often added to the diet in purified form (e.g., DL-methionine and L-lysine) to minimize the total protein level as well as the cost of the diet. This has the added advantage of minimizing nitrogen excretion and digestibility generally (Okamoto *et al.*, 2009).

2.3.4 Vitamins and Minerals Requirements

Minerals can be classified as macro-minerals (calcium, phosphorus, sodium, potassium, and magnesium), micro-minerals (copper, zinc, iron, iodine and manganese) and trace minerals (cobalt and selenium) (Mahendra, 2017). Vitamins includes; fat soluble (vitamin A, D, E and K) and the other; water soluble (vitamin B complex group). Balanced diet with essential minerals and vitamins are imperative for good health of poultry birds. The deficiency of these nutrients in feed can lead to several health problems, which can severely affect the poultry production, causing economic losses (Mahendra, 2017).

Table 2.1: Nutritional Requirements of Broiler Chicks (Starter)

Nutrients	Requirements
Metabolizable Energy (ME)	3000 kcal/kg
Crude Protein (CP)	21-22%
Crude Fat	5% (max)
Crude Fibre (CF)	5% (max)
Calcium	0.85% (min)
Phosphorus, available	0.45% (min)
Lysine	1.10% (min)
Methionine	0.37% (min)
Methionine + Cystine	0.88% (min)
Threonine	0.77% (min)
Tryptophan	0.18% (min)

Source: BIS, 2007

Table 2.2: Nutritional Requirements of Broiler Chickens (Finisher)

Nutrients	Requirements
Metabolizable Energy (ME)	3100-3200 kcal/kg
Crude Protein (CP)	18-19%
Crude Fat	6% (max)
Crude Fibre (CF)	8% (max)
Calcium	0.80% (min)
Phosphorus, available	0.45% (min)
Lysine	0.95% (min)
Methionine	0.30% (min)
Methionine + Cystine	0.74% (min)
Threonine	0.65% (min)
Tryptophan	0.17% (min)

Source: BIS, 2007

2.4 Trial Solution to Tackle the Challenges of Poultry Production

Despite the numerous challenges faced by the poultry production industry, they have not stopped functioning, although the challenges might have caused reduction in the total production rate (Anosike *et al.*, 2018). In order to tackle these challenges and reduce some of its effect, numerous researches have been carried out regarding the use of conventional feedstuffs like (maize and soybean) which are highly demanded by human for consumption, to the use of non-conventional feedstuffs that are cheaper, locally available and less competitive but also contain

the adequate nutrients required by the animal. These conventional feedstuffs includes; sesame seed cake, palm kernel cake, wheat offal etc. (Adedeji *et al.*, 2011; Anitakumar *et al.*, 2010).

To find solution to mitigate the challenges of high cost and boost productivity, the use of growth promoters to effectively boost the performance of the animal to rapidly meet market rate has been introduced through the use of feed additives that has been utilized over the decades (Hernandez *et al.*, 2004). Feed additives are substances incorporated into the feed of livestock to increase feed digestibility, palatability and general acceptability by the animal for adequate growth and development. Feed additives commonly used includes; antibiotics, spice, phytogenics, prebiotics, probiotics, enzymes and zoo-technical etc. (Barreto *et al.*, 2008; Al-Kassie *et al.*, 2011).

2.5 Feed Additives in Poultry Nutrition

Feed additives are products used in animal nutrition for the purpose of improving the quality of feed or improve the animals' performance and health by providing enhance digestibility of the feed materials.

2.5.1 Functions and Classes of Feed Additives

Feed additives do not only supplement vitamins and minerals. They are also used to make the food more palatable to ensure that the animals continue eating them. They make the animal feed more easily digestible so as to avoid bowel stress and other digestion issues that can often occur to livestock (Barreto *et al.*, 2008). Classes of feed additives include vitamins, minerals, amino acid, binders, enzymes, antibiotics, spices, acidifiers and oxidants.

2.5.2 Antibiotics as Feed Additives Poultry Nutrition

Antibiotics are simply medications used to prevent microbial infections (Dibner, 2015). Antibiotics as a feed additive was geared to treat illness and infections in livestock and later

discovered to have growth promoting properties (Hassan *et al.*, 2018). The use of antibiotics as feed additive to promote growth was a huge discovery in the field of animal nutrition that changed the phase of animal production leading to increased poultry chicken production and achievement of high feed efficiency goal (Hassan *et al.*, 2004).

2.5.3 Challenges Associated with Antibiotics Usage

The use of anti-microbial feed additives (antibiotic and chemotherapeutics drugs) have been used as growth promoters with generalized used in animal (poultry) nutrition allowing improved productivity of animals raised under intensive condition (Maitel *et al.*, 2004). Despite the observed improvement in broiler performance, the use of antibiotics growth promoters have been criticized due to its possible role in the occurrence of anti-microbial resistance in humans consumers (Barreto *et al.*, 2008). Due to the adverse effect and health threat on humans, the use of antibiotics feed additives in poultry nutrition have been restricted and banned in several countries with recommendation in poultry production, except for the use of antibiotics whose action have been reported to be negative and threat to human health (Barreto *et al.*, 2008).

Researches has been conducted in an intensified manner in the last two decades for developing antibiotics alternative in maintaining human health as well as performances of animals (Dhama *et al.*, 2014 Hassan *et al.*, 2004). Amongst the discovered alternative growth promoters are; probiotics, prebiotics, phytobiotics, essential oils, enzymes and are proven for their ability to replace antibiotics as feed additives (Hassan *et al.*, 2007, Thacker, 2013).

2.6 Other Classes of Feed Additives

2.6.1 Probiotics

Probiotics are the live microbial feed supplement which are used for balancing the microbial population in the intensive through the production of various compounds, competitive exclusion and displacement of pathogens from enterocytes as well as maintenance of gut pH and thereby improving the health and immune system of the birds (Dhama *et al.*, 2014). This helps in the production of healthy meat without any drug residue (Alavi *et al.*, 2012). Probiotics improve digestion and utilization of nutrient and also helps in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin B10, B2, B12 and k) which are responsible for proper growth and metabolism (Dhama *et al.*, 2007, 2014).

2.6.2 Prebiotics

Prebiotics was first introduced by Gibson and Roberfroid (1995) who defined them as non-digestible food ingredient/supplement that beneficially affects the host by selectively stimulating the growth of some or all of the non-pathogenic organism (bacteria) in the gut or colon (Gibson and Roberfroid, 1995; Roberfroid, 2007). Supplementation of the diets with small fragments of carbohydrates (such as oligosaccharides) is another method used to manipulate the gut ecosystem. They are selectively fermented by beneficial micro flora into short chain fatty acid (SCFA), which effectively excludes the pathogenic microbes due to a lower pH in the gastro-intestinal tract through lactic acid production and this inhibiting colonization of pathogenic bacteria (Roberfroid, 2007). The beneficial effect of prebiotics is specific for the types and dose of carbohydrates and also the rate of fermentation by the beneficial organism (Dhama *et al.*, 2014). Aside this, prebiotics are beneficial in increasing the performances, productivity and meat quality of broiler chickens (Giannenas *et al.*, 2012; Liu *et al.*, 2012).

2.6.3 Phytobiotics

Phytobiotics are otherwise known as phyto-extracts, they are simply plants derived feed additives and growth promoters (Wenk, 2003). They are compounds from herbs, spices and those extracts can stimulate appetite, endogenous secretions such as enzymes, have antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals (Wenk 2013). Nowadays, there is an increasing interest in the use of medicinal plants as feed additives in poultry diet to enhance the performance of poultry birds (Khan et al., 2012).

There are several researches and reports, regarding the use of medicinal herbs to boost broilers products. Some of the major plant additives which have been extensively reported in poultry nutrition and its positivity in enhancing growth in birds include: Garlic (*Allium Sativum*), Turmeric (*Curcuma longa*), Thyme (*Thymus vulgans L*), Aloe vera, onions (*Allium sepa*), Ginger (*Zingiber officinale*), black pepper (*Piper nigrum*) (Auose et al., 2005; Sunder et al., 2013; Dhama et al., 2014).

2.7 Spices, Types and General Properties in Animal Nutrition

Spices are non-leaf parts of plants, including seeds, fruits, bark or root with intensive taste or smell (Applegate et al., 2010). The use of spices as feed additives have been largely tested in animal production with the aim to identify their effects on the gastrointestinal function and health, and their implications on animals' systemic health and welfare, the production efficiency of flocks, food safety, and environmental impact (Mohammadi et al., 2018). These feed additives originating from spices, include many different bioactive ingredients. A wide variety of herbs and spices (thyme, oregano, cinnamon, rosemary, marjoram, yarrow, garlic,

ginger, green tea, black cumin, coriander, among others) as well as essential oils (from thymol, carvacrol, cinnamaldehyde, garlic, anise, citrus, clove, ginger) have been used in animal production, individually or mixed, for their potential application as growth promoter (Tollba, 2010). However, there are contradicting results on the action of spices on animals. Some authors reported no positive effects of spices inclusion (Gadde *et al.*, 2017). This discrepancy may be attributed to several factors, including the inherent variability of the botanic composition, as well the variability of the animal scenarios, environmental, management and sanitary conditions (i.e., including the likely presence of a pathogen challenge). The technique of treatment (cold, steam distillation, extraction or maceration with non-aqueous solvents) of some spices has been also reported to change the active substances and related compounds in the final product which is capable of giving negative effect (Gadde *et al.*, 2017). The positive effect of spices on animal performance can be attributed to its chemical composition and bioactive substances. Plant active principles are chemical compounds present in the entire plant or in specific parts of the plant that confers them therapeutic activity or beneficial effects (Martins *et al.*, 2000).

2.8 Black Pepper (*Piper nigrum*)

2.8.1 Taxonomy and Botanical Description of Black Pepper (*Piper nigrum*)

Black pepper (*Piper nigrum*) is a flowering vine in the family “*piperaceae*” cultivated for its fruit, known as pepper corolla which is usually dried and used as spice and seasoning (Dhama *et al.*, 2014) the fruit is a drupe which is about 5mm (0.20 inches) in diameter (fresh and fully matured) and dark red in colour. The flowers are small, produced on pendulous spikes of about 4 to 8cm (1.6 to 3.1 inch) at the leaf nodes. The spikes lengthening up to 7.15cm (2.8 -5.9 inch) as

the fruit matures. A single stem of the plant bears up to 20-30 fruiting spikes (Alkassie *et al.*, 2011; Thompson, 2014)

2.8.2 Taxonomical Classification of Black Pepper (*Piper nigrum*)

Kingdom	-	Plantae
Order	-	Piperales
Family	-	Piperaceae
Genus	-	Piper
Species	-	<i>Piper nigrum</i> L.

Safa *et al.*, 2014

2.8.3 Nutritional Component of Black Pepper (*Piper nigrum*)

Black pepper is an important spice throughout the world. It is used as a traditional medicine to treat cough (Al-Kassie *et al.*, 2011). Black pepper (*Piper nigrum* L.) is rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase, it has been shown that piperine can increase the absorption of selenium, vitamin B complexes, beta carotene and curcumin as well as other nutrients (Khalaf, 2008). Piperine in black pepper enhances the thermogenesis of lipid and accelerates energy metabolism in the body and also increases the serotonin and beta-endorphin product in the brain (Nalini *et al.*, 2006). Black pepper is found to have antioxidant properties and anticarcinogenic effect (Nalini *et al.*, 2006). The outer fruit layer contains important odor-contributing terpenes including; pinene, sabinene and limonene which gives a tasty properties of the black pepper (Al-kassie *et al.*, 2011).

The consumption of black pepper (*Piper nigrum*) exerts several health beneficial effects by the virtue of their innumerable therapeutic potential such as; fever, asthma, cold, cough and other general health disorders (Rakesh and Sushil, 2003).

2.8.4 Feeding Trial of Spices and Black Pepper (*Piper nigrum*) in Broiler Nutrition

Al-kassie *et al.*, 2011 illustrates the effect of different levels of black pepper on haematological parameters treatments. His study showed that treatment groups fed black pepper had significantly lowered cholesterol, H/L ratio, RBC, PCV and Hb as compared with the control group, but had no significant effect on WBC among treatments. H/L ratio is regarded as a good indicator to examine the stress level of birds. The fact that active compounds in black pepper having receptors on adrenal gland may affect the nervous system and decrease ACTH secretion that causes stress which may lead to increase blood glucose concentration. The reduction of the parameters (PCV, Hb and RBC) may be due to the activity of black pepper which may act on oestrogen hormone.

Singh (2015) reported that the use of black pepper (*Piper nigrum*) was found to improve feed digestibility in broilers. Improve feed digestibility could be attributed to the bioactive ingredient in black pepper “*piperine*” which can dramatically increase absorption of selenium, vitamin B complex, β carotene and curcumin as well as other nutrients. Piperine also enhances the thermogenesis of lipids and accelerates energy metabolism in the body and also increases the serotonin and β -endorphin production in the brain.

Tollba *et al.*, (2007) reported zero effect on growth performance and low digestibility of black pepper in broiler chickens. He further concluded that the digestibility property is attributed to the level of black pepper used that reflects the high activity of piperazine citrate included in the broiler diet which may have affected the flow of digestive juices across the stomach. This report was similar to the findings of Ghazalah *et al.*, (2007).

The apparent digestibility of nutrients reported by Javed *et al.* (2009) indicates that the black pepper (*Piper nigrum*) at certain levels improved digestibility, which is due to the

concentration of the bioactive ingredient in black pepper “*piperine*”, which enhances the release of digestive enzymes.

Lee *et al.* (2001) and Windisch *et al.* (2008) also reported that black pepper and its essential oils could be used to aid digestion in monogastric animals attributed to the better nutrient digestion to antimicrobial property of the essential oil in black pepper.

Abdel-Ghaney *et al.*, (2017) reported that the addition of 0.5% of thyme (*Thymus vulgaris*) powder to broiler chickens improved the immune status and antioxidant activities in broilers while the lipid per oxidation of meat was reduced. This action was due to antimicrobial activity found in the oils against gram-positive and gram-negative bacteria, as thyme extract in agar can have antimicrobial activity against *Salmonella typhimurium*, *Clostridium perfringens*, and *helicobacter pylori*.

The addition of black pepper (*piper nigrum*) in animal diet has been reported to improve hematological and composition, piperine (*piper nigrum*) which is characterized by antimicrobial and anti-inflammatory properties (Al-kassie *et al.*; 2011; Awad *et al.*, 2011). Investigation of (Abou-Eljhair *et al.*; 2014) showed that black pepper in broiler nutrition had influence on improved health status through increase of serum globulin concentration.

Piperine possibly reduces LDL, triglyceride and cholesterol in serum and tissue (Nikola *et al.*, 2015). Black pepper has been found to lower serum and liver cholesterol and reduce oxidative stress in monogastric animals (Al-kassie *et al.*; 2011; Nikola *et al.*, 2015). Hence, black pepper impact on animal blood parameters positively.

2.9 Nutrient Digestibility in Broiler Birds

Nutrient digestibility refers to the amount of nutrient absorbed by the individual animal. It is expressed as; $100 \times (\text{intake} - \text{excreted}) / \text{intake}$

2.9.1 Methods of Estimating Nutrients Digestibility

One of the method commonly employed to estimate digestibility of animal is the Total collection method. It is a quantitative research method where the animal is kept in a collection pen or cage. Some of other methods include: the difference technique and the Marker technique.

2.9.2 Proximate Composition of Broiler Feed

- **Nitrogen Free Extract (NFE):** This consists of carbohydrates, sugars, starches and a major portion of materials classed as hemicellulose in feed.

It is expressed as $\% \text{NFE} = 100 \% - (\% \text{EE} + \% \text{CP} + \% \text{Ash} + \% \text{CF})$. (Mohammadi *et al.*, 2018).

- **Crude Protein:** It is a measure of the amount of protein in a feed determined as the amount of nitrogen multiplied by 6.25 (Nikola *et al.*, 2015).
- **Ether extract (Crude Fat):** This refers to free lipids that can be extracted into less polar solvents such as petroleum ether (Awad *et al.*, 2011).
- **Crude fiber:** This refers to the cellulose material obtained as a residue in the chemical analysis.
- **Dry matter (DM):** This represents everything contained in a feed sample except water such as protein, fiber, fat, minerals etc. It is expressed as total weight of feed minus the weight of water in the feed (Mohammadi *et al.*, 2018).

2.10 Functions and Factors that Affect the Serum Biochemical Parameters of Animals

Blood clots and separates into two parts – a clear pale yellow liquid called serum and a small solid portion composed of blood cells (Al-kassie *et al.*, 2011). Serum is collected by placing a tube of clotted blood in a machine called a centrifuge, which spins the blood very quickly in a small circle, forcing the cells to the bottom of the tube and allowing the serum to sit on top. There are many substances in serum, including proteins, enzymes, lipids, hormones, etc. Testing for these various substances provides information about the organs and tissues in the body as well as the metabolic state of the animal. If a test result is abnormal, it may indicate that disease is present. Further assessment of the test results may offer clues about which organ system is affected and also the nature and severity of the disorder (Love, 2000).

2.10.1 Alanine Aminotransferase (ALT)

Alpha-Ketoglutarate reacts with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The enzyme ALT been found to be in highest concentration in the liver, with decreasing concentrations found in kidney, heart, skeletal muscle, pancreas, spleen, and lung tissue. Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases. Elevated levels of the transaminases can indicate myocardial infarction, hepatic disease, muscular dystrophy, or organ damage (Glick and Ryder, 2001). Serum elevations of ALT activity are rarely observed except in parenchymal liver disease, since ALT is a more liver-specific enzyme than asparate aminotransferase (AST).

2.10.2 Albumin (ALB)

Albumin constitutes about 60% of the total serum protein in normal, healthy individuals. Unlike most of the other serum proteins, albumin serves a number of functions which include

transporting large insoluble organic anions (e.g., long-chain fatty acids and bilirubin), binding toxic heavy metal ions, transporting excess quantities of poorly soluble hormones (e.g., cortisol, aldosterone, and thyroxine), maintaining serum osmotic pressure, and providing a reserve store of protein (Glick and Ryder, 2001). Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys (Love, 2000).

2.10.3 Aspartate Aminotransferase (AST)

Alpha-Ketoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate (Glick and Ryder, 2001). The enzyme AST has been demonstrated in every animal tissue studied. Although the enzyme is most active in the heart muscle, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys of some animals. AST measurements are used in the diagnosis and treatment of certain types of liver and heart disease. AST is present in both the cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of serum AST is from the cytoplasm, with smaller amounts from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, or organ damage (Glick and Ryder, 2001).

2.10.3 Blood Urea Nitrogen

Urea is hydrolyzed by urease to form CO_2 and ammonia. Urea is synthesized in the liver from ammonia produced as a result of deamination of amino acids. The determination of serum urea nitrogen is the most widely used test for the evaluation of kidney function (Glick and Ryder, 2001). High urea levels are associated with impaired renal function, increased protein catabolism, nephritis, intestinal obstruction, urinary obstruction, metallic poisoning, cardiac

failure, peritonitis, dehydration, malignancy, pneumonia, surgical shock, Addison's disease, and uremia. Low urea levels are associated with amyloidosis, acute liver disease, and nephrosis (Glick and Ryder, 2001).

2.10.4 Creatinine

In an alkaline medium, creatinine forms a yellow-orange-colored complex with picric acid. The rate of color formation is proportional to the concentration of creatinine present and may be measured photometrically (Awad *et al.*, 2011). Creatinine measurement serves as a test for normal glomerular filtration. Elevated levels are associated with acute and chronic renal insufficiency and urinary tract obstruction. Levels below 0.6 mg/dL are of no significance (Glick and Ryder, 2001).

2.10.5 Total protein (TP)

Peptides of low molecular weight are present in serum, but their concentration is too low to cause interference. Serum proteins perform a number of different functions in the body (Glick and Ryder, 2001). In addition to being major structural components of cells, proteins are involved in transport, enzymatic catalysis, homeostatic control, hormonal regulation, blood coagulation, immunity, growth and repair, and heredity. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders (Glick and Ryder, 2001).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Experimental Site

The experiment was conducted at the poultry research unit of the department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State. The area lies between latitude 4⁰30'N and 5⁰ 00'N and longitudes 70⁰ 30'E and 80⁰ 00'E. The climatic condition of the experimental site was a tropical rain forest characterized with high temperature (average of 30⁰C), high rainfall (about 1500mm) and relative humidity of 70% on average (SLUS-AK, 1989).

3.2 Sources and Processing of Experimental Materials

The feed ingredients were purchased from Abak market in Akwa Ibom State. A whole soybean was used for the experiment which was processed by roasting it for about 25 minutes and grind to eliminate the anti-nutritional factors, maize used was purchased and grind to a texture suitable for animal absorption. The Black pepper (*Piper nigrum*) used as additive in the experiment was purchased from the Abak market in dry form, grind into powder and stored in an air-tight plastic container free from moist until used. The zinc Bacitracin antibiotics was purchased from a veterinary shop at No.226 Abak road, Uyo, and stored in a polythene bag. All other feed ingredients was purchased and included in the basal diet used for the research.

3.3 Experimental Birds and Management

One hundred (110) Arbor Acre plus strain of day-old broiler chicks used for this experiment were purchased from a local distributor outlet (Brilliance Livestock Enterprise) in

Abak Local Government Area in Akwa Ibom State. The experiment lasted for 49 days (7 weeks). The brooding of the birds for the first 14 days was done using heat sources like the charcoal pot and kerosene stove with an average temperature of 32-35°C to enable feather development. The birds were managed intensively using deep litter system using wood shavings as litter material. The starter broiler basal diet was given to the birds for the first 21 days, thereafter the finisher broiler basal diet was given from day 22 to day 49 of the termination of the experiment. The feed and water was given ad-libitum. The birds were vaccinated against the most common diseases such as; Newcastle Disease and Infectious Bursal Disease (Gomboro). The initial weight of each of the birds was recorded using a scale before they were divided into treatment and replicate respectively.

3.4 Experimental Design

Completely Randomized Design (CRD) was used for the research where the birds were distributed into five (5) treatment group with two replicate each and each replicate housing 11 birds. Each of the five treatment groups was assigned to one of the five experimental basal diets.

3.5 Experimental Diets

Feed additives were added to experimental basal diets both starter and finisher respectively such that treatment group one (1) received only basal diet (control), treatment group two (2) received basal diet with inclusion of feed additive (Zinc Bacitracin) at the rate of (0.5%), treatment group three (3) received basal diets with inclusion of Zinc Bacitracin at the rate of (0.2%), treatment group four (4) received basal diet with inclusion of Black Pepper at the rate of (0.5%) and treatment group five (5) received basal diet with inclusion of Black Pepper at the rate of (0.2%).

Table 3.1: Ingredient and Nutrient Composition of the Experimental Starter Broiler Diet

Ingredient	Treatments				
	T1(control)	T2 (0.5%ZB)	T3 (0.2%ZB)	T4 (0.5%BP)	T5 (0.2% BP)
Maize	52.10	52.10	52.10	52.10	52.10
Soybean meal	29.40	29.40	29.40	29.40	29.40
Fish meal	5.00	5.00	5.00	5.00	5.00
Wheat offal	8.00	8.00	8.00	8.00	8.99
Palm Oil	3.00	3.00	3.00	3.00	3.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.00	1.00	1.00
Salt	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Premix	0.20	0.20	0.20	0.20	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100

*ZB – Zinc Bacitracin *BP – Black Pepper

Calculated Composition:

Crude Protein – 22%

Metabolizable Energy – 3000kcalME/kg

Table 2: Ingredient and Nutrient Composition of the Experimental Finisher Broiler Diet

Ingredient	Treatments				
	T1(control)	T2 (0.5%ZB)	T3 (0.2%ZB)	T4 (0.5%BP)	T5 (0.2% BP)
Maize	56.36	56.36	56.36	56.36	56.36
Soybean meal	19.14	19.14	19.14	19.14	19.14
Fish meal	8.00	8.00	8.00	8.00	8.00

Wheat offal	10.00	10.00	10.00	10.00	10.00	*ZB –
Palm Oil	4.00	4.00	4.00	4.00	4.00	
Bone meal	1.00	1.00	1.00	1.00	1.00	Zinc
Limestone	1.00	1.00	1.00	1.00	1.00	
Salt	0.10	0.10	0.10	0.10	0.10	Bacitrac
Lysine	0.10	0.10	0.10	0.10	0.10	in *BP –
Premix	0.20	0.20	0.20	0.20	0.10	
Methionine	0.10	0.10	0.10	0.10	0.10	Black
Total	100	100	100	100	100	Pepper

Calculated Composition:

Crude Protein – 20%

Metabolizable Energy – 3200kcal/MEkg

3.6 Data Collection

3.6.1. Blood collection for Serum Analysis

At day 56, three birds from each treatment were randomly selected for blood collection. Blood samples were collected from their jugular veins and transferred into gel activated plain sample tubes. The samples were labeled according to treatment and replicate. The blood samples was allowed to coagulate to produce sera for determination of serum biochemical parameters. The biochemical parameters determined were total protein, albumin, globulin, urea, creatinine, AST and ALT using colorimetric method as prescribed by Chronolab Commercial Kits (Barcelona,Spain).

3.6.2. Feecal Collection for Nutrient Digestibility

Two birds from different groups were selected randomly and transferred to a metabolism cage. The bids were acclimatized for four days in the cages with their irrespective diets. Their feaces was collected separately and dried at the temperature of about 60°C to constant weight.

The Chemical composition of the feed and feces samples were determined according to the method described in AOAC (2000) and the metabolizable energy of feed and feces were also calculated.

3.7 Data Analysis

All the data obtain was expressed as mean \pm standard error (X+SE) and subjected to one way analysis of Variance (ANOVA). Significant means was separated by applying Duncan multiple range test as outlined by Duncan (1955).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Nutrient Digestibility

Table 4.1: Results of Nutrient Digestibility of Broiler Birds Fed Diets Containing Zinc Bacitracin (ZB) and Black Pepper (BP)

Parameters	T ₁ (Control)	T ₂ (0.5%) ZB	T ₃ (0.2%) ZB	T ₄ (0.2%) BP	T ₅ (0.5%) BP	SEM	P-Value (P<0.05)
Crude protein (CP)	^b 52.53	^a 57.43	^b 50.85	^a 57.8	^a 58.53	0.92	0.006
Crude Fat (CFAT)	^{ab} 47.68	^a 48.25	^b 47.18	^a 47.90	^a 47.90	0.11	0.015
Crude Fibre (CF)	^c 48.25	^a 52.93	^c 49.03	^b 50.70	^b 50.20	0.43	0.000
Nitrogen Free Extract (NFE)	42.0	40.43	40.43	41.63	41.63	0.42	0.638
ASH	^a 59.38	^a 58.85	^a 58.85	^b 52.75	^b 52.75	0.93	0.000
Dry Matter (DM)	55.73	55.13	55.13	57.13	57.13	0.36	0.197
Energy	56.6	56.45	56.2	57.55	57.88	0.35	0.33

***ZB - Zinc Bacitricin *BP - Black Pepper *SEM – Standard Error of Mean**

4.1.1 Crude Protein (CP)

There was significant difference ($P < 0.05$) in crude protein digestibility across the treatment groups as shown in Table 4.2: Birds in the control group (T₁) had the least crude protein digestibility value while birds that received 0.5% Black pepper inclusion (T₅) in their diet had the highest crude protein digestibility. Also, the birds in the control treatment group was significantly lower ($P < 0.05$) compared to birds that received 0.5% zinc Bacitracin inclusion (T₂) and 0.2% Black pepper (T₃) inclusion in their diets respectively. The result obtained was

similar to that of Javed *et al.*, (2009) and Al-Kassie *et al.*, (2011) who reported that black pepper bio-active component “piperine” promote pancreatic digestive enzymes such as lipase, amylase and proteases which played important role in digestion. The significantly lower ($P<0.05$) value of the crude protein digestibility of the birds in the control treatment groups could be due to the anti-nutritional factors in the diets such as trypsin inhibitors due to the method of processing.

4.1.2 Crude Fat (CFAT)

There was significant difference ($P<0.05$) observed across the treatment group on crude fat digestibility as in the Table 4.2 above. The birds in the treatment group (T_3) that was fed with 0.2% of Zinc Bacitracin inclusion had the least crude fat digestibility value. While birds that received 0.5% zinc Bacitracin (T_2) had the highest crude fat digestibility value. The crude fat digestibility in treatment group (T_3) was significantly lower ($P<0.05$) compared to birds that received 0.5% black pepper in their diet (T_5). The results obtained was in contrast to the findings of Lee *et al.*, (2001) and Windisch *et al.*, (2007) that reported the high digestibility of black pepper. The effect of low digestibility of crude fat in this research was due to potential of the bio-active component of ‘*Piperine*’ in black pepper to inhibit fat cell differentiation as used in human nutrition for obesity.

4.1.3 Crude Fibre

There was significant difference ($P<0.05$) in the crude digestibility of birds across the treatment groups. Birds in the control group (T_1) had the least crude fibre digestibility value while the bird that received 0.5% zinc Bacitracin in their diet (T_2) had the highest crude fibre digestibility value. The crude fibre digestibility of birds in the control group was also significantly lower ($P<0.05$) compare to birds that received black pepper at 0.5% and 0.2% inclusion rate in their diets.

4.1.4 Ash

There was a significant difference ($P < 0.05$) in the ash nutrients digestibility across the groups. Birds that received 0.5% Black pepper inclusion in their diet (T_5) was least significant difference ($P < 0.05$) compared to the birds in the control treatment group that had the highest ash digestibility value. Birds that received 0.5% Black pepper inclusion in their diet (T_3) was significant lower compared to the birds that received 0.5% zinc Bacitracin inclusion in their diets.

There was no significant difference ($P > 0.05$) in the gross energy, nitrogen free extract and dry matter between the treatment groups. The results obtained were similar to the findings of Mohan *et al.*, (1996), Hassan *et al.*, (2004).

4.2 Serum Biochemical Parameters

Table 4.2: Results of Serum Biochemical Parameters of Broiler Birds Fed Diets Containing Zinc Bacitracin (ZB) and Black Pepper (BP)

	TP	ALB	GLB	CRT	UREA	AST	ALT
	(g/dl)	(g/dl)	(g/dl)				
T₁	41.3 ^a	14.7	30.0 ^a	21.3	0.83 ^a	327.0 ^b	10.0 ^b
T₂	27.7 ^b	12.7	15.0 ^c	20.3	0.93 ^a	444.0 ^a	13.3 ^a
T₃	31.3 ^b	14.3	17.0 ^c	20.0	0.76 ^a	306.7 ^b	11.7 ^{ab}
T₄	33.0 ^b	14.0	19.0 ^c	22.0	0.86 ^a	318.3 ^b	10.3 ^b
T₅	38.3 ^a	12.7	25.7 ^b	21.3	0.50 ^b	260.3 ^b	10.0 ^b
SEM	1.45	0.37	1.58	0.36	0.04	18.18	0.43
P-Value	0.001	0.294	0.000	0.452	0.024	0.002	0.025
(P<0.05)							

SEM - Standard Error Mean

P-Value - ($P < 0.05$)

4.2.1 Total protein (TP)

There was a significant difference ($P<0.05$) in the total protein across the treatment groups. Birds in the control treatment groups (T_1) had the highest total protein, while the birds that received 0.5% zinc Bacitracin inclusion in their diet (T_2) had the least total protein value. The birds received 0.5% zinc Bacitracin inclusion in their diet (T_2) was significantly difference ($P<0.05$) compared to the birds that received 0.5% of Black pepper inclusion in their diets. The least value of the birds that received 0.5% zinc Bacitracin inclusion in their diet (T_2) may be due to negative action on osmotic balance of the kidney, as lower protein values reflect chronic effect especially to the liver and kidney disorders. Additionally, from the result it can be deducted that black pepper improves total protein, hence kidney and liver health as total protein maintains osmotic balance between circulatory blood and tissue spaces.

4.2.2 Globulin (GLB)

A significant difference ($P<0.05$) was observed in globulin across the treatment groups as shown in Table 4.2. The birds that received zinc bacitracin at 0.5% inclusion in their diet (T_2) had the least globulin value, while the control group had the highest amount of globulin in their serum.

The birds that received zinc bacitracin at 0.5% inclusion in their diet (T_2) was significantly lower ($P<0.05$) compared to birds that received black pepper at 0.5% inclusion in their diet (T_5). Zinc Bacitracin action in lowering globulin concentration in the serum could be due to it amelioration, since globulin plays an important role in liver function, blood clotting and fighting infections. Hence, Black pepper has a beneficial effect on globulin and health of the

birds additionally, which is attributed to its bioactive constituent “piperine”, this result is similar to that Al-kassie *et al.*, 2011.

4.2.3 Urea

There was a significant difference ($P < 0.05$) in urea across the treatment groups as birds. In the treatment group that received 0.5% inclusion of black pepper in their diets was the least, while the birds in treatment group that received 0.5% inclusion of zinc bacitracin in their diets had the highest urea value as shown in Table 4.2. Birds in treatment group that received 0.5% inclusion of black pepper in their diets was significantly lower ($P < 0.05$) compared to the birds in treatment group that received 0.5% inclusion of black pepper in their diets. This result is in contrast to the findings and report of Al-kassie, who reported an increased urea value.

4.2.4 Aspartate Transaminase (AST)

There was a significant difference ($P < 0.05$) in AST across the treatment groups. The birds that received zinc bacitracin at 0.5% inclusion in their diet (T_2) had the highest AST value, while the birds that received 0.5% inclusion in their diet had the least AST value. The least value of AST signals a non-toxicity effect of black pepper. Liver enzymes (AST) are liberated into the blood whenever liver cells are damaged and enzyme activity in the blood is increased.

4.2.5 Alkaline Phosphatase (ALT)

There was a significant difference ($P < 0.05$) in ALT across the treatment groups. The birds in treatment group that received zinc Bacitracin at 0.5% inclusion was higher (T_2), while the birds that received 0.5% inclusion of black pepper in their diets. The birds that received 0.5% inclusion of black pepper in their diets (T_5) was significantly lower ($P < 0.05$) compared to the

birds that received 0.2% inclusion Zinc Bacitracin in their diet (T3). There was no significant difference ($P>0.05$) in albumin (ALB) and Creatinine across the treatment groups.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Exploring the benefit of broiler production and the need to boost production due to increased demand and in a quest to resolve high cost of broilers feed, spices, such as; Black pepper (*Piper nigrum*) have been exploit. Additionally, regarding the detrimental effect of antibiotics on human consumer, spices have comparatively improved performance and safer than antibiotics. Spices such as black pepper (*Piper nigrum*) from this study has improved serum parameters and increased protein digestibility which is essential for broilers growth and productivity.

5.2 Recommendation

Reference to the medicinal and nutritive value of black pepper (*Piper nigrum*), it is a better substitute for black pepper as it has been proven from this study, to increased serum parameters which is essential to maintain proper health of broilers. Black pepper have also increased protein digestibility, a nutrient that is essential for efficient growth and development of broilers. Hence, Black pepper is recommended at 0.5%/10kg in broilers diet in order to invariably provide safe chicken meat for the human consumers and increase poultry production.

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