DETERMINATION OF OPTIMUM TRYPTOPHAN AND CRUDE PROTEIN REQUIREMENTS OF BROILER CHICKENS REARED IN THE TROPICAL ENVIRONMENT

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BY

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JUNE, 2016

DECLARATION

I declare that the work in the thesis entitled DETERMINATION OF OPTIMUM TRYPTOPHAN AND CRUDE PROTEIN REQUIREMENTS OF BROILER CHICKENS REARED IN THE TROPICAL ENVIRONMENT has been performed by me in the Department of Animal Science. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any University.

| --- | --- | --- |
| Emmanuel OPOOLA | Signature | Date |

CERTIFICATION

This thesis entitled DETERMINATION OF OPTIMUM TRYPTOPHAN AND CRUDE PROTEIN REQUIREMENTS OF BROILER CHICKENS REARED IN THE TROPICAL ENVIRONMENT, by Emmanuel OPOOLA meets the regulations governing the award of Doctor of Philosophy Degree in Animal Science of Ahmadu Bello University, Zaria, Nigeria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, my wife, Dr (Mrs) Oluyinka Opoola, my beloved son, Joseph Ayonioluwa Opoola and my parents: Mr and Mrs S.A. Opoola.

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ABSTRACT

Three experiments were conducted to determine tryptophan requirements of broiler chickens under tropical environment. Experiments 1 and 2 were conducted to determine the requirements for the cool and hot seasons respectively. The third experiment was conducted to determine the effect of diet formulations on the basis of four critical amino acid requirements (lysine, methionine, tryptophan and threonine) on the crude protein requirements of broiler starter and finisher chickens. The first and second experiments had 19 birds and 18 birds per replicate for the starter and finisher phases respectively. The minimum daily temperature was from $14^{o}C$ - $20^{o}C$ during the cool season while the maximum daily temperature is from $19^{0}C$- $40^{0}C$ during the hot season. Diet 1 had *insitu* tryptophan content of 0.15%. Diets 2 to 5 contained graded levels of supplemental tryptophan at 0.04% intervals giving total dietary tryptophan levels of 0.15, 0.19, 0.23, 0.27 and 0.31- percent levels for the starter phase studies conducted during the cool and hot seasons. For the finisher phases, there were five experimental diets. Diet 1 had *insitu* tryptophan content of 0.13% while diets 2 to 5 contained graded levels of supplemental tryptophan at 0.04% intervals resulting in total dietary tryptophan levels of 0.13, 0.17, 0.21, 0.25 and 0.29- percent respectively in both cool and hot season experiments. A total of two hundred and eighty five day old broiler chicks were used for each of the starter phase experiments while two hundred and seventy chicks were used for the finisher experiments. Experiments 1 and 2 had five dietary treatments. Each experiment lasted from 0 to 4 weeks for the starter phases and 5 - 8 weeks for the finisher phases. For the third experiment, four iso-caloric starter diets were formulated with the dietary crude protein levels increasing by 1% intervals from the 20% CP to 23% CP while the finisher diets had the crude protein increasing by 1% intervals from 18% CP to 21%.CP. The three experiments were of completely randomized designs, each treatment had three replications.

The third experiment had 16 birds and 15 birds per replicate for the starter and finisher phases respectively. The results for the cool season starter study showed that chicks fed starter diet containing 0.23% dietary tryptophan gave significantly (P<0.05) higher final body weight and average daily gain than those fed other dietary tryptophan levels. For the finisher phase, the birds fed 0.21% tryptophan diet performed significantly (P<0.05) better in terms of final weight, average daily gain and feed conversion ratio than all other treatments. Chickens fed diets containing supplemental tryptophan had better carcass values such as %breast, % thigh and % drumstick compared to chickens fed diets with no supplemental tryptophan in the diet. For experiment 2, conducted during hot season, starter chicks fed 0.23% total tryptophan diet gave better results in terms of final body weight, average daily gain and feed cost (₦) /kg gain compared to chicks fed other tryptophan diets. The results for the finisher phase showed that birds fed 0.21% tryptophan diet performed significantly (P<0.05) better in terms of final weight, average daily gain and feed cost (₦)/kg gain compared to other dietary tryptophan levels. Birds fed 0.21% total tryptophan diet also had significantly (P <0.05) better carcass weight, dressing percentage and % breast. It was also observed that broiler chickens fed diet high in essential amino acids (lysine, methionine, tryptophan and threonine) had slightly low abdominal fat and tissue fat. Results for the starter phase for the third experiment indicated 21% CP diet gave similar results as obtained with the higher crude protein diets in terms of the final weight, and average daily gain of chicks across treatments. The FCR also indicated similar results for the 21 and 23 % CP diets. For the finisher phase chicks fed 19 %CP or 20%CP diets had similar results for final weight, weight gain and average daily gain as those fed the 21% CP diet. Also the digestibility for ash and NFE were significantly better for the 21% CP diet. The 21% CP diet was however regarded to be the optimum crude protein requirement due to slightly better results for FCR and feed cost per kg gain. It was concluded that dietary tryptophan requirements during the cool and hot seasons for the

starter phase was 0.23% and for the finisher phase was 0.21%. The crude protein of broiler starter diets can also be reduced from the current practice of 24 or 23 percent to 21% while the optimum for the finisher phase stood at 21% CP.

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ABBREVATIONS

AA	Amino acids
ADWG	Average daily weight gain
ALP	Alkaline Phosphatase
ALT	Alanine Amino-Transferase
AOAC	Association of Official Analytical Chemists
AST	Aspartate Amino-Transferase
CK	Craetine kinase
CP	Crude protein
EE	Ether Extract
FCR	Feed conversion ratio
Hb	Haemoglobin count
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin count
MCV	Mean cell volume
ME	Metabolizable energy
NE	Net energy
NFE	Nitrogen Free Extract
NRC	National Research Council
Avail P	Available Phosphorus
PCV	Packed cell volume
RBC	Red blood cells
SBM	Soyabean meal
SEM	Standard Error of Means
TP	Total protein
Try	Tryptophan
TSAA	Total sulphur amino acid
WBC	White blood cells

CHAPTER ONE

1.0 INTRODUCTION

Poultry production represents a large section in animal production. The world poultry production has increased significantly over the past sixty years with broiler production making up a large part of the industry. The chicken meat production account for 88% of the world poultry meat output (Economic Research Service/ USDA, 2001). There are many facets of production, and hence many areas that are of potential concern for the welfare of animals. These areas may include nutrition, genetics and breeding, pre-slaughter, slaughter practices among others (Mench and Siegel, 1997).

Feed cost is a major factor in animal production, representing 50-70 percent of the total cost of animal production (Bawa *et al*., 2003; Ogundipe *et al*., 2003), as it is well understood that a large portion of costs associated with poultry production involves meeting the protein or amino acids requirements of birds (Corzo *et al*., 2005a). Protein and amino acids are generally considered as one of the major cost components of poultry diets and the trend of researches among broiler nutritionist is to formulate diets for maximum broiler performance. Therefore, providing balanced dietary crude protein and essential amino acids according to the requirements is very important (Ogundipe *et al*., 2003: Eits *et al*., 2004). All stakeholders in poultry production and business are interested in the appropriate quantity and quality of a formulated feed that results in an optimum economic return (Onimisi *et al.,* 2008). Feed intake is one of the important factors that may influence animal live performance as well as health and carcass characteristics. The nutrient content of a diet may affect voluntary feed intake and growth performance of broiler chickens. Feed intake depends largely on energy density (Noblet and Van Milgen, 2004). On the other hand, feed intake can be depressed both

by a severe deficiency in the limiting amino acids or an excessive supply of some essential amino acids.

Protein synthesis requires that all necessary amino acids are available to the tissues (Boisen *et al*, 2000). Therefore the dietary intake of essential and the non-essential amino acids needs to meet the requirement. Requirements of dietary amino acid can be classified as qualitative and quantitative. Qualitative requirements are related to the question of "*what* amino acids are required for maintenance, optimum performance (e.g., growth, lactation, reproduction, and sports competition), and optimum health (e.g., prevention of chronic metabolic disorders, resistance to infectious disease, and recovery from illness)?" Quantitative requirements refer to the question of "*how much* of an amino acid (AA) is required for maintenance, optimum growth, and optimum health?" Feeding experiments have traditionally been employed to determine both qualitative and quantitative requirements of dietary AA by animals. The ideal amino acid is useful in several ways; it results to more efficient use of protein, reduction in overfeeding and reduction in nitrogen excretion (NRC, 1994). There is still a common belief that whenever crude protein levels are lowered, performance is negatively affected. This is a result of a number of studies where researchers have lowered crude protein levels beyond practical formulation levels and then failed to supplement all limiting amino acids to sufficient levels (Abebe and Morris 1990; Khaled, 2001). In some cases crude protein levels were lowered without supplementing amino acids other than lysine and methionine. Although several theories for why performance is negatively affected have been proposed including amino acids imbalance, increase in blood ammonia level and change in the ratio of net energy to metabolizable energy (Aftab *et al*., 2006). Results from different studies about performance of broilers fed low crude protein diets are contradictory. This may be due to differences based on the

basal diets, assay length, age of chicks, environment, genotype or other factors. Furthermore results pooled from researches carried out on the manipulation of dietary protein showed that protein can have various effects on broiler performance, body weights, feed intake and especially carcass composition (Waldroup *et al*., 2005). The increased concentration of animals together with environment constraints (i.e. reduction of land and water pollution by nitrogen and phosphorus from manure spread) have forced the nutritionist to propose new feeding strategies for animals. The most efficient technique involves reducing the amount of nitrogen intake and keeping the amino acid supplies adequate for meeting the animal's requirements (Dean *et al*., 2006).

Tryptophan is an essential amino acid in poultry and is required for a wide variety of metabolic activities, because its concentration in organisms is among the lowest of all amino acids, it can easily play a rate-limiting role in protein synthesis. Tryptophan is a well known protein- forming (proteogenic) amino acid. Tryptophan which is the third or fourth limiting amino acid in poultry is the precursor of serotonin, which plays an important role in feed intake, weight gain, animal performance and feed conversion ratio. Its levels influence behaviour and carcass composition (MacKenzie *et al*., 2004; Corzo *et al.,* 2005b). Tryptophan was found to have a sedative effect because of its influence on levels of the neurotransmitter serotonin (Newberry and Blair, 1993). Several studies have indicated that an increase in tryptophan levels causes a decrease in the total liver lipids of broiler and layer chickens. According to Peganova and Eder (2003), tryptophan is significant in the formulation of feed with reduced content of protein in the nutrition of laying hens. NRC (1994) pointed out the requirement of tryptophan for broilers for the age of 0-3week as 0.20%, 3-6 week as 0.18% and 6 – 8weeks as 0.16%. Increased dietary tryptophan resulted in improvement of blood plasma parameters such as glucose, serum concentration of total protein and albumin

(Corzo *et al*., 2005b). Amino acids like lysine, methionine, threonine and tryptophan are indispensable amino acids for body protein deposition and growth in broiler production. Thus, deficiency in tryptophan affects the utilization of dietary lysine and threonine, reduction in feed intake and consequently animal growth (Peganova and Eder, 2003). L-Tryptophan supplementation also contributes to a better utilization of the other amino acids and of the feed in general. Besides its utilization for protein synthesis, tryptophan is involved in other biological functions such as appetite regulation, immune response and health maintenance. The depressive effect of tryptophan deficiency (Uttrecht *et al*., 1991) is aggravated in the presence of excess protein in the form of large neutral amino acids.

There is little information in the literature about tryptophan requirements for broilers and egg laying hens and some of the results are controversial. Reasons for this may be due to genotype, seasons of rearing, protein levels used in the diets and tryptophan digestibility of the feedstuffs used in chickens. Uttrecht *et al*. (1991) estimated that the requirement of tryptophan for male broilers, from 1 to 20 d of age, is not greater than 0.16%. The level of tryptophan recommended for broiler chickens by the NRC (1994), from 0 to 3 wk of age, is 0.20% and from 4-8 weeks is 0.18% of the diet. Larbier and Leclercq (1994) reported that the tryptophan requirement, expressed in relation to the lysine level of the diet is 19.2% and 18.0%, respectively.

In a tropical climate such as in Nigeria, there are four distinct seasons in a year; the early dry season which covers the period between October and January, the late dry season which covers the period between February and March, the early wet season which covers the period between April- July and the late wet season which covers the period between August - September, (Institute for Agricultural Research (2011). The

studies which have been on the use of synthetic amino acids, dietary crude protein and temperature were limited to 35^0C and in most cases in the temperate regions. On the other hand, the temperature rises to 45 to 50^0C in the tropical regions. As a result, the nutrient requirements may differ in the tropical regions. Not much compilation of nutrient requirements of chickens has been done in the tropics. Most nutrient requirements compilations are being determined in the temperate regions. Therefore the following experiments were aimed at evaluating diet formulation on the basis of four critical amino acids on the crude protein requirements of broiler chickens and to determine the requirements of tryptophan for broiler chickens reared at different phases of production under the tropical environment. This will enable the modern broiler chickens in the tropical environment to fully express their genetic potentials and possibly to permit further reduction of dietary protein levels.

1.1 OBJECTIVES OF THE STUDY

The Objectives of this study were as follows; to determine

1. The optimum level of tryptophan in practical broiler rations during the cool season under tropical environment.

2. The optimum level of tryptophan in practical broiler rations during the hot season under tropical environment.

3. The effect of varying dietary tryptophan levels on abdominal fat content, tissue fat and tissue crude protein of broiler chickens in the cool and hot seasons under tropical environment.

4. The effect of diet formulation on the basis of four critical essential amino acid requirements on the crude protein requirement of broiler chickens.

5. The effect of diets formulated on the basis of four critical essential amino acids on carcass characteristics and nutrient digestibilities of broiler chickens at each level of crude protein.

1.2 HYPOTHESIS

H_o: Dietary tryptophan levels have no significant effects on broiler growth performance, during the cool and hot seasons under the tropical environment.

H_o: Dietary levels of crude protein fed on the basis of essential amino acids at each crude protein level has no significant effects on broiler growth performance, during the cool and hot seasons under the tropical environment.

H_a: Dietary tryptophan has significant effects on broiler growth performance, haematological parameter, carcass characteristics, nutrient digestibility, tissue crude protein and tissue fat of broiler chickens.

H_a: Diets formulated on the basis of four critical essential amino acids requirements on the crude protein requirements has significant effects on broiler growth performance, haematological parameter, carcass characteristics, nutrient digestibility, tissue crude protein and tissue fat of broiler chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Protein in Poultry Ration

Protein is generally considered as one of the major cost components of the poultry diets (Ogundipe *et al*., 2003; Bawa *et al*., 2003). When formulating a broiler's diet the main

emphasis is placed on the crude protein (CP), because the protein level in the broilers' diet strongly affects performance, feed cost and profitability of a broiler production enterprise (Eits *et al*., 2004). To meet the protein requirements of broilers is much difficult as compared to other nutrients (Ojano-Dirain and Waldroup, 2002). Broilers have high dietary crude protein needs because of their genetic tendency of growing fast, also because optimizing feed conversion and other performance traits require diets that are quite high in protein. Dietary protein level, therefore, has major effects on growth, carcass composition and overall cost of the finished product (Bregendahl *et al*., 2002). The production of lean meat has become more important as the trend has changed from the sale of live birds to processed meat now with abdominal fat having much lower price.

It is well known that the crude protein and amino acids status of a diet affects the carcass composition of broilers with increased carcass protein and decreased carcass fat accompanying increases in dietary protein or essential amino acid contents because protein has the ability to act as a lipotrophic agent (Neto *et al*., 2000; Si *et al*., 2001). Therefore dietary composition has a major effect on body composition of broilers (Collin *et al*., 2003). Excess dietary crude protein results in lean birds but reduces feed efficiency whereas less than optimal protein content increases fat retention (Si *et al*., 2001). Therefore, importance of using appropriate amounts of balanced dietary crude protein and amino acid for poultry is a high priority issue for several reasons like feed efficiency, cost and environmental concerns. The concept of formulating broiler diets on an ideal amino acid basis decreases the main problems related to protein and amino acid nutrition.

2.2 History, Constraint and Application of Ideal Amino Acids in Poultry Feed Formulation

Initially, animal diets especially the poultry diets were formulated on total crude protein basis while the amino acid requirements of birds were not considered NRC (1994). Due to this limitation, nutritionists then started to formulate poultry rations on an amino acid requirement basis. Although enough data were not available on the amino acids requirements and composition of feed ingredients, it was still a major development towards fulfilling the true requirements of the bird. After that, there was an increased trend of routine laboratory analyses of common feedstuffs for amino acids and formulas were developed to predict amino acid contents based on the crude protein content of the feedstuffs. Presently, diets are being formulated on amino acid digestibility values to have the same amino acid content using different feedstuffs. The ideal amino acid is useful in several ways; more efficient use of protein, reduction in overfeeding, rapid determination of all amino acid requirements as genetics change are of increasing importance, reduction in nitrogen excretion. It also allows us to formulate very low protein diets. The use of the ideal amino acid however has some difficulty based on the apparent lack of practicality in formulation. Attempt to meet requirements exactly is expensive as it means that very low protein diets need to be supplemented with high amounts of essential amino acids (Firman and Boling, 1998).

2.3 Importance of Amino Acids in Poultry Production

Amino acids are important in poultry nutrition not only for protein deposition, but also for other metabolic functions; such as improving immune status, improving growth rate and gut functions. Amino acids requirements may vary due to changes in dietary composition, environmental factors (change in temperature and relative humidity) and

breeding and selection (Baker and Han, 1994). Thus amino acids requirements have remained an ongoing research topic. It has been reported that formulating diets with optimal amino acids will not only reduce the feed costs and improve the production efficiency, but also have environmental benefits such as reducing nitrogen excretion. Protein is a critical component of poultry rations, and along with the other major nutrient classes such as carbohydrates, fats, vitamins, minerals, and water, is essential for life (NRC, 1994).

Proteins are polymers that are made up of α-amino acids in covalent linkage by peptide bonds, and the structure of individual α-amino acids includes an amino group and a carboxyl group linked to an α- carbon, as well as a side chain that differs for each amino acid. Proteins are broken down by hydrolysis during digestion to yield these amino acids, which are then utilized in the body to fulfill a variety of functions in poultry, including as structural components of skin, feathers, and muscle, as well as filling important metabolic roles as blood plasma proteins, enzymes, hormones, and immune antibodies which are all individually involved in specific functions in the body (Han *et al*., 1992). Dietary protein level has major effects on growth, carcass composition and overall cost of the finished products as production of lean meat has become more important (Collin *et al*., 2003).

It is well known that imbalance in amino acids profile will result in reduced growth rate and feed utilization efficiency. A deficiency in dietary amino acids impairs immune function. Protein and amino acids are some of the most expensive nutrients in feed formulation; thus selecting the correct level of amino acids needed in feed formulation has economical impact. In addition, diets formulated with balanced amino acids profile will reduce nitrogen losses in animal waste and lead to an environmental benefit. The

amino acids requirements are influenced by many factors including dietary, environmental, genetics and productive purposes.

2.4 Importance of Ideal Amino Acids in Broiler Production

The ideal amino acid is the exact balance of amino acid that precisely meets the animal's needs for lean tissue growth. In ideal amino acid conditions, the essential and non-essential amino acid levels exactly provide the requirement, leaving no extra amino acids nitrogen (N) for elimination (NRC, 1994). Antagonistic interaction effects on overall birds' performance can occur by increasing a specific amino acid if other amino acids are at deficient level. Therefore, the concept of ideal amino acids is based upon achieving a perfect dietary amino acid balance i.e. all amino acid are present in amounts that exactly meet the animals' requirements. Thus amino acid excesses are eliminated and consequently, protein accretion can occur with maximum efficiency (Chung and Baker, 1992). Baker and Han (1994) reported that the amino acid requirements cannot be applied to all birds under all dietary, sex, and body compositional circumstances.

2.5 Importance of Ideal Balance of Amino Acids in Low Crude Protein Diets

One of the advantages of feed formulation on the basis of ideal amino acid concept is seen in the formulation of low protein diets, allowing for the same animal performance if compared to unbalanced high protein diets. However, in practice, it is very difficult and sometimes not possible to formulate diets with natural feed ingredients that will provide the entire amino acid needed by birds in adequate quantities and also to maintain an optimal amino acid balance with minimal excesses (Han *et al*., 1992). However, use of commercially available synthetic essential amino acid in broiler feeds can satisfy broiler requirements for essential amino acids (Moran and Stilborn, 1996).

In this way, excesses of dietary amino acids are decreased, making the balance of dietary crude protein more ideal with reduced dietary crude protein content (Moran and Bushong, 1992; Bregendahl *et al*., 2002).

It is known that broiler chicks fed diets lower in protein and supplemented with limiting amino acids like lysine and methionine show similar performance to those fed high crude protein diets (Mozdeh *et al*., 2010). This would reduce feed cost in both starting and growing broilers (Bregendahl *et al*., 2002). In recent time, lysine, methionine, threonine and in some cases, tryptophan are being used in poultry feed formulation in a cost-effective way (NRC, 1994). Waldroup *et al*. (2005) reported that this will cause the development of more crystalline amino acids for use in feed formulation leading to more reductions in dietary crude protein. There are great discrepancies, however, in results of feeding broiler chicks, low protein diets supplemented with essential amino acids. These discrepancies may be due to, among other factors, the extent of reduction of crude protein contents, the inclusion or otherwise of the crude protein and metabolizable energy (ME) contributions of the AA supplements, the class and age of the chickens used and whether or not the intact protein sources are kept at constant ratios to minimize amino acid imbalances.

Lowering dietary crude protein content may result in growth performance similar to that of a normal or high crude protein diet (Parr and Summer, 1991; Han *et al*., 1992; Moran and Stilborn, 1996). Conversely, in some previous studies, feeding low crude protein diets to broilers reduced growth performance and carcass characteristics (Jensen and Colgano, 1991; Jacob *et al*., 1994; Ferguson *et al*., 1998). Some studies with broiler chickens have also shown that performance in terms of growth and carcass composition is adversely affected with low crude protein diets (Pinchasov *et al*., 1990; Aletor *et al*.,

2000). Recently, Bregendahl *et al*. (2002) concluded that chicks fed low crude protein (19%) diets had lower growth rate, feed efficiency and N retention and more ether extract than chicks fed the control (23%) diets despite increased supplementation of crystalline essential and non essential amino acids. Several potential reasons for the decreased performance were reported. This include changing the dietary net energy concentration (Leeson *et al*., 2000), addition of several crystalline non essential amino acids (Moran and Stilborn, 1996; Aletor *et al*., 2000; Sohail *et al*., 2003; Dean *et al*., 2006), increasing dietary concentration of essential amino acids than standard requirements (Waldroup *et al*., 2005) and considering the dietary acid base balance (Waldroup *et al*., 2005). Previously reported studies showed that decreasing the crude protein content of broiler diets is possible only to a certain limit after which the performance is affected even if the requirements for the entire essential amino acids have been met. Therefore, although decrease in crude protein content of broilers diets is possible, there are biological limits to the replacement of crude protein with synthetic amino acids. Therefore, crude protein is still very important factor to get good performance.

2.6 The Effect of Amino Acid Interactions, Imbalances, and Antagonisms in Poultry Production

The interactions between amino acids when attempting to meet poultry amino acid requirements need some consideration. This is to avoid imbalances or antagonisms. A deficiency of one amino acid is enough to cause problems with the entire diet and birds may attempt to make up for the deficiency by consuming more feed, thereby reducing the efficiency of the diet (Corzo *et al*., 2005a). Conversely, an amino acid imbalance arises with changes in the proportion of amino acids in the ration, usually because one amino acid will be deficient and others provided in excess. Imbalances cause

deleterious effects in performance resulting from reduced feed intake likely due to changes in the pattern of amino acids in the plasma which may affect satiety, and this may be overcome by supplementation of the most limiting amino acids(s). Two main theories have been proposed to more precisely explain the mechanisms behind the reduced feed intake seen in birds consuming imbalanced rations.

The anabolic theory, proposed by NRC (1994), suggests that an excess of amino acids will stimulate synthesis or suppress breakdown of protein in the liver, leaving more of the limiting amino acid there. This then leads to a reduced concentration of the first-limiting amino acid in the blood. The resulting altered amino acid pattern may be detected by the brain, consequently depressing feed intake (Waldroup *et al*., 2005). An alternative explanation is presented in the catabolic theory, proposed by Lewis and D'Mello (2004). This theory states that an excess of one amino acid will enhance the catabolism and excretion of the other amino acids and consequently encourage loss of the target amino acid. This disrupts the pattern of free amino acids in the plasma and tissue causing reduced intake and performance. Amino acid antagonisms involve interactions in which an increase in the requirement of one indispensable amino acid results from the addition of another amino acid that is structurally related. Common examples of amino acid antagonisms in poultry include the lysine and arginine antagonism, and the branched-chain amino acid antagonisms between leucine, isoleucine, and valine (NRC, 1994). The interaction between lysine and arginine arises from excessive lysine in relation to arginine, increasing the requirement for arginine through intensified competition for re-absorption in the renal tubules and enhanced activity of renal arginase, which degrades arginine to ornithine and urea.

2.7 Protein and Amino Acid Requirements of Broiler chickens

Poultry actually have protein requirements as much as an amino acid requirement; this is because there are biological limits to the replacement of crude protein diets with synthetic amino acids although it is these amino acids that are used in the body for a variety of functions. The National Research Council (1994), states that poultry do not have a protein requirement as much as an amino acids requirement and it is these amino acids that are used in the body for a variety of functions. Nutrient requirements are often defined as the minimum dietary concentration required for maximum performance, and nutritionists frequently provide a margin of safety above the requirement when formulating to avoid deficiencies and the resultant decreases in performance (Sterling *et al.*, 2006).

A protein deficiency, caused by either one or more limiting amino acids or an overall inadequate consumption of protein, will result in decreases in parameters such as rate of growth, nitrogen retention, feed consumption and feed utilization, while an over-consumption of protein results in the catabolism of amino acids through deamination and excretion as uric acid which is both energetically and economically inefficient, or, in severe cases, ammonia toxicity may result. It is essential to try to meet the requirement of the bird as closely as possible in order to maximize production and profitability.

2.8 The Importance of Essential and Non-essential Amino Acids in Broiler Production

The importance of essential and non essential amino acids as opposed to crude protein in feeding of animals has been investigated and recognized by researchers mostly in the temperate region. The works of Rosa *et al*. (2001), Shan *et al.* (2003), Corzo *et al*. (2005a), Hsia *et al*. (2005) and Mozdeh *et al*. (2010), demonstrated the essentiality of

amino acids such methionine, lysine, threonine, tryptophan and arginine. They reported significant decreases in growth of broiler chickens fed low concentrations of these EAA, similar results were reported in pullets. The use of essential and non-essential amino acids will allow a relative feed cost reduction together with the lowering of nitrogen excretion which is now a global concern (Namroud *et al*., 2008).

Twenty amino acids have been be reported to be present in protein, while all of the twenty amino acids are required in the body, only some, termed the essential or indispensable amino acids, must be provided in the diet. Essential amino acids are those that cannot be synthesized by the body either at all or in amounts great enough to meet physiological needs. They include valine, tryptophan, threonine, isoleucine, leucine, lysine, arginine, histidine, phenylalanine, methionine and, in some cases for poultry, glycine and serine (NRC, 1994). The nonessential amino acids, which include proline, alanine, cystine, tyrosine, glutamic acid and aspartic acid, can be synthesized from other amino acids or nitrogen in the bird, and so are not necessarily required in the diet.

2.9 Limiting Amino Acids for Poultry

The limiting amino acid of a protein or whole feed can be defined as the essential amino acid found in the smallest quantity relative to its requirement (NRC, 1994). The order of limitation can vary among individual ingredients or, in a complete feed, the level and combination of ingredients as well as overall protein level. Corn and soybean meal (SBM) are the two most commonly utilized ingredients for broiler diets in most countries and a great deal of research has been conducted in an effort to determine the limiting amino acids in such rations.

Typically, the order of limitation in corn-SBM broiler diets containing 20-23% crude protein and approximately 75-85% of the total dietary amino acids provided by SBM is methionine, lysine, threonine, tryptophan and valine (Baker *et al*., 1993; Fernandez *et al*., 1994). This commonly utilized level of crude protein in corn-SBM diets usually will not result in a deficiency of the less limiting amino acids (Kidd *et al*., 2001). However, interest in reducing the protein level in poultry diets has grown as nutritionists and producers attempt to decrease diet costs, environmental impact, and excess dietary amino acids which are utilized inefficiently and can cause reduced feed intake and body weight gain (Waldroup *et al*., 2005). The use of synthetic amino acids has made it possible to decrease crude protein to a degree, but has also furthered the need for the assessment of the limiting amino acids in low protein corn-SBM diets. The two most common methods of determining limiting amino acids are addition assays. This involves a process in which amino acids are added individually and in combination to a low protein diet or one that is deficient in a particular amino acid and deletion assays which employs a ration that initially meets all amino acid requirements and then from it individual amino acids are systematically removed and the effects on performance are measured. Inconsistent results have been reported for the order of amino acid limitation in low protein corn and soybean meal diets. Han *et al*, (1992) fed broiler chicks a 19% crude protein diet, and determined that methionine and lysine were first and second limiting, respectively. Threonine, tryptophan, Arginine, and valine were limiting as well.

2.10 Factors Affecting Protein and Amino Acid Requirements

A number of factors influence the protein and amino acid requirements of poultry. Changes in protein and amino acids requirements occur as a result of bird variation in

age, gender, production status, size, species, and strain (NRC, 1994; Kidd *et al*., 2001), as well as variation in protein quality and digestibility. Season of rearing may also affect the amino acid requirements, as intake is often decreased in excessive heat season and increased during periods of cold. As a result, adjustments to amino acid levels in the ration may be required in periods of heat and cold stress, although contradictions concerning this approach exist in the literature. When the effects of increasing essential amino acid levels to 110% of the expected requirement while maintaining constant crude protein were tested, researchers found no differences in live performance; however, abdominal fat was increased in the treatment receiving increased levels of amino acids suggesting an improvement in recovery of productive energy from the dietary metabolizable energy, which was deposited as fat rather than muscle. However, other research indicates that reducing the heat increment of the diet by reducing crude protein and providing a well-balanced amino acid supply that closely matches the requirement of the bird can alleviate the poor performance associated with heat stress. Other factors that affect both feed and amino acid consumption include health status of the bird, the form of the feed, (such as mash versus pellets) and a variety of environmental stressors.

2.11 The Relationship between Amino Acid Requirements, Protein and Energy

Early use of fat in poultry rations as a means of boosting the energy content and corresponding decreases in feed intake that were observed encouraged researchers to investigate the relationship between energy and protein and several groups came to the conclusion that if fat was added to a ration, the percentage of protein should also be adjusted. Fat additions to a high protein diet will result in improved growth, indicating the existence of a relationship between protein and energy. Changes in dietary

metabolizable energy will affect feed intake, and therefore the intake of amino acids (NRC, 1994).

2.12 Amino Acid Digestibility in Broiler Chickens

The term "digestibility" as it is used in animal nutrition refers to the percentage of a nutrient or a feed that is available for absorption and use by the body and is therefore one of the determining factors of the nutritive value of a feedstuff (Chung and Baker, 1992).Amino acid requirements such as those found in the NRC (1994) are provided on a total basis, and so do not account for endogenous loss in the bird or those that are passed through to the excreta. In 1986, Sibbald published the methodology for an assay which may be used to determine the amino acid digestibility in feed ingredients or mixed feeds using adult cecectomized roosters, and since then a number of studies have been conducted that present the importance of formulating rations based on digestible amino acids rather than total amino acids (Fernandez *et* al., 1994; Rostagno *et al*., 1995).

A number of factors can affect digestibility and these include; processing of the feedstuff, age of the animal, species, strain, sex and physiological state. Digestible amino acid values are also very useful and are superior to total amino acid values when formulating diets containing animal byproducts such as meat and bone meal, which can vary widely in digestibility, or when feedstuffs with differing amino acid digestibility values are used to replace higher quality ingredients such as corn and soybean meal (Rostagno *et al*., 1995).

2.13 Amino Acid Requirements as Related to Protein Levels

The levels of total protein in the diet of poultry may affect their requirements for amino acid. Amino acid requirements have been shown to fluctuate with the level of protein in the diet; specifically, the amino acid requirement as a percentage of the diet will increase with the concentration of dietary crude protein. It is also apparent that amino acid supplementation can affect the requirement for protein. Early research with turkeys concluded that the 28% crude protein requirement for turkeys could be reduced to 20% with proper amino acid supplementation with similar performance (Corzo *et al*., 2005a). Similar work in broilers has shown that crude protein in diets can be reduced to a point without harming performance with the addition of lysine and methionine (Corzo *et al*., 2005b) or a combination of essential amino acids and a source of non-essential amino acids/nitrogen (Corzo *et al*., 2005b). It has been suggested that when the level of total dietary protein (amino acids) is reduced, the requirement for each amino acid also decreases due to the depression in growth resulting from a single or many amino acid deficiencies and that by supplementing each amino acid individually to a low protein diet it is possible to improve the overall balance of the ration.

2.14 Effect of Low Crude Protein Diets on Broiler Performance

2.14.1 Effect of Low Crude Protein Diets on Broiler Feed Intake

The fate and effect of feed intake of broiler chickens fed low crude protein diets is evident in their growth performance. Some studies reported that, there was no effect or even increased feed intake (Han *et al*., 1992; Bregendahl *et al*., 2002; Fatufe and Rodehutscord, 2005; Elmutaz *et al*., 2014) while in other studies; feed intake decreased with low crude protein diets (Si *et al*., 2004; Jiang *et al*., 2005). It is possible that, reduced growth performance with low crude protein diets may be due to decreased feed

intake in some cases. Some researchers have found that feed intake increased during starter period due to the deficiency of any nonessential amino acids or too low nonessential to essential amino acids ratio during early age as the crude protein requirements are high during this period. However, Araujo *et al*. (2004) and Dean *et al*. (2006) found no differences in feed intake during this period with low crude protein diets.

Another major factor is that feed intake depends on the calorific value of the feed because birds primarily eat to maintain their energy intake (Leeson *et al*., 2000). It was reported that the effect of dietary energy on the growth performance depends on the animal's ability to alter feed intake with the change in dietary energy (Ogundipe *et al*., 2003). Hence, equal feed intakes may be expected if low crude protein diets with same ME values are supplemented with limiting amino acids. Han *et al*. (1992) found no differences in feed intake of broilers when the crude protein content of the diet was decreased from 23 to 20%. Kidd *et al*. (2001) reported an increase in feed intake of chicks fed diets containing 19% crude protein as compared to those fed diets containing 22.5% crude protein. Bregendahl *et al*. (2002) found significantly increased feed intake of broilers fed 20% amino acids supplemented diet as compared to those fed a 23% crude protein diet. A few reasons that might change the feed intake of broilers fed low crude protein diets can be following:

1. The dietary electrolyte balance and salts like potassium (K) may influence the feed intake by promoting the water intake particularly during heat-stress (Aftab *et al*., 2006).

2. Net energy (NE) of low crude protein diets: In low crude protein diets, NE:ME ratio is found to be increased. This increased ratio may lead to an increased feed intake as a result of lower heat increment.
3. Changed amino acid contents of low crude protein diets: In low crude protein diets, level of non essential amino acids is reduced with minimized excess of the essential amino acids. The deficiency of one or more amino acids may result in increased feed intake.
4. Ingestion of diet containing free amino acids may cause a rapid/unsynchronized flux of amino acids into the blood stream. This may reduce feed intake by unbalancing plasma amino acid contents (Austic *et al*., 2000).

2.14.2 Effect of Low Crude Protein Diets on Weight Gain of Broiler Chickens

An experiment conducted by a team of researchers (Si *et al*., 2001) on the effect of tryptophan to large neutral amino acid ratios and overall amino acid levels on utilization of diets low in crude protein by broilers showed that overall birds fed diets with less than 22% CP and a minimum of 100% of NRC recommended amino acid concentration weighed significantly less than those fed the diets with 22% CP. It was reported by Parr and Summer, (1991) and also by Moran and Stilborn, (1996) that reducing dietary crude protein by 2% did not affect body weight gain. There were no adverse effects on the weight gain with low crude protein diets during first six weeks of age (Han *et al*., 1992; Moran and Bushong 1992). It was pointed out that perhaps the utilization of protein is better with low crude protein diets (Temim *et al*., 2000; Zarate *et al*., 2003). However, some researchers have reported reduced growth performance with low crude protein

diets. Bregendahl *et al*. (2002) reported that chicks fed low crude protein (20%) amino acids supplemented diet had lower weight as compared to those fed high crude protein diet (23%).

In another study, Colnago *et al*. (1991) observed a depression in body weight when the crude protein was reduced from 23 to 18% and supplemented with essential amino acids. Kerr and Kidd (1999a) also reported a significant depression in body weight when the crude protein was reduced. Some workers have reported reduced growth performance (Aletor*et al*., 2000) when the dietary crude protein content is lowered by more than three to four percentage points. It was reported that broiler performance was not affected even when crude protein of amino acids supplemented diet was decreased by 20-25% of positive control (Fatufe and Rodehutscord, 2005; Dean *et al*., 2006). It can be argued that if balance of essential amino acid is according to the birds' requirements, the dietary crude protein may significantly be reduced.

2.14.3 Effect of Low Crude Protein Diets on Feed Conversion Ratio (FCR)

A research was conducted by Araujo *et al*., (2004) and Rezaei *et al*., (2004) who fed broiler chickens with low dietary protein during the starter, grower and finisher periods. They observed no differences in FCR of the birds when fed low protein diets supplemented with amino acids according to an ideal amino acids ratio. However, Moran and Bushong (1992) found an increase in FCR with the decrease in crude protein (23 to 20% and 20 to 17% in starter and grower periods, respectively) by amino acids supplementation. Ferguson *et al*. (1998) noted significant increase in FCR when crude protein was reduced from 21.5 to 19.6% at 6 weeks of age. Neto *et al*. (2000) also reported a 13% increase in FCR of birds fed 17% crude protein diets as compared to

those fed 24% crude protein diet. The increased FCR might be the result of the birds fed low crude protein diets consuming more feed and growing more slowly. This increased feed intake is considered to be due to the deficiency of some critical amino acids. Hence, the efficiency of protein utilization is decreased. There may also be differences in amino acids digestibility of diets because there are various levels of corn, soybean meal and synthetic amino acids resulting in different availabilities of amino acids and increased feed intake and FCR.

2.14.4 Effect of Low Crude Protein Diets on Whole Body Composition

In recent years, the influence of diet on carcass composition has attracted an increased attention. Dietary manipulation may be better utilized for improving carcass composition and efficiency of nutrient utilization. The amino acids status of a diet influences the carcass composition of broilers and if essential amino acids are adequate, there is no effect on the protein and fat content of the carcass even if the crude protein is reduced (Si *et al*., 2001). Increasing dietary crude protein has been shown to increase carcass protein (Whitehead, 1990). However, Parr and Summers (1991) reported no effect on carcass protein of chicks fed low protein amino acids supplemented diets (21-16.5% crude protein) as compared to those fed a 23% crude protein diet.

Similarly, Neto *et al*. (2000) also found no effect on breast muscle protein when the dietary crude protein was reduced from 24 to 17% by supplementing essential amino acids and methionine. Hai and Balha, (2000) conducted experiments to study the effects

of isocaloric low crude protein diets adequate in levels of essential amino acids on broiler chicken performance. They concluded that decrease in dietary crude protein level had no negative effect on carcass characteristics and carcass protein. In another experiment, Bregendahl *et al*. (2002) did not find any significant difference in the whole body crude protein content of chicks fed low crude protein diets as compared to the control. However, they failed to get similar results in two other experiments of the same study despite increased dietary concentration of crystalline essential and non essential amino acids. Bregendahl *et al*. (2002) found that chicks fed low crude protein diets retained more body fat than the chicks fed control diet. This is mainly due to the fact that birds fed diets deficient in any nutrient consume more to meet their requirements for gain, resulting in increased fat deposition. The increase in total body fat as dietary CP and AA depart from the optimum levels could therefore be explained in terms of protein as well as AA deficiency and imbalance.

2.14.5 Effect of Low Crude Protein Diets on Efficiency of Nutrient Utilization

There has been growing evidence that performance improvement may be associated with nutrient digestibility improvement (El- Husseiny *et al*., 2007; Ratiyanto *et al*., 2012). Efficiency of nutrient utilization for the production of edible protein is equally as important as performance or carcass composition in determining optimum dietary levels due to high cost of nutrients. Although, efficiency of energy utilization has been shown to improve with decrease in dietary protein, results on efficiency of protein utilization have been less consistent. The efficiency of protein utilization is better with low CP diets (Whitehead, 1990). However, in situations where low energy diets are used or where maximized returns of feed protein versus food protein are desirable, lower protein diets should be considered. It would appear that increased weight gain in high

density diets is being achieved by an increase in carcass fat with high energy diets and an increase in carcass water with high protein diets with little increased protein deposition in the carcass.

The amino acid (AA) digestibility of feed ingredients by broiler chickens has most often been determined using older birds. However, these values are unlikely to predict AA digestibility in chicks during the initial period post-hatch because of their immature digestive tracts and lower nutrient utilization. Beyond this, the chick has an immature intestinal mucosa, which also could limit both the digestion and absorption of nutrients (Geyra *et al*., 2001; Sklan, 2001 Parsons, 2004). Research examining the digestion of nutrient by young broilers has found that the digestion of proteins and absorption of amino acids is the most limiting of the nutrients (Noy and Sklan, 2001).

Research in AA digestibility in young birds has shown that ingredient digestibility is variable (Batal and Parsons, 2002). Additionally, the degree of improvement with age for ingredients is quite different while chicks do not digest some protein sources very well. Other protein sources are highly digestible and do not increase with age (Batal and Parsons, 2002; Parson, 2004). In the hatchling there are numerous factors that could reduce protein digestion and absorption. These factors include reduced pro-ventricular acid secretions, reduced pancreatic and intestinal mucosa secretions such as bile and proteolytic enzymes (Noy and Sklan, 1995) and juvenile intestinal AA transporters (Noy and Sklan, 2001). Variations can be explained by the natural variability of these ingredients due to ingredient quality and processing that can affect digestibility (Adedokun *et al*., 2007). Huang *et al*. (2005) examined the apparent ileal AA digestibility at 14, 28 and 42 days of age and found that, in general, digestibility increased with age however the increase was not as dramatic as the current research

result have shown from 5 to 21 days of age. This suggests that the major changes in digestibility occur before 14 days of age. Therefore using birds that are 14 days old or older is not appropriate when trying to determine the AA digestibility coefficients for young birds.

2.14.6 Effect of Low Crude Protein Diets on Carcass Characteristics

Lowering the dietary CP level may not affect carcass characteristics like carcass yield, breast meat yield and thigh yield of the birds. This might be due to better utilization of protein in well balanced low CP diets because efficiency of utilization of protein may increase in low CP, AA supplemented diets (Si *et al*., 2001; Baker *et al*., 2002). Sterling *et al*. (2002) found that dietary CP level had no significant effect on carcass yield. Similarly, Rezaei *et al*. (2004) found that carcass yield was significantly decreased with low CP diets in starter and grower periods.

Rezaei *et al*. (2004) and Sterling *et al*. (2002, 2006) found no differences in the breast meat yield of the broiler chicks when the CP level was reduced and formulated to satisfy the essential AA needs. Kerr and Kidd (1999ab) concluded that lowering the dietary CP with AA supplementation had no effect on breast meat yield of broilers. It may be concluded that if the essential AA are balance then decreasing the dietary CP does not decrease the breast meat yield (Si *et al*., 2001). Although, result from the research conducted by Moran *et al*. (1992), indicated a significant decrease in breast meat yield with the reduction in CP level even when the essential amino acids were at the recommended levels.

The most consistent problem of low CP diets is that abdominal fat is increased which deteriorates the quality of carcass. As a consequence of enhanced lipogenesis, birds fed

low protein diets are expected to deposit more abdominal fat. Smith and Pesti (1998), Smith *et al*. (1998) and Sterling *et al*. (2002) found significantly increased abdominal fat content with low CP diets. Neto *et al*. (2000) observed that abdominal fat pad weight was increased by 104% when the birds were fed 17% CP diet as compared to those fed 24% CP diet. Hai and Blaha (2000) also observed that low CP diets resulted in a higher abdominal fat deposition although it was formulated to satisfy the needs of essential AA. One major factor responsible for increased abdominal fat with low CP diets is the increased ME:CP. Hidalgo *et al*. (2004) reported no differences in carcass yield, breast meat yield and abdominal fat pad in broilers fed low CP diets with constant ME:CP. However, Dozier and Moran (2001; 2002) reported that feeding broiler diets formulated with lower concentrations of CP and ME adversely affected the amount and yield of carcass parts. The liver is the main lipogenic organ in chickens. As a consequence of enhanced lipogenesis in the liver of birds fed the low CP diets, it is expected that liver weights be increased due to increase ME:CP (Swennen *et al*., 2006). However, Sterling *et al*. (2006) did not find any effect of dietary CP levels on liver weights.

2.14.7 Effect of Low Crude Protein Diets on Excretion

Maximum N losses through excreta are due to the inadequacy of dietary protein to meet the AA requirements of the birds and particularly the imbalances between different AA (Morris *et al*., 1999).In recent years animal nutritionists have shown interest in reducing N excreted in the litter and the amount of ammonia (NH_3) produced. Dietary protein in excess of requirements causes an increased heat increment and water intake which results in an elevated litter moisture level (Alleman and Leclercq, 1997). It has been shown that NH_3 production is positively correlated with the litter pH and moisture content (Carr *et al*., 1990; Ferguson *et al*., 1998). Some of the methods used to control

NH_3included dietary manipulations (Jacob *et al*., 1994; Ferguson *et al*., 1998). The most efficient and possibly the cheapest method of controlling NH_3 production is to reduce the amount of N excreted from the birds and lowering dietary CP is effective in reducing N excretion (Ferguson *et al*., 1998). N excretion in litter is reduced by 28% with 2.5% reduction in dietary CP (Jacob *et al*., 1994).

2.15 Effect of Dietary Tryptophan on Broiler Chickens

2.15.1 Effect of Dietary Tryptophan on Feed Intake of Broiler Chickens

Tryptophan is the precursor for synthesis of neurotransmitter serotonin in the hypothalamus which plays an important role in the regulation of appetite and feed intake (Denbow *et al*., 1993; Harms and Russels 2000; Xi *et al*., 2009). Birds fed tryptophan deficient diets exhibited a declined level of serotonin in the body which is directly associated with the reduced availability of tryptophan for serotonin biosynthesis thus resulting in appetite depression and decreased feed intake. However, dietary tryptophan supplementation can be useful to improve that circumstance. Shan *et al*. (2003) showed that an increase of feed intake due to dietary tryptophan supplementation was observed in young broiler chicks raised at moderate (25°C) and warm (35°C) temperatures. Harms and Russell (2000) reported that feed consumption of the commercial layer was significantly enhanced as the dietary level of Tryptophan increased which is in agreement with the present research in Yangzhou goslings.

Inadequate tryptophan may be a factor that could be reducing the amount of feed consumed by broiler chickens especially those fed diet low in crude protein.

Tryptophan serves as a precursor to serotonin, a neurotransmitter highly involved in feed intake regulation. A study carried out by Corzo *et al*. (2005b) reported a reduced feed intake of broiler chickens fed the lowest concentration of tryptophan, and also a research conducted by Mozhdeh *et al*. (2010) reported that the feed consumed by broiler chickens which received high concentration of dietary tryptophan was higher compared to those receiving low concentration. This is in agreement with Harms and Russell (2000). This may be due to the function of tryptophan as a precursor of the neurotransmitter serotonin involved in feed intake (Peganova and Eder, 2003). A research conducted on the effect of varying levels of tryptophan on growth performance of growing and finishing broiler chickens showed that broiler feed intake was not significantly influenced by the levels of tryptophan of 0.136% and 0.257%. This result agreed with the report of Tabiri *et al*. (2002) which stated there was a dramatic reduction of feed intake when tryptophan level dropped to half of the NRC (1994) requirement. According to Rosebrough (1996), diets with low protein levels (12%) supplemented with tryptophan excess caused a reduction in feed intake, which did not happen with diets high in crude protein (30%) for 28-day-old male broilers.

2.15.2 Effect of Dietary Tryptophan on Body Weight of Broiler Chickens

Several reports revealed that tryptophan improved body weight gain in poultry (Fatufe and Rodehutscord 2005; Emadi *et al*., 2010b; Yu *et al*., 2010). This increase in body weight gain may be partly due to the elevated feed intake as the tryptophan content of the diet increased. It was also reported that birds fed optimal tryptophan diet dramatically gained more daily body weight than those in tryptophan-deficient diet. The influence of tryptophan on daily body weight gain in broiler chickens can be explained by a phenomenon of amino acid balance. An adequate tryptophan content brought about

optimal amino acid balance while excess tryptophan supplementation dramatically disrupt the balance of amino acid especially when tryptophan is limited or marginal thus resulting in low utilization rate of nitrogen and poor growth performance.

Several research conducted showed that supplementation of tryptophan in the diet may be responsible for improved growth performance of broiler chickens. Mozhdeh *et al*. (2010) observed a depressed body weight in chickens fed diets containing low concentration of tryptophan. Corzo *et al*. (2005a) reported that broiler chicks fed tryptophan deficient diets had poor body weight gain. There was reduction in body weight gain with dietary tryptophan at 0.12% and 0.16% but weight gain improved as tryptophan increased with maximum overall performance being attained at 0.17% which agreed with the NRC (1994) recommendation of 0.16% tryptophan estimated from modeling for this feeding period. Consequently, the observed body weight decline in the control birds could be related to possible stress-induced insufficiency of dietary tryptophan. This result also agreed with the report of Rostagno *et al*. (2007) that supplemental tryptophan alleviated body weight decline.

2.15.3 Effect of Dietary Tryptophan on Feed conversion ratio of Broiler Chickens

Tryptophan inadequacy in a research conducted by Corzo *et al*. (2005a) was found to increase feed conversion ratio of broiler chickens. This result was similar to the findings of Mozhdeh *et al*. (2010) and Rostagno *et al*. (1995) who reported a significant increase in feed conversion ratio and a gradual decrease in feed conversion. In a study conducted by Karina *et al*. (2013) digestible tryptophan levels did not influence feed conversion and no appropriate fit was obtained by the quadratic, exponential models for bird performance data. Since these models were not significant ($P>0.05$) by regression

analysis of variance. The equation obtained from regression analyses in this study was not reliable for obtaining an optimal digestible tryptophan level in the analyzed variable. Although there was no difference between the evaluated levels, they concluded that 0.2255% digestible tryptophan level indicated numerical improvement in feed conversion, disagreeing with the 0.18% suggested by NRC (1994) and Rostagno *et al*. (2007) for broilers from 22 to 42 days of age. This result agrees with the findings of Rosa and Pesti (2001). Based on their analysis, the current NRC (1994) tryptophan requirement estimate of 0.20% is probably too low for broilers. These analyses suggest that at least 0.25% tryptophan is necessary to maximize feed efficiency.

2.15.4 Effect of Dietary Tryptophan on Blood parameters and Serum Biochemistry of Broiler Chickens

Haematological parameters, blood biochemical indices and their knowledge can be used to assess the health, physiological and nutritional status of broiler chickens (Muhammad *et al*., 2004). Normally, the values of blood constituents are affected by many factors such as genotype, age, physiological condition, gender, diet, micro- and macro-climatic conditions, the method of rearing, season and pathological factors. Under tropical conditions, birds are exposed to marked seasonal changes in the thermal environment (Szabo *et al*., 2005; Lloyd and Gibson, 2006).

The analyses of blood haematological parameters are discussed in several studies with domestic animals (Jain, 2000), but only a few specifically refer to broilers (Kaneko *et al*., 1997; Simarak, 2004).The evaluation of the levels of total protein and its fractions supply the information required to interprete the occurrence of dehydration, infections, immune diseases, and inflammatory responses. The determination of blood component values using laboratory examination is an important procedure to aid the diagnosis of

several diseases and dysfunctions, as they provide reliable result, and may also give inputs for research studies on nutrition, physiology, and pathology (Mirtuka and Rawnsley 1977; Bounous *et al*., 2000; Swennen *et al.*, 2006). Commonly used haematological parameters measured during blood studies include red blood cell count (RBC), haemoglobin concentration (Hb), leucocytes counts (WBC), erythrocyte; absolute (i.e. mean corpuscular haemoglobin (MCH)) and derived (i.e. mean corpuscular heamoglobin concentration (MCHC)) values. Dietary differences affect erythrocyte values among group of animals (Akpodiete and Ologhobo, 1998, Diarra and Usman, 2008). This could be due to poor nutritional status, alongside sub clinical diseases. Haematological parameters are those parameters that are related to the blood and blood-forming organs (Waugh and Grant, 2001; Mohammed*et al*., 2009).

Reports from research revealed some of the normal range for haematological parameters and serum biochemical indices, RBC (1.58-3.82 x 10^6l), WBC (2.05 X 10^6 l), Hb (8.9 g/dl), PCV (21 - 45%), MCHC (27.97g/dl), MCH (39.11Pg), MCV (90-140fl), TP (3.0-4.08g/dl) Diarra and Usman (2008), Urea (1.50-6.30mmol/l) and ALT (9.50- 37.20 i.u/l) Mirkuta and Rawnsley (1977). Numerous investigations have shown that tryptophan depletion and tryptophan utilization are related to immune response modulation (Widner *et al*., 2000; Moffet and Namboodini, 2003). However, many aspects of immune- related tryptophan metabolism remain to be elucidated and pooled data do not afford to present complete explanation of the observed decline of spleen weight, since it was not related with higher corticosterone level. Mozhdeh *et al*. (2010) reported that tryptophan supplementation significantly increased the serum concentration of total protein, albumin, urea and uric acid at 27 and 49 days of age. Dietary Try had no significant effect ($P>0.05$) on creatine, ALT and AST at 27 and 49 days of age.

2.15.5 Effect of Dietary Tryptophan on Carcass Characteristics of Broiler Chickens

Numerous factors may affect meat quality in broiler chickens. Pre-slaughter excitement results in darker breast meat color, while struggling during slaughter increases the rate of postmortem glycolysis (decreased muscle pH) and consequently the time required for the onset of rigor mortis. Research conducted by Kerr and Kidd (1999a) on the effect of tryptophan on live weights at the time of intubation reported slight difference between treatments ($P<0.05$) that corresponded to differences in carcass weight, fat pad weight, and empty carcass weight ($P < 0.05$). Weights of wings, thighs, drums, and breast, and carcass and part yields did not differ between treatments ($P > 0.05$). There were no consistent effects of tryptophan supplementation on any carcass defects measured ($P > 0.05$). Kerr and Kidd (1999b) further reported that addition of 0.15% crystalline L-tryptophan to a withdrawal feed containing 0.17% total tryptophan in broilers from 46 to 49 d of age had no discernable impact on carcass quality. It is well known that crude protein and amino acids status of a diet affects the carcass composition of broiler with increased carcass protein and decreased carcass fat accompanying increases in dietary protein or essential amino acids contents because protein has the ability to act as a lipotrophic agent (Hai and Blaha 2000; Neto *et al*., 2000; Si *et al*., 2001).

Additional research is needed on the length and level of tryptophan supplementation required to observe any growth, potential carcass quality improvements through metabolic or behavioral modifications in the tropical region. In a research conducted by Wei et al. (2011) they reported no significant difference in abdominal fat due to tryptophan in dietary treatments fed to goslings. These finding is in contrast to those previously reported in chickens. In general, body fat deposition may be considered as the net result of the balance among dietary absorbed fat, endogenous fat synthesis

(Lipogenesis) and fat catabolism via Beta- oxidation (lipolysis). Thus lowering body fat deposition can be achieved either by increasing fat catabolism or diminishing endogenous fatty synthesis or both processes. Research has shown that feed alteration (changing protein to energy ratio) and balancing the addition of individual amino acids like DL- methionine, lysine, L-tryptophan and L-threonine amongst others can be used as a measure to reduce fat deposition in broiler chickens. Rosa *et al*. (2001) reported a significant reduction in fat pad weights in broiler chickens fed elevated tryptophan level. Corzo *et al*. (2005a) reported that dietary tryptophan supplementation dramatically increased the weight and percentage fat deposition in male broilers fed tryptophan at 0.12%. Such differences in abdominal fat observed between different studies may be partially explained by higher feed consumption with tryptophan addition.

2.15.6 Effect of Dietary Tryptophan on Mortality

Denbow *et al*. (1993) tested the effect of supplemental tryptophan (0.1, 0.2, and 0.3%) in a turkey finisher diet containing 17.9% CP and 0.18% tryptophan prior to market (112 to 120 days) and reported no differences in bird performance, mortality, or condemnations during transportation when fed diets varying in supplemental tryptophan.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENT 1: Determination of Optimum Tryptophan Requirement for Broiler Chickens Reared during Cool Season under Tropical Environment

3.1.1 Starter Phase: Determination of Optimum Tryptophan Requirement for Broiler Chicks Reared during the Cool Season Under Tropical Environment.(0 - 4 Weeks)

3.1.2 Experimental Site

The experiment was carried out at the Poultry Unit of Kogi State Ministry of Agriculture, Kabba. Kabba is located within the Southern Guinea Savannah Zone on latitude $7^05^{'}$N, longitude $6^0 4^{'}$E and altitude of 640m above sea level. It has an annual rainfall of 1500mm and rain starts between late April and early May to mid October. The dry season begins around the middle of November, with cool weather that ends in February. This is followed by relatively hot-dry weather between March and April just before the rain begins. The minimum daily temperature is from 14^oC - 20^oC during the cool season while the maximum daily temperature is from 19^0C- 40^0C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72%, respectively. Kabba, College of Agriculture Metrological Section (2015).

3.1.3 Source of Experimental Birds and Testing Ingredient (Tryptophan)

The broiler chicks (Arbor Acre) used in this experiment were obtained from a commercial hatchery with good records of birds' performance. Synthetic tryptophan was purchased from Hi- Nutrient Ltd, Nigeria.

3.1.4 Experimental Diets

Five broiler starter diets were formulated. Diet 1 was formulated without synthetic tryptophan but had 0.15% dietary tryptophan which was practically below 0.20% which is the NRC (1994) tryptophan requirement for broiler starter chickens while diets 2, 3, 4 and 5 contained graded levels of supplemental tryptophan at: 0.04, 0.08, 0.12 and 0.16% to give total tryptophan dietary levels of 0.19, 0.23, 0.27 and 0.31% respectively (Table3 .1). The diets were formulated to be isocaloric and isonitrogenous (CP=23%, ME=3008 Kcal/Kg). Diets were also formulated to meet the NRC (1994) nutrient requirement. The birds were fed the experimental diets from 0-4 weeks. All the diets were chemically analyzed according to AOAC (1990) methods for their proximate composition (Table 3.2)

3.1.5 Experimental Design and Management of Birds

A total of two hundred and eighty five day old broiler chicks were used in this study. The mean weights of birds for all the pens were made to be approximately equal. There were three replicates, with 19 birds each. The experiment was a completely randomized design. The birds were reared in a deep litter system. Heat and light were provided throughout the brooding period, using kerosene stoves, charcoal pots, lanterns and electricity. Routine vaccinations (gumboro and lasota) and all necessary medications

were administered as follows. Day 1-5 Neoteramycine + vitalyte in water orally, day7, first dose of gumboro, day 14, NDV-Lasota, day 21, second dose of gumboro; day24 coccidiostat in water. Feed and water were provided *ad libitum*. The experiment was conducted during the cool season between November (2014) and January (2015).

Table 3.1: Composition of Broiler Starter Diets Containing Graded Supplemental Levels of Tryptophan During the Cool Season (0 - 4 Weeks)

Ingredient (kg)	Treatments				
	1	**2**	**3**	**4**	**5**
	Try 0.00%	**Try 0.04%**	**Try 0.08%**	**Try 0.12%**	**Try 0.16%**
Maize	50.66	50.66	50.66	50.66	50.66
Groundnut cake	25.00	25.00	25.00	25.00	25.00
Soya cake	12.59	12.59	12.59	12.59	12.59
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	3.69	3.69	3.69	3.69	3.69
Limestone	0.90	0.90	0.90	0.90	0.90
Bone meal	2.75	2.75	2.75	2.75	2.75
Common salt	0.30	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30	0.30
Lysine	0.32	0.32	0.32	0.32	0.32
Methionine	0.21	0.21	0.21	0.21	0.21
Tryptophan	0.00	0.04	0.08	0.12	0.16
Threonine	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	3008	3008	3008	3008	3008
Crude protein (%)	23.00	23.00	23.00	23.00	23.00
Ether extract (%)	8.11	8.11	8.11	8.11	8.11
Crude fibre (%)	3.70	3.70	3.70	3.70	3.70
Calcium (%)	1.28	1.28	1.28	1.28	1.28
Lysine (%)	1.26	1.26	1.26	1.26	1.26
Methionine (%)	0.56	0.56	0.56	0.56	0.56
Available P (%)	0.57	0.57	0.57	0.57	0.57
TSAA (%)	0.90	0.90	0.90	0.90	0.90
Tryptophan (%)	0.15	0.19	0.23	0.27	0.31
Threonine (%)	0.95	0.95	0.95	0.95	0.95
Glycine (%)	1.69	1.69	1.69	1.69	1.69
Arginine (%)	1.69	1.69	1.69	1.69	1.69
Phenylalanine (%)	1.07	1.07	1.07	1.07	1.07
Leucine (%)	1.85	1.85	1.85	1.85	1.85
Isoleucine (%)	0.99	0.99	0.99	0.99	0.99
Valine (%)	1.05	1.05	1.05	1.05	1.05
Feed cost/kg (₦)	99.44	105.44	111.44	117.44	123.44

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000 iu; Vit E, 23mg;Vit.K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin,

0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20 mg; Manganese, 40 mg; Selenium, 0.2 mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 3.2: Proximate Composition of Broiler Chicks Diets Fed Under the Cool and Hot Seasons (0 – 4 weeks)

	Treatments				
Parameters (%)	0.15	0.19	0.23	0.27	0.31
Dry matter	93.01	89.01	93.44	91.01	89.91
Crude protein	22.92	23.01	22.86	21.96	21.41
Crude fibre	4.01	3.82	3.68	3.62	3.71
Ether extract	7.99	8.21	8.01	7.89	7.86
Ash	8.55	7.39	8.05	8.50	8.24
Nitrogen free extract	56.56	57.87	57.40	56.96	57.35

The maximum temperatures varied from 14^0C to 20^0C. Drugs and vaccinations were administered as at when due. The chicks were fed the experimental diets from 0-4 weeks.

3.1.6 Parameters Measured

The parameters measured include: Final body weight and feed intake. From the primary data collected for feed intake and weight gain, other data for feed conversion rate and feed cost per unit gain in weight were generated. Mortality record was recorded as it occurred.

3.1.7 Haematological and Blood Serum Evaluations

At the end of the starter phase, 2ml of blood samples were collected from each of three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determination of haematological parameters. Packed Cell Volume (PVC) was determined by the microhaematocrit method, haemoglobin concentration (HB) by photometrically at the wavelength of 540nm, the erthtocyte (RBC) and leucocytes (WBC) were done using the improved Neubauer haemocytometer. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were derived by calculation using the following formulae:

$$MCHC=\frac{Hb}{} \times 100$$

$$MCH = \frac{Hb}{RBC} \times 100$$

$$MCV = \frac{PCV}{RBC} \times 100$$

2ml of blood was also allowed to clot and then centrifuged. The serum was separated and stored at -20°C until analyzed for serum parameters (albumin, total protein, glucose, cholesterol, triglyceride, urea, uric acid, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), Alkaline Phosphatase (ALP) and Creatine Kinase (CK) according to the methods described by Bush (1975) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates.

3.1.8 Nutrient Digestibility Trial

A nutrient digestibility trial was carried out at the end of the starter phase (4 weeks) of the experiment, using metabolic cages. This was done by randomly selecting 3 birds of approximately equal weight from the each replicate. The birds were placed in alternated cages, with polythene bags attached beneath the cages. The birds were fed with the control diet for 7 days of adjustment period and fasted for 24 hours with only water given to them. 40g of experimental diets was allocated to each bird by 8:00am daily. Faecal samples were collected daily, separated from feeds and other extraneous materials, weighed, bulk together and kept in a deep freezer. At the end of the 6^{th} day the birds were not fed. The remaining faeces were then collected by 8.00am on the 7^{th} day. The total samples were thawed and weighed after it has been thoroughly mixed together and oven dried for 72 hours at 65^0C. The dried samples were then weighed and ground, after which samples were taken for proximate analysis along with the sample of the feed fed at the Department of Animal Science Laboratory, Ahmadu Bello

University, Zaria. Nutrient retention was determined for crude protein, crude fibre, ether extract, ash, and nitrogen free extract.

$$\text{Nutrient Retention} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

3.2. Finisher Phase: Determination of Optimum Tryptophan Requirement for Broiler Finisher Chickens Reared during the Cool Season under Tropical Environment (5- 8 Weeks)

3.2.1 Experimental Diets

Five broiler finisher diets were formulated. Diet 1 was formulated without synthetic tryptophan but had 0.13% dietary tryptophan which was practically below 0.18% NRC (1994) tryptophan requirement for broiler finisher chickens while diets 2, 3, 4and 5 contained graded levels of supplemental tryptophan at: 0.04, 0.08, 0.12 and 0.16% to give total dietary levels of tryptophan of 0.17, 0.21, 0.25, and 0.29% respectively (Table 3.3). The diets were formulated to be isocaloric and isonitrogenous (CP=21%, ME=3056 Kcal/Kg). Diets were also formulated to meet the NRC (1994) nutrient requirements. The birds were fed the experimental diets from 5 - 8 weeks. All the diets were chemically analyzed according to the standard of AOAC (1990) methods for their proximate composition (Table 3.4).

3.2.2 Experimental Design and Management of Birds

At the end of the chick phase, all the birds in the various groups were pooled together and fed a common diet for one week, after which they were randomly re-allocated to five groups on the basis of approximately equal weights with eighteen (18) chickens per replicate. The experiment was a completely randomized design. The birds were housed in pens under the deep litter system.

3.2.3 Parameters measured

The parameters measured include: Final body weight and feed intake. From the primary data collected for feed intake and weight gain, other data for feed conversion rate and feed cost per unit gain in weight were generated. Mortality record was recorded as it occurred.

3.2.4 Nutrient Digestibility Trial: As described for the starter phase.

3.2.5 Carcass Analysis

Three birds per replicate with weights closest to the mean body weight of the replicate were used for the carcass study. The birds were slaughtered after being starved for twenty four hours. The birds were weighed, slaughtered, de-feathered and eviscerated. Weights of the prime cut, the giblets and the abdominal fat were taken and expressed as percent of the dressed weight.

3.2.6 Tissue Fat and Tissue Protein Analyses

Three birds per replicate used for carcass analysis were used also for the determination of tissue fat composition and crude protein. Representative samples of the carcass cut-up parts (breast, thigh, drumstick, wings and back) from each bird were chopped and minced together and taken to the Department of Animal Science Laboratory, Ahmadu Bello University laboratory for tissue fat and tissue protein analyses according to the standard of AOAC (1990).

Table 3.3: Composition of Broiler Finisher Diets Containing Graded Supplemental Levels of Tryptophan During the Cool Season (5- 8 Weeks)

	Treatments				
	1	**2**	**3**	**4**	**5**
Ingredient (kg)	**Try 0.00%**	**Try 0.04%**	**Try 0.08%**	**Try 0.12%**	**Try 0.16%**
Maize	56.33	56.33	56.33	56.33	56.33
Groundnut cake	22.00	22.00	22.00	22.00	22.00
Soya cake	10.00	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm oil	3.50	3.50	3.50	3.50	3.50
Limestone	1.00	1.00	1.00	1.00	1.00
Bone meal	2.80	2.80	2.80	2.80	2.80
Common salt	0.30	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.21	0.21	0.21	0.21	0.21
Tryptophan	0.00	0.04	0.08	0.12	0.16
Threonine	0.29	0.29	0.29	0.29	0.29
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	3056	3056	3056	3056	3056
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Ether extract (%)	7.79	7.79	7.79	7.79	7.79
Crude fibre (%)	3.43	3.43	3.43	3.43	3.43
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Lysine (%)	1.14	1.14	1.14	1.14	1.14

Methionine (%)	0.54	0.54	0.54	0.54	0.54
Available P (%)	0.57	0.57	0.57	0.57	0.57
TSAA (%)	0.85	0.85	0.85	0.85	0.85
Tryptophan (%)	0.13	0.17	0.21	0.25	0.29
Threonine (%)	0.88	0.88	0.88	0.88	0.88
Glycine (%)	1.52	1.52	1.52	1.52	1.52
Arginine (%)	1.51	1.51	1.51	1.51	1.51
Phenylalanine (%)	0.98	0.98	0.98	0.98	0.98
Leucine (%)	1.72	1.72	1.72	1.72	1.72
Isoleucine (%)	0.90	0.90	0.90	0.90	0.90
Valine (%)	0.96	0.96	0.96	0.96	0.96
Feed cost/kg (₦)	96.88	102.69	108.69	114.69	120.69

Total sulfur amino acid =TSSA, Try= Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 3.4: Proximate Composition of Broiler Finisher Chickens Diets Fed Under the Cool and Hot Seasons (5 – 8 weeks)

	Treatments				
Parameters (%)	0.13	0.17	0.21	0.25	0.29
Dry matter	84.51	90.85	92.91	89.41	91.89
Crude protein	20.01	20.94	20.05	19.89	19.99
Crude fibre	3.05	3.89	3.20	3.48	3.38
Ether extract	6.98	7.24	6.99	7.34	7.80

Ash	7.81	7.69	8.33	7.14	7.63
Nitrogen free extract	62.15	60.28	61.43	62.15	61.30

3.2.7 Haematological and Blood Serum Evaluation

At 8 weeks of age, 2ml blood samples was collected from each of three birds per replicate via the wing veins and put into ethylene Di-amine Tetra Acetic Acid (EDTA) treated Bijou bottles (1mg/ml) for haematological assay. Blood samples were analyzed

within three hours of collection for Packed Cell Volume (PCV), hemoglobin concentration (Hb) and total protein (TP) according to the methods described by Lamb (1991) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was determined in triplicates.

Two millilitres (2mls) blood from three birds per replicate were allowed to clot and then centrifuged and serum was separated and stored at -20°C until analyzed for serum parameters (albumin, total protein, glucose, cholesterol, triglyceride, urea, uric acid, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), Alkaline Phosphatase (ALP) and Creatine Kinase (CK) according to the methods described by Bush (1975) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates.

3.3 EXPERIMENT 2. Determination of Optimum Tryptophan Requirements for Broiler Chickens Reared in the Hot Season Under Tropical Environment

3.3.1 Starter Phase: Determination of Optimum Tryptophan Requirement for Broiler Chicks Reared During the Hot Season Under Tropical Environment (0-4 weeks)

3.3.2 Experimental Site

Same as described under 3.1.2 for the cool season.

3.3.3 Source of Experimental Birds

Same as described in section 3.1.3 for the cool season.

3.3.4 Experimental Diets

Same as described under section 3.1.4and Table 3.1 for the cool season.

3.3.5 Experimental Design and Management of Birds

A total of two hundred and eighty five (285) day old broiler chicks were used in this study. The mean weights of birds for all the pens were made to be approximately equal. There were three replicates, each replicate having 19 birds. The experiment was a completely randomized design. The birds were reared in a deep litter system. Heat and light were provided throughout the brooding period, using kerosene stoves, charcoal pots, lanterns and electricity. Routine vaccinations (gumboro and lasota) and all necessary medications were administered as follows. Day 1-5 Neoteramycine + vitalyte in water orally, day 7, firstdose of gumboro, day 14, NDV-Lasota, day 21, second dose of gumboro; day 24 coccidiostat in water. Feed and water were provided *ad libitum*. The experiment was conducted during the hot season between March (2015) and May (2015). The maximum temperatures varied from 32^0C to 34^0C. Drugs and vaccinations were administered as at when due. Feed and water were provided *ad libitum*. The chicks were fed the experimental diets from 0 - 4 weeks.

3.3.6 Parameters Measured

Same as described in section 3.1.6 for the cool season

3.3.7 Haematological and Blood Serum Evaluations

Same as described in section 3.1.7 for the cool season.

3.3.8 Nutrient Digestibility Trial

Same as described in section 3.1.8 for the cool season.

3.4 Finisher Phase: Determination of Optimum Tryptophan Requirement for Broiler Finisher Chickens Reared During the Hot Season Under Tropical Environment (5-8 weeks)

3.4.1 Experimental Diets

Same as described in Section 3.2.1 and Table 3.2 respectively.

3.4.2 Experimental Design and Management of Birds

At the end of the starter phase, all the birds in the various pen groups were pooled together and fed a common diet for one week, after which they were randomly allocated to five dietary treatments. There were three replicates, each replicate having 18 birds. The experiment was a completely randomized design. The birds were housed in pens under the deep litter system. Routine medications and vaccinations were administered.

3.4.3 Parameters measured

Same as described under Section 3.2.3.

3.4.4 Nutrient Digestibility Trial

Same as described for the starter phase under section 3.2.4.

3.4.5 Carcass Analysis

Same as described under Section 3.2.5.

3.4.6 Tissue Fat and Tissue Protein Analyses

Same as described under Section 3.2.6.

3.4.7 Haematological and Blood Serum Evaluations

Same as described under Section 3.2.7.

3.5 EXPERIMENT 3: Effect of Diet Formulated on the Basis of Four Critical Essential Amino acids on the Crude Protein Requirements of Broiler Chickens

3.5.1 Starter Phase: Effect of Diets Formulated on the Basis of Four Critical Amino Acids on the Crude Protein Requirement of Broiler Chicks (0 – 4 Weeks)

3.5.2 Experimental Site

The same as described for experiment 1.

3.5.3 Source of Experimental Birds

The same as described for experiment 1.

3.5.4 Experimental Diets

Four broiler starter diets were formulated. Diets, 1, 2, 3, and 4 contained 20, 21, 22 and 23% crude protein, respectively (Table 3.5). The diets were formulated to be isocaloric and with the same amino acid profile across treatments. Diets were also formulated to meet the NRC (1994) nutrient requirements for the first four critical essential amino acids which are lysine, methionine, tryptophan and threonine. The chicks were fed the experimental diets from 0-4 weeks. All the diets were chemically analyzed according to the standard of the AOAC (1990) methods for their proximate composition Table 3.6.

3.5.5 Experimental Design and Management of Birds

A total of one hundred and ninety two (192) day old chicks were used in this study. The mean weights of birds for all the pens were approximately equal. There were four treatment groups each containing three replicates. Each replicate had 16 birds in a completely randomized design. The birds were reared on a deep litter system.

Table 3.5: Composition of Broiler Starter Diet Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement (0- 4 Weeks)

	Treatments			
Ingredient (kg)	**1 20%CP**	**2 21%CP**	**3 22%CP**	**4 23%CP**
Maize	61.41	57.46	53.47	49.57
Groundnut cake	15.70	18.98	22.36	25.60
Soya cake	10.00	10.00	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00
Palm oil	3.30	4.07	4.85	5.56
Limestone	0.80	0.80	0.80	0.80
Bone meal	2.90	2.90	2.90	2.90
Common salt	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30
Lysine	0.51	0.46	0.41	0.37
Methionine	0.32	0.29	0.27	0.24

Tryptophan	0.12	0.11	0.09	0.08
Threonine	0.35	0.33	0.31	0.28
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (Kcal/kg)	3101	3102	3103	3101
Crude protein (%)	20.00	21.00	22.00	23.00
Ether extract (%)	7.48	8.26	9.10	9.84
Crude fibre (%)	3.18	3.08	3.12	3.25
Calcium (%)	1.31	1.32	1.33	1.33
Lysine (%)	1.27	1.27	1.27	1.27
Methionine (%)	0.64	0.63	0.65	0.65
Available P (%)	0.60	0.60	0.60	0.60
TSAA (%)	0.92	0.92	0.92	0.92
Tryptophan (%)	0.23	0.23	0.23	0.23
Threonine (%)	0.91	0.91	0.91	0.91
Glycine (%)	1.31	1.44	1.57	1.59
Arginine (%)	1.31	1.43	1.44	1.48
Phenylalanine (%)	0.89	0.94	0.99	1.05
Leucine (%)	1.62	1.68	1.72	1.80
Isoleucine (%)	0.84	0.88	0.91	0.95
Valine (%)	0.84	0.93	0.98	1.02
Feed cost/kg (₦)	104.15	105.71	105.87	104.36

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 2 mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 3.6: Proximate Composition of Broiler Chicks Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement (0 – 4 weeks)

	Level of Crude Protein (%)			
Parameters	20	21	22	23
Dry matter	90.81	92.19	91.76	90.48
Crude protein	18.99	20.98	21.01	21.95
Crude fibre	2.98	3.14	3.20	3.98
Ether extract	7.01	7.89	8.70	8.10

Ash	4.10	5.58	4.32	3.18
Nitrogen free extract	66.92	62.41	62.77	62.79

Heat and light were provided throughout the brooding period, using stoves, charcoal pots, lanterns and electricity. Drugs and vaccinations were administered as at when due. Feed and water were provided *ad libitum*.

3.5.6 Parameters Measured

The parameters measured include: feed intake, initial weight, final weight, weight gain, feed conversion ratio and mortality. From the primary data collected for feed intake and weight gain, other data were generated for feed conversion ratio and feed cost per unit gain in weight.

3.5.7 Haematological and Blood Serum Evaluations: As described in Experiment 1.

3.5.8 Nutrient Digestibility Trial: As described in Experiment 1.

3.6 Finisher Phase: Effect of Diet Formulated on the Basis of Four Critical Essential Amino Acids on the Crude Protein Requirement of Broiler Finisher Chickens (5 – 8 Weeks)

3.6.1 Experimental Diets

Four broiler finisher diets were formulated. Diets 1, 2, 3 and 4 contained 18, 19, 20, and 21% crude protein respectively (Table 3.7). The diets were formulated to be isocaloric. Diets were also formulated to meet the NRC (1994) nutrient requirements for essential amino acids. All the diets were chemically analyzed according to the standard of AOAC (1990) methods for their proximate compositions (Table 3.8).

Table 3.7: Composition of Broiler Finisher Diet Formulated on the Basis of Four Critical Essential Amino on the Crude Protein Requirement (5 – 8 Weeks)

Ingredient	Treatments			
	1 18%CP	**2 19%CP**	**3 20%CP**	**4 21%CP**
Maize	65.78	62.76	58.01	53.28
Groundnut cake	14..00	16.56	20.50	24.50
Soya cake	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00
Palm oil	3.50	4.10	4.97	5.87
Limestone	1.00	1.00	1.00	1.00

Bone meal	2.90	2.90	2.90	2.90
Common salt	0.30	0.30	0.30	0.30
Premix **	0.30	0.30	0.30	0.30
Lysine	0.49	0.45	0.40	0.34
Methionine	0.30	0.28	0.25	0.22
Tryptophan	0.11	0.10	0.09	0.07
Threonine	0.32	0.31	0.28	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (Kcal/kg)	3151	3152	3150	3151
Crude protein (%)	18.00	19.00	20.00	21.00
Ether extract (%)	7.53	7.56	7.65	7.79
Crude fibre (%)	3.00	3.07	3.12	3.25
Calcium (%)	1.33	1.33	1.34	1.35
Lysine (%)	1.15	1.15	1.15	1.15
Methionine (%)	0.60	0.57	0.60	0.60
Available P (%)	0.57	0.57	0.57	0.57
TSAA (%)	0.85	0.85	0.85	0.85
Tryptophan (%)	0.21	0.21	0.21	0.21
Threonine (%)	0.83	0.83	0.83	0.83
Glycine (%)	1.16	1.26	1.42	1.52
Arginine (%)	1.17	1.25	1.40	1.54
Phenylalanine (%)	0.81	0.85	0.91	0.98
Leucine (%)	1.50	1.54	1.62	1.70
Isoleucine (%)	0.76	0.79	0.83	0.88
Valine (%)	0.82	0.85	0.90	0.95
Feed cost/kg (₦)	109.60	109.44	109.39	108.95

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 3.8: Proximate Composition of Broiler Finisher Chickens Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement (5 – 8 weeks)

	Level of Crude Protein (%)			
Parameters (%)	18	19	20	21
Dry matter	90.14	89.49	92.84	90.90
Crude protein	17.01	18.22	18.99	20.64
Crude fibre	2.04	2.91	2.98	3.01

Ether extract	6.68	7.41	8.41	8.98
Ash	4.11	5.21	3.14	4.89
Nitrogen free extract	70.16	66.25	66.48	62.48

3.6.2 Experimental Design and Management of Birds

At the end of the chick phase, all the birds in the various groups were pooled together and fed a common diet for one week, after which they were randomly re-allocated to four groups on the basis of approximately equal weights with fifteen (15) chickens per

replicate. The experiment was a completely randomized design. The birds were housed in pens under the deep litter system.

3.6.3 Parameters Measured: As described in experiment 1.

3.6.4 Haematological and Blood Serum Evaluations: As described in experiment 1.

3.6.5 Nutrient Digestibility Trial: As described in Experiment 1.

3.6.6 Carcass Analysis

Three birds per replicate with weights closest to the mean body weight of the replicate were used for the carcass study. The birds were slaughtered after being kept off-feed for twenty four hours. The birds were weighed, de-feathered and eviscerated. Weights of the conventional cut up parts, the giblets and the abdominal fat were taken and expressed as percent of the live weight.

3.6.7 Tissue Fat and Tissue Protein Analysis

Three birds per replicate used for carcass analysis were also used for the determination of tissue fat composition and for tissue protein analysis. Representative samples of the carcass cut-up parts (breast, thigh, drumstick, wings and back) from each bird were chopped and minced together and taken to the Department of Animal Science Laboratory, Ahmadu Bello University laboratory for tissue fat and tissue protein analyses.

3.7 Statistical Analyses:

All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of SAS software, (2001) for the analysis of variance. Duncan's (2001) multiple range tests were used to determine differences among treatment means. Means were considered different at $P<0.05$. The growth response estimates, of broiler chickens to varying levels of tryptophan was determined by regression analysis. To determine optimum tryptophan requirements, polynomial regression models was used which was the best fit obtained for each variable studied.

General Linear Model

$Y_{ij} = \mu + K_{i+} e_{ij}$

Y_{ij}= Observation of the i^{th} level of tryptophan as shown by broilers performance

μ= Overall mean

K_i =i^{th} effect of tryptophan

e_{ij} = Random error

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment 1: Performance of Broiler Chickens Fed Graded Levels of Dietary Tryptophan During the Cool Season

4.1.1 Performance of Broiler Starter Chicks Fed Graded Dietary Levels of Tryptophan During the Cool Season (0-4 weeks)

The performance of broiler chicks fed graded dietary levels of tryptophan, (0.15%, 0.19%, 0.23%, 0.27% and 0.31%) respectively during the cool (harmattan) season (November- January) is shown in Table 4.1. Final body weights, weight gains, feed intake, feed conversion ratio and feed cost per kg gain were significantly ($P<0.05$) affected by dietary treatments. A trend was observed for final body weight, weight gain and average daily weight gain. Dietary treatments however had no significant ($P>0.05$) effect on the rate of mortality.

Birds fed diet containing 0.23% tryptophan performed significantly ($P<0.05$) better in most of the parameters measured (final body weight, weight gain, average daily weight gain and feed cost per Kg weight gain.) compared to chicks fed other tryptophan diets. The final weight and weight gain increased as the levels of dietary tryptophan increased and began to decrease at 0.27% level of dietary tryptophan. It can be inferred from the graph in Figure 4.1, 4.2 and 4.3, that 0.23% dietary tryptophan level indicated an improvement in final body weight, weight gain and average daily weight gain.

Table 4.1: Performance of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Cool Season (0- 4 Weeks)

	Tryptophan Levels%					
Parameters	0.15	0.19	0.23	0.27	0.31	SEM
Initial weight (g)	52.06	52.07	52.09	52.05	52.06	0.02
Final weight(g)	1073.47^{c}	1177.97^{ab}	1215.78^{a}	1179.83^{ab}	1159.65^{b}	14.84
Weight Gain (g)	1021.00^{c}	1125.97^{ab}	1163.69^{a}	1127.77^{ab}	1106.59^{b}	14.88
Ave daily gain (g)	36.48^{c}	40.21^{ab}	41.56^{a}	40.28^{ab}	39.52^{b}	0.53
Feed Intake (g)	1783.39^{a}	1569.20^{b}	1682.46^{ab}	1587.72^{b}	1687.91^{ab}	57.82
Feed Intake (g/b/d)	63.69^{a}	56.04^{b}	60.09^{ab}	56.70^{b}	60.28^{ab}	2.06
FCR	1.74^{b}	1.39^{a}	1.44^{a}	1.41^{a}	1.53^{a}	0.05
Feed cost/kg Gain(₦)	97.41^{a}	93.71^{a}	95.77^{a}	104.17^{b}	111.57^{c}	1.36
Mortality (%)	3.51	1.75	0.00	0.00	3.52	1.92

a, b, c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
Try = Tryptophan
FCR = Feed conversion ratio

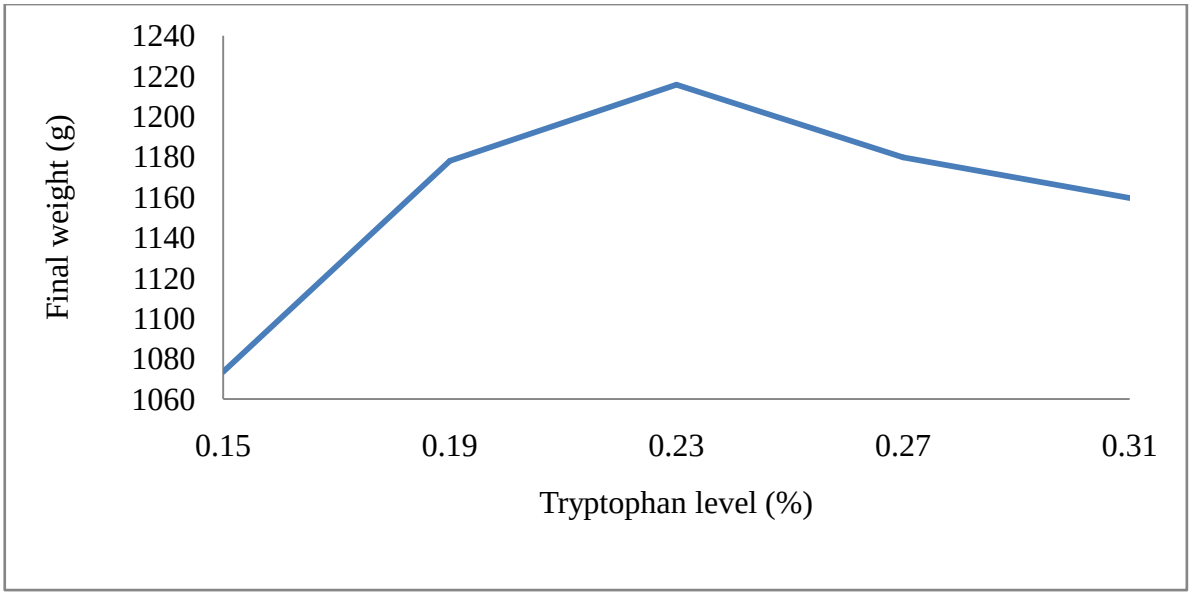


Figure 4.1: Optimum dietary tryptophan level to maximize final weight for broiler chicks reared in the cool season under tropical environment. (0 - 4 weeks).

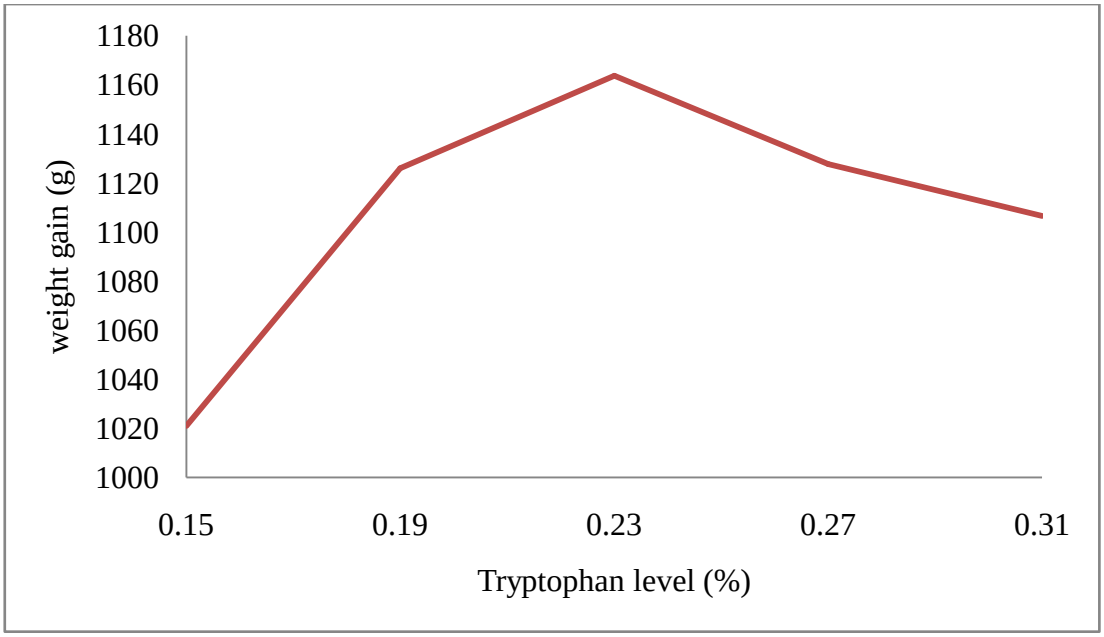


Figure 4.2: Optimum dietary tryptophan level to maximize weight gain for broiler chicks reared in the cool season under tropical environment. (0 -4 weeks).

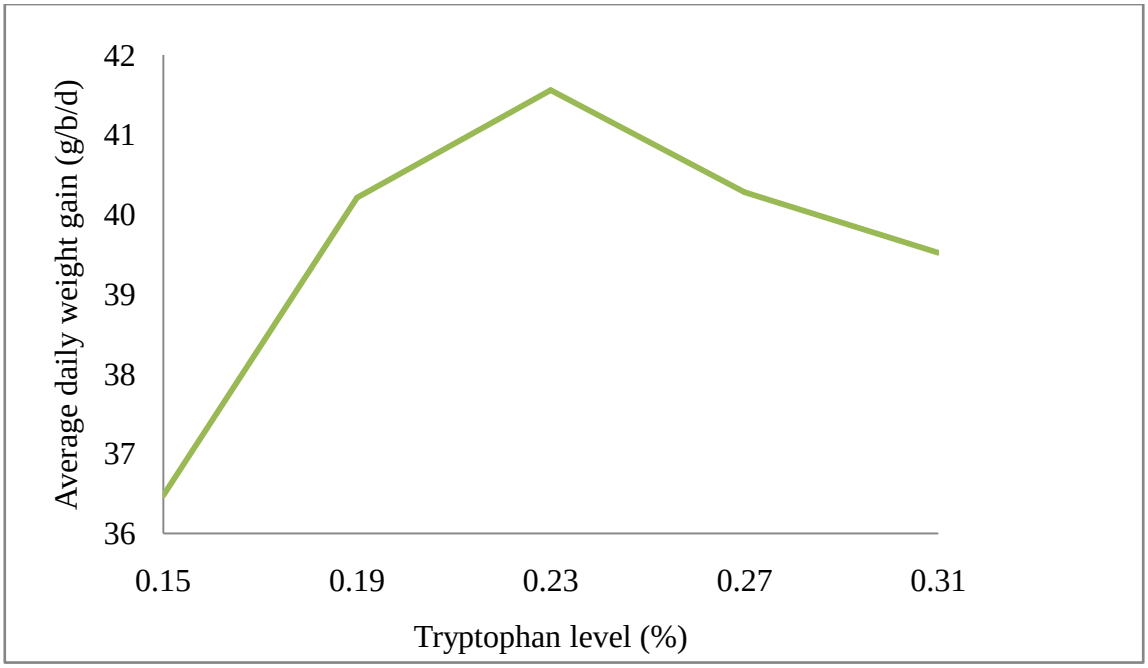


Figure 4.3: Optimum dietary tryptophan level to maximize average daily weight gain for broiler chicks reared in the cool season under tropical environment. (0 – 4weeks).

It was visually observed that chicks that consumed low tryptophan exhibited abnormal behavior characterized by an increase in feed spillage. Chicks fed 0.15% dietary tryptophan had the highest feed spillage followed by birds fed 0.31, 0.23, 0.27 and 0.19% dietary tryptophan respectively. It was also observed that chicks fed 0.15% dietary tryptophan had the highest feed conversion ratio value compared to chicks fed other tryptophan levels. Chicks fed 0.23% total tryptophan had similar results for feed conversion ratio and feed cost (₦)/ kg weight gain compared to chicks fed other tryptophan levels.

4.1.2 Performance of Broiler Finisher Chickens Fed Graded Dietary Levels of Tryptophan During the Cool Season. (5 – 8 Weeks).

Table 4.2 shows the performance of broiler finisher chickens fed graded dietary levels of tryptophan, (0.13%, 0.17%, 0.21%, 0.25% and 0.29%) respectively during the cool (harmattan) season (November to January). It was observed that dietary treatments had significant ($P<0.05$) effects on final weights, weight gains, average daily gain, feed intake, feed conversion ratio and feed cost per kg gain. Dietary treatments however had no significant ($P>0.05$) effects on mortality rate.

Chickens fed diets containing 0.21% dietary tryptophan level performed significantly better than other dietary levels in terms of final weight, weight gain, average daily gain, feed conversion ratio and feed cost per kg gain. Figure 4.4, 4.5 and 4.6 indicated that the level of 0.21% dietary tryptophan improved final weight; weight gain and average daily weight gain were optimal. Birds fed 0.13% supplemental dietary tryptophan had the highest feed intake while birds fed 0.21% supplemental dietary tryptophan had the lowest feed intake. It was also observed that birds fed 0.21% and 0.25% levels of tryptophan had the lowest feed conversion ratios.

Table 4.2: Performance of Broiler Finisher Chickens fed Graded Dietary Level Tryptophan During the Cool Season (5 – 8 Weeks)

	Tryptophan Levels%					
Parameters	0.13	0.17	0.21	0.25	0.29	SEM
Initial weight (g)	1420.73	1420.70	1421.42	1420.76	1421.16	0.29
Final weight(g)	2993.97^{c}	3161.67^{b}	3378.95^{a}	3364.91^{a}	3243.51^{ab}	51.89
Weight Gain (g)	1575.00^{b}	1741.00^{ab}	1957.50^{a}	1944.20^{a}	1535.90^{b}	106.07
Ave daily gain (g)	74.92^{b}	82.90^{ab}	93.22^{a}	92.58^{a}	73.12^{b}	5.05
Feed Intake (g)	4559.45^{a}	4414.03^{ab}	4352.63^{b}	4523.69^{ab}	4378.95^{ab}	59.87
Feed Intake (g/b/d)	217.12^{a}	210.19^{ab}	207.27^{b}	215.42^{ab}	208.52^{ab}	2.85
FCR	2.90^{b}	2.54^{a}	2.23^{a}	2.32^{a}	2.99^{b}	0.23
Feedcost/kg Gain(₦)	61.59^{a}	59.12^{a}	58.69^{a}	55.59^{a}	82.45^{b}	6.24
Mortality (%)	1.75	0.00	1.75	0.00	0.00	1.11

a, b, c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
Try = Tryptophan
FCR = Feed conversion ratio

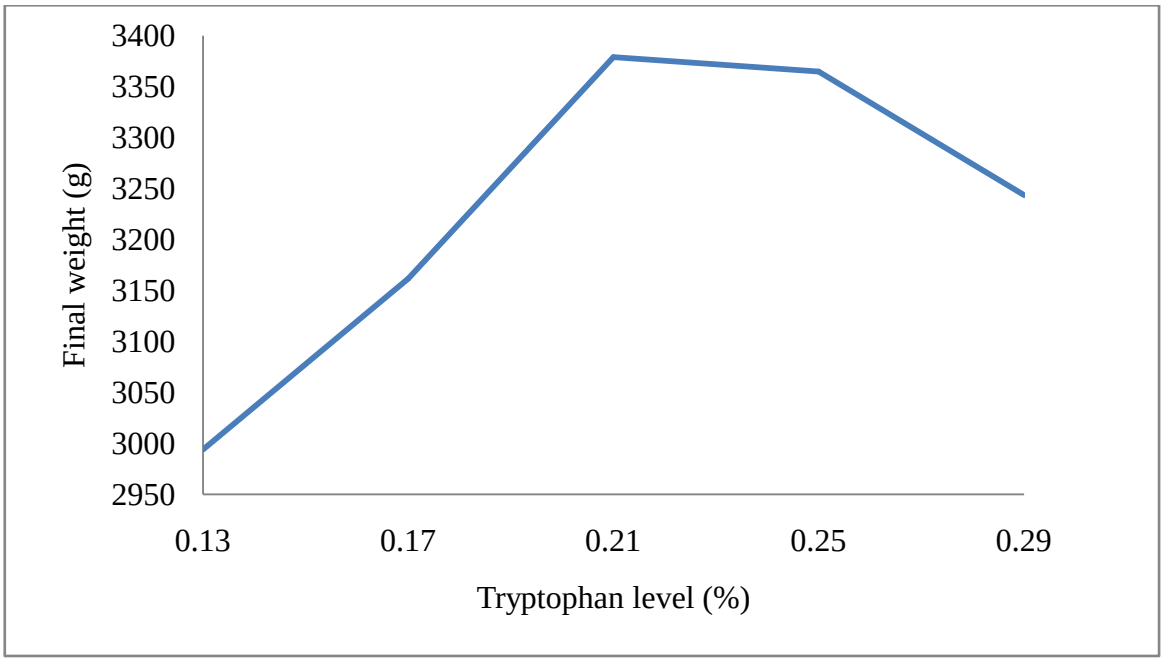


Figure 4.4: Optimum dietary tryptophan level to maximize final weight for broiler finisher chickens during the cool season under tropical environment (5-8 weeks).

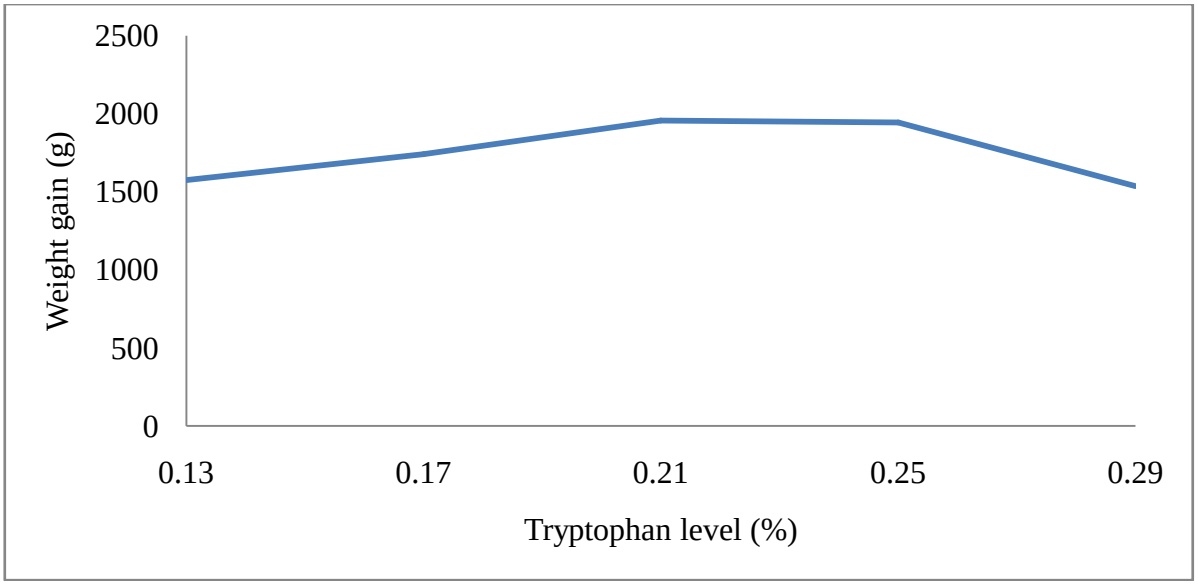


Figure 4.5: Optimum dietary tryptophan level to maximize weight gain for broiler finisher chickens during the cool season under tropical environment (5-8 weeks).

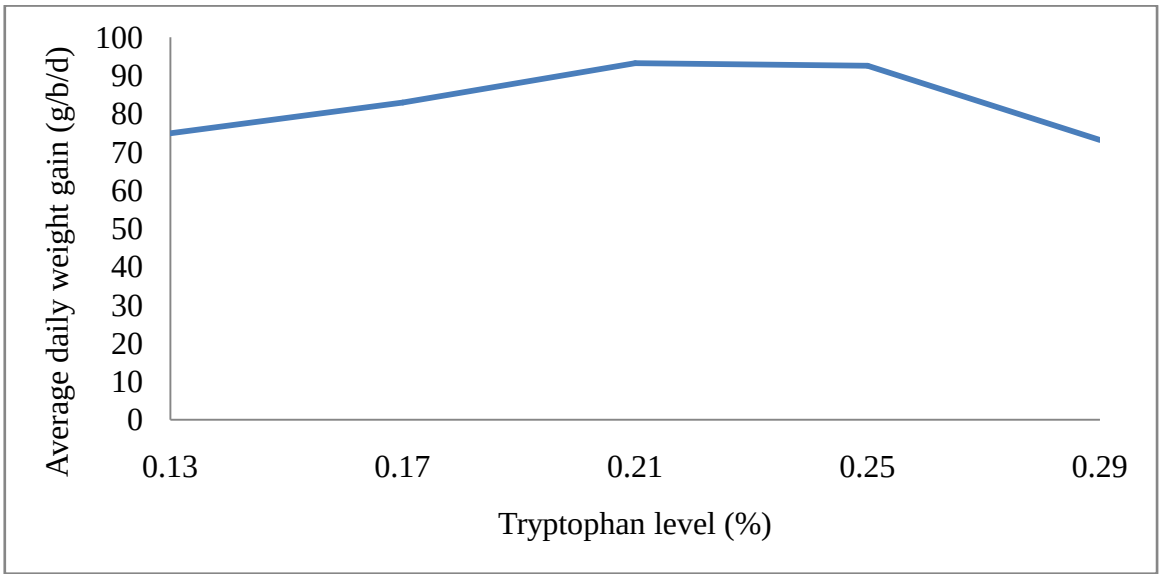


Figure 4.6: Optimum dietary tryptophan level to maximize average daily weight gain for broiler chicks during the cool season under tropical environment (5-8 weeks).

Feed conversion ratio however became poorest at 0.29% dietary level of tryptophan. Feed cost per kg weight gain for chicks fed 0.13, 0.17, 0.21 and 0.25% dietary tryptophan were statistically similar. Feed cost per kg weight gain was highest for birds fed 0.29% dietary tryptophan level.

4.1.3 Effect of Graded Dietary Levels of Tryptophan fed During the Cool Season on the Haematological Parameters and Serum Biochemical Indices of Broiler Starter Chicks. (0 - 4 Weeks)

Table 4.3 shows the effect of graded dietary levels of tryptophan on haematological parameters and serum biochemical indices of broiler starter chicks reared during the

cool season. Dietary tryptophan levels significantly ($P < 0.05$) affected packed cell volume, haemoglobin count, red blood cells, mean cell heamoglobin concentration, total protein, albumin, alkaline phosphate, cholesterol, blood urea and uric acid levels of broiler chicks. Dietary treatments had no significant ($P > 0.05$) effects on white blood cell, mean cell haemoglobin, mean cell volume, creatine, glucose, aspartate amino-transferase (AST), alanine amino-transferase (ALT) and triglyceride. Chicks fed 0.31% dietary tryptophan level had the highest values for haemoglobin count, packed cell volume, red blood cells and mean cell haemoglobin concentration. It was observed also that birds fed 0.27% tryptophan had the highest value for total protein and cholesterol.

Chicks fed the lowest dietary tryptophan level of 0.15% had the highest value of mean cell haemoglobin and mean cell volume while birds fed 0.23% dietary tryptophan level had the highest urea and uric acid values. It was observed that the values obtained for most of the blood parameters and serum biochemical indices were similar. These similarities were observed in the packed cell volume where chicks fed 0.15%, 0.19% and 0.23% had similar values.

Table 4.3: Effect of Graded Dietary Levels of Tryptophan fed During the Cool Season on the Haematological Parameters and Serum Biochemical Indices of Broiler Chicks (0 - 4 Weeks)

	Tryptophan Levels (%)					
Parameters	0.15	0.19	0.23	0.27	0.31	SEM
PCV (%)	34.00^{ab}	31.67^{ab}	32.67^{ab}	30.67^{b}	34.50^{a}	1.10
Hb (g/dl)	10.70^{b}	10.03^{b}	10.47^{ab}	9.87^{b}	11.33^{a}	0.38
RBC (x 10^6/l)	3.40^{b}	3.30^{b}	3.40^{b}	3.42^{b}	4.25^{a}	0.31
WBC (x 10^3/l)	6.93	7.27	9.47	7.53	6.67	1.60
MCH (pg)	32.23	30.55	30.95	29.66	26.78	2.45
MCV (fl)	102.52	96.32	96.59	92.38	81.61	7.99
MCHC (g/dl)	31.47^{b}	31.69^{b}	32.04^{ab}	32.17^{ab}	32.83^{a}	0.27
Urea (mmol/l)	1.45^{b}	2.00^{ab}	3.40^{a}	2.80^{ab}	2.10^{ab}	0.84
Uric acid (mg/dl)	359.67^{b}	496.33^{ab}	599.00^{a}	459.67^{ab}	317.00^{b}	61.15
Creatine (mg/dl)	30.33	45.33	44.00	47.33	51.00	6.48
Glucose (mg/dl)	5.13	3.10	4.53	5.01	4.30	0.65
AST (i.u/l)	5.00	4.20	5.27	4.77	4.33	0.74
ALT (i.u/l)	3.33	4.87	3.87	4.00	4.50	0.71
ALP (i.u/l)	73.67^{a}	64.00^{ab}	58.00^{ab}	48.00^{b}	55.33^{ab}	6.24

Cholesterol (mg/dl)	4.77[a]	3.07[b]	4.27[a]	4.70[a]	4.03[ab]	0.32
Triglyceride (mg/dl)	1.70	1.70	2.10	1.43	2.30	0.31
Albumin (g/dl)	3.80[a]	4.03[a]	3.70[b]	2.43[c]	3.53[b]	0.60
Total protein (g/dl)	3.20[c]	5.53[b]	5.00[b]	6.27[a]	6.20[a]	0.70

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
TRY = Tryptophan
PCV= Packed cell volume
Hb=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

The same was observed for most of the parameters measured. An increase in the blood total protein was observed as the concentration of dietary tryptophan increased. Average values for blood total protein of broiler chicks fed 0.27 and 0.31% dietary tryptophan levels were observed to be higher (6.27 and 6.20 g/dl) than those fed diets containing lower dietary tryptophan levels. Average albumin value was higher for chicks fed 0.15% and 0.19% dietary tryptophan diets while the values were significantly lower for the birds fed higher diets containing higher dietary tryptophan levels. The results for the blood cholesterol were similar across treatment groups (4.03 to 4.77mg/dl) except for the 0.19% tryptophan diet that had slightly reduced blood cholesterol value of 3.07mg/dl. Chicks fed 0.23% dietary tryptophan diet had the highest value for blood urea and uric acid levels. Blood urea and uric acid levels

increased gradually as dietary tryptophan levels increased from 0.15% up to 0.23% and thereafter decreased.

Tryptophan Levels (%)

4.1.4 Effect of Graded Dietary Levels of Tryptophan fed During the Cool Season on the Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens. (5-8 Weeks)

The effect of graded dietary levels of tryptophan on haematological parameters and serum biochemical indices of broiler finisher chickens reared during the cool season are shown in Table 4.4. Dietary treatments had significant ($P<0.05$) effects on packed cell volume, haemoglobin count, red blood cell, white blood cells, urea, creatine, glucose, triglyceride, albumin and total protein. Chickens fed 0.21% total dietary tryptophan diets had the highest value for packed cell volume, haemoglobin count, red blood cells, white blood cell, uric acids, creatine and triglyceride. Dietary treatments had no significant ($P>0.05$) effects on the blood cholesterol, ALP, ALT, AST, mean cell volume, mean cell haemoglobin and mean cell heamoglobin count.

Table 4.4. Effect of Graded Dietary Levels of Tryptophan fed During the Cool Season on the Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens (5 - 8Weeks)

Parameters	0.13	0.17	0.21	0.25	0.29	SEM
PCV (%)	27.67^{ab}	23.33^{b}	30.33^{a}	25.67^{ab}	27.33^{ab}	1.79
Hb (g/dl)	9.40^{ab}	7.33^{b}	10.07^{a}	8.33^{ab}	9.07^{ab}	0.64
RBC (x 10^{6}/l)	4.63^{ab}	3.90^{b}	5.00^{a}	4.27^{ab}	4.57^{ab}	0.31
WBC (x 10^{3}/l)	6.33^{ab}	5.07^{b}	7.63^{a}	5.13^{b}	5.70^{ab}	0.86
MCH (pg)	20.27	19.85	20.16	20.01	19.84	0.32
MCV (fl)	59.77	59.94	60.74	60.20	59.81	0.77
MCHC (g/dl)	33.92	33.12	33.19	33.25	33.17	0.33
Urea (mmol/l)	1.33^{c}	1.83^{bc}	2.00^{bc}	3.00^{a}	2.17^{b}	0.23
Uric acid (mg/dl)	315.33^{b}	453.33^{ab}	590.00^{a}	440.00^{ab}	326.67^{b}	60.49
Creatine (mg/dl)	28.33^{b}	43.00^{ab}	54.33^{a}	47.00^{a}	39.67^{ab}	5.35
Glucose (mg/dl)	4.90^{a}	2.93^{b}	3.77^{ab}	5.17^{a}	4.24^{ab}	0.83
AST (i.u/l)	6.07	3.67	4.13	4.67	6.67	1.77
ALT (i.u/l)	4.67	3.33	2.07	3.33	4.67	1.35
ALP (i.u/l)	66.33	49.00	69.00	45.67	59.67	8.17
Cholesterol (mg/dl)	4.50	3.87	5.17	4.73	4.60	0.52
Triglyceride (mg/dl)	1.70^{ab}	1.50^{b}	2.13^{a}	1.37^{b}	1.10^{b}	0.18
Albumin (g/dl)	3.13^{ab}	3.93^{a}	3.87^{ab}	2.27^{b}	2.53^{ab}	4.75
Total protein (g/dl)	4.00^{a}	4.27^{a}	3.07^{b}	3.83^{ab}	3.73^{ab}	0.27

a, b, c = Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means
TRY = Tryptophan
PCV= Packed cell volume
Hb=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

It was observed that chickens fed 0.17% dietary tryptophan had the highest value for total protein while those fed 0.21% tryptophan diet had the least value for the total protein.

4.1.5 Effect of Graded Dietary levels of Tryptophan on Apparent Nutrient Digestibility of Broiler Chicks During the Cool Season. (0-4 Weeks)

Table 4.5 shows the effect of graded levels of dietary tryptophan on apparent nutrient digestibility of broiler chicks in the cool season (0-4 weeks). It was observed that dietary treatments had significant ($P < 0.05$) effects on the apparent dry matter, crude protein (CP),crude fibre (CF), ether extract (EE), ash and the nitrogen free extract (NFE) digestibilities. It was observed that chicks fed higher concentrations of dietary tryptophan (0.19, 0.23 and 0.27 %) had better apparent dry matter digestibility values than chicks fed the control diet. Chicks fed 0.23% dietary tryptophan had the best apparent dry matter digestibility of 87.55% followed by chicks fed 0.27 and 0.19% dietary tryptophan levels respectively. The chicks fed 0.19, 0.23 and 0.27 percent dietary tryptophan levels had the highest value for apparent CP digestibilities of 84.93, 82.49 and 84.08 percent respectively. The least apparent CP digestibility was obtained with the control diet that had the lowest tryptophan level of 0.15%. Average apparent ether extract digestibility increased as the dietary tryptophan level increased from 0.15 to 0.23% in the diet and thereafter remained at the higher level. The highest ether extract digestibility of 89.01% was obtained at the 0.23% tryptophan level. Apparent NFE digestibility values also increased as the dietary tryptophan levels increased from 0.15 up to 0.27% tryptophan level. The highest NFE digestibility of 72.05% was obtained from the diet containing 0.27% tryptophan level. It was also observed that chicks fed higher concentrations of dietary tryptophan had better apparent ash and NFE digestibility values than chicks fed lower tryptophan concentrations.

Table 4.5: Nutrient Digestibility of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Cool Season (0-4 weeks)

	Tryptophan Levels (%)					
Parameters (%)	0.15	0.19	0.23	0.27	0.31	SEM
Dry matter	81.88^{c}	85.50^{b}	87.55^{a}	85.56^{b}	84.28^{b}	0.90

Crude protein	74.20^{c}	84.93^{a}	82.49^{a}	84.08^{a}	80.59^{b}	2.51
Crude fibre	77.75^{ab}	77.57^{b}	73.37^{c}	72.71^{c}	80.68^{a}	6.92
Ether extract	81.39^{b}	84.33^{ab}	89.01^{a}	86.94^{ab}	87.15^{ab}	2.20
Ash	74.48^{c}	79.13^{b}	87.62^{a}	87.10^{a}	80.38^{b}	4.17
NFE	64.46^{b}	67.05^{ab}	70.28^{ab}	72.05^{a}	69.44^{ab}	1.80

a, b,c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
NFE = Nitrogen free extract

4.1.6 Effect of Graded Dietary levels of Tryptophan on Nutrient Digestibility of Broiler Finisher Chickens Reared During the Cool Season (5- 8 Weeks)

The results of the nutrient digestibilities of broiler finisher chickens fed graded dietary levels of tryptophan during the cool season is shown in Table 4.6. The results indicated that dietary treatments had significant ($P<0.05$) effects on the apparent NFE digestibility of chickens. Chickens fed 0.17% tryptophan diet had the highest value for NFE digestibility, although this value was similar to those obtained for chickens fed other higher tryptophan diets. Dietary treatments had to significant effects on the apparent dry matter, Crude protein, Crude fibre, Ether extract and Ash digestibilities. Although it was observed that chickens fed 0.17% tryptophan diet recorded the highest apparent Crude protein and Crude fibre digestibilities of 75.09% each. Chickens fed 0.29% tryptophan diet had the highest value for apparent ether extract digestibility of 84.73% compared to other treatments. The highest apparent ash digestibility of 86.04% was recorded in birds fed 0.21% tryptophan diet compared to other treatments.

4.1.7 Effect of Graded Dietary Levels of Tryptophan on Carcass Characteristics of Broiler Finisher Chickens During the Cool Season (5- 8 Weeks).

The results for the carcass study of broiler finisher chickens fed graded dietary levels of tryptophan during the cool season are presented in Table 4.7. The results showed that live weights, carcass weights, dressing percentage, breast, wings, thigh, drum sticks, liver, heart, gizzard, abdominal fat, lung and back expressed as percentage of the carcass weights were significantly ($P <0.05$) affected by dietary treatments. The spleen and kidney expressed as percentage of carcass weight were however not significantly ($P >0.05$) affected by dietary treatments.

Table 4.6. Nutrient Digestibility of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Cool Season (5 – 8 Weeks)

	Tryptophan Levels (%)					
Parameters (%)	0.13	0.17	0.21	0.25	0.29	SEM
Dry matter	80.22	86.32	84.06	86.78	87.69	5.67
Crude protein	72.76	75.09	74.71	74.48	72.37	2.68
Crude fibre	67.43	75.09	74.71	74.48	72.37	5.67
Ether extract	69.77	77.03	79.78	82.35	84.73	4.51
Ash	74.60	84.83	86.04	75.91	84.62	3.67
NFE	64.77^{b}	71.90^{a}	67.98^{ab}	$65.9b^{a}$	68.62^{ab}	1.64

a, b= Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means
NFE = Nitrogen free extract

Table 4.7: Carcass Characteristics of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Cool Season. (5 - 8 weeks)

Parameters	Tryptophan Levels (%)					SEM
	0.13	0.17	0.21	0.25	0.29	
Live weight (g)	2990[b]	3091[b]	3342[a]	3334[a]	3242[a]	0.03
Carcass weight (g)	2391[c]	2652[b]	2853[a]	2821[a]	2673[b]	1.09
Dressing (%)	79.73[b]	85.87[a]	85.60[a]	84.43[a]	82.38[a]	0.05
Prime cuts and organ weights expressed as % of dressed weights						
Breast	17.25[b]	21.12[ab]	21.54[a]	23.80[a]	17.53[b]	1.54
Wings	5.59[c]	7.45[b]	7.84[ab]	9.33[a]	8.15[ab]	0.52
Thigh	8.24[b]	10.80[ab]	11.75[a]	13.12[a]	11.10[ab]	0.87
Drumsticks	6.49[b]	9.30[a]	9.64[a]	10.13[a]	8.70[a]	0.52
Back	9.76[b]	13.51[a]	12.26[ab]	13.05[a]	11.13[ab]	0.92
Liver	1.32[c]	1.44[bc]	1.57[bc]	1.95[a]	1.86[b]	0.13
Heart	0.35[b]	0.45[ab]	0.57[a]	0.50[ab]	0.38[b]	0.05
Kidney	0.37	0.37	0.31	0.40	0.34	0.05
Gizzard	1.47[b]	1.99[a]	2.09[a]	2.22[a]	1.96[a]	0.13
Abdominal fat	1.27[b]	0.65[a]	0.30[a]	0.36[a]	0.25[a]	0.16
Spleen	0.07	0.08	0.12	0.08	0.05	0.02
Lung	0.38[b]	0.40[b]	0.56[a]	0.44[ab]	0.42[ab]	0.03

a, b, c= Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means

The results showed that the breast, wings and thigh improved with increase in the level of supplemental tryptophan in the diets up to 0.25% tryptophan level. Abdominal fat also decreased significantly as the level of tryptophan increased in the diet. Birds fed

diets containing 0.25 and 0.29% dietary tryptophan showed significantly ($P<0.05$) higher values of breast, wings, thighs, drumsticks, liver, kidney and back.

4.1.8. Effect of Graded Dietary Levels of Tryptophan on Tissue Crude Protein and Tissue Fat Deposition of Broiler Finisher Chickens During the Cool Season. (5-8 Weeks).

The results of the tissue fat and tissue crude protein analysis of broiler finisher chickens fed graded levels of tryptophan during the cool season are presented in Table 4.8. This results showed that tissue fat were significantly ($P<0.05$) affected by dietary treatments. Dietary treatments however had no significant ($P>0.05$) effect on tissue protein. Tissue fat were significantly lower for the birds fed higher dietary tryptophan diets than those fed the control diet containing the lowest dietary tryptophan level. This result therefore showed that the tissue fat of broiler birds partially decreased with increase in the dietary level of tryptophan up to 0.27% dietary tryptophan level.

Table 4.8: Effect of Graded Dietary Levels of Tryptophan on Tissue Fat Deposition and Tissue Crude Protein of Broiler Finisher Birds Reared During the Cool Season (5-8) weeks

	Tryptophan Levels %					
Parameters	0.15	0.19	0.23	0.27	0.31	SEM
Crude protein (%)	20.54	21.86	22.84	22.41	22.41	1.41
Ether extract (%)	2.96[a]	2.86[a]	1.98[ab]	1.68[b]	2.39[ab]	0.25

a, b,= Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means

4.2 Experiment 2: Performance of Broiler Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season

4.2.1 Performance of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Hot Season (0-4 Weeks)

The performance of broiler starter chicks fed graded dietary levels of tryptophan is shown in Table 4.9. Dietary levels of tryptophan had significant ($P < 0.05$) effects on final weight, weight gain, average daily weight gain, feed intake and feed cost per kg gain. Chicks fed 0.23% dietary tryptophan had the significantly better results in terms of the final weight, weight gain, average daily weight gain and feed cost per kg weight gain compared to the chicks fed other dietary tryptophan levels (Figures 4.7, 4.8 and 4.9). A steady increase in final weight, weight gain and average daily weight gain was observed as the levels of dietary tryptophan increased and began to decrease at 0.27% dietary tryptophan (Figure 4.7, 4.8 and 4.9).

In general, tryptophan supplemented diets performed significantly better than the control diets in terms of final weights, weight gains, average daily gain, feed intake and feed cost per Kg gain. Although dietary tryptophan, levels had no significant ($P<0.05$) effects on feed conversion ratio and mortality. Chicks fed tryptophan supplemented diets gave slightly better values than un-supplemented diet.

Table 4.9: Performance of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Hot season (0- 4 Weeks)

	Tryptophan Levels %					
Parameters	0.15	0.19	0.23	0.27	0.31	SEM
Initial weight (g)	40.02	40.02	40.01	40.00	40.01	0.01
Final weight(g)	725.99^{c}	824.98^{b}	905.27^{a}	904.95^{a}	904.01^{a}	14.21
Weight Gain (g)	685.98^{c}	784.96^{b}	865.25^{a}	864.95^{a}	864.00^{a}	14.21
Ave daily gain (g)	24.50^{c}	28.03^{b}	30.91^{a}	30.89^{a}	30.86^{a}	0.51
Feed Intake (g)	1062.31^{b}	1099.56^{ab}	1159.26^{ab}	1218.52^{a}	1177.78^{ab}	42.95
Feed Intake (g/b/d)	37.94^{b}	39.27^{ab}	41.40^{ab}	43.52^{a}	42.06^{ab}	1.53
FCR	1.55	1.41	1.34	1.41	1.36	0.06
Feed cost/kg Gain(₦)	145.38^{c}	134.46^{ab}	128.80^{a}	135.78^{ab}	142.89^{bc}	2.49
Mortality (%)	5.60	1.85	0.00	3.70	1.85	2.90

a, b, c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
FCR = Feed conversion ratio

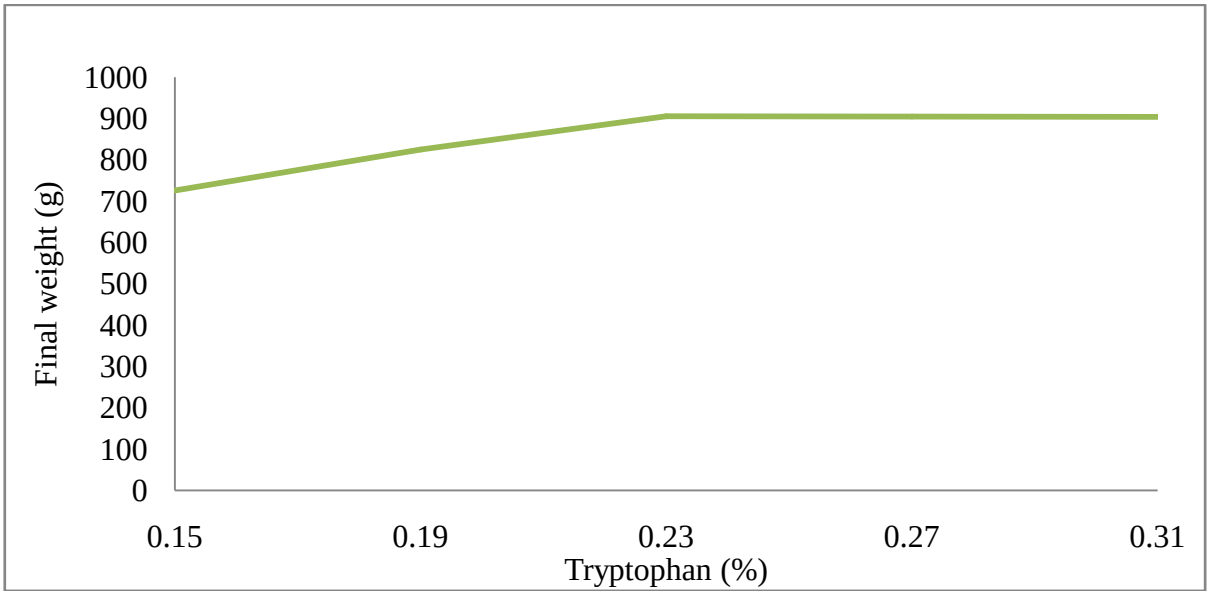


Figure 4.7: A graph showing optimum dietary tryptophan level to maximize final weight for broiler chicks during the hot season under tropical environment (0-4 weeks).

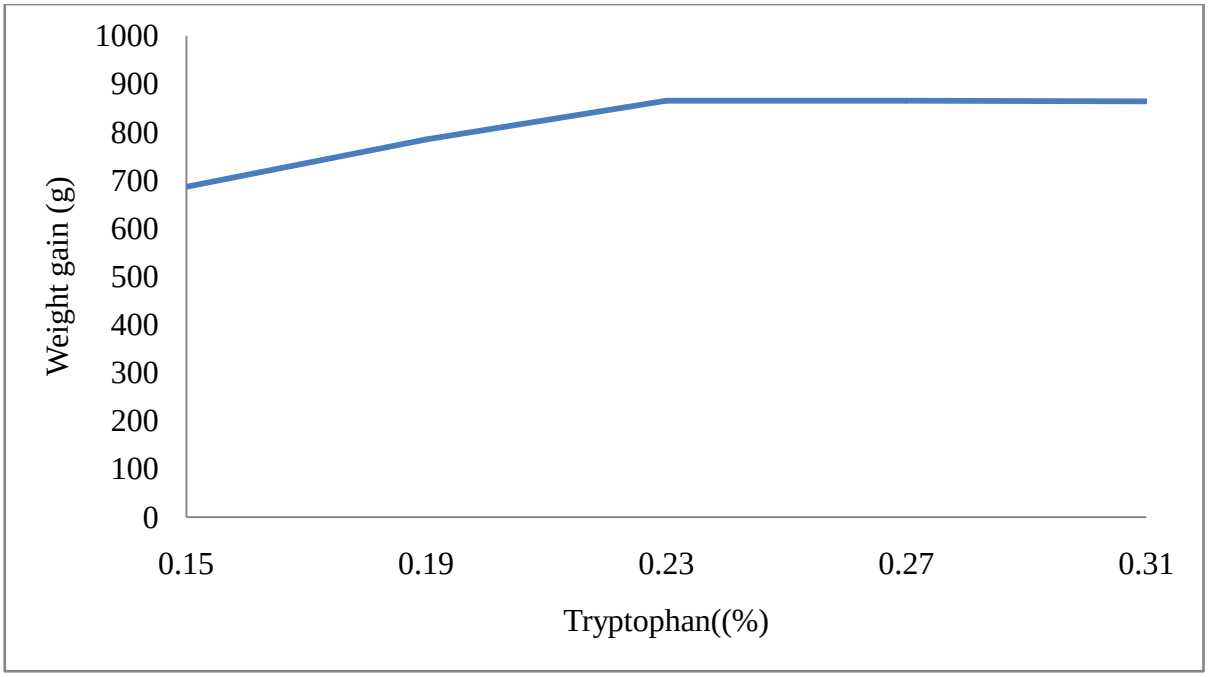


Figure 4.8: Optimum dietary tryptophan level to maximize weight gain for broiler chicks during the hot season under tropical environment (0-4 weeks).

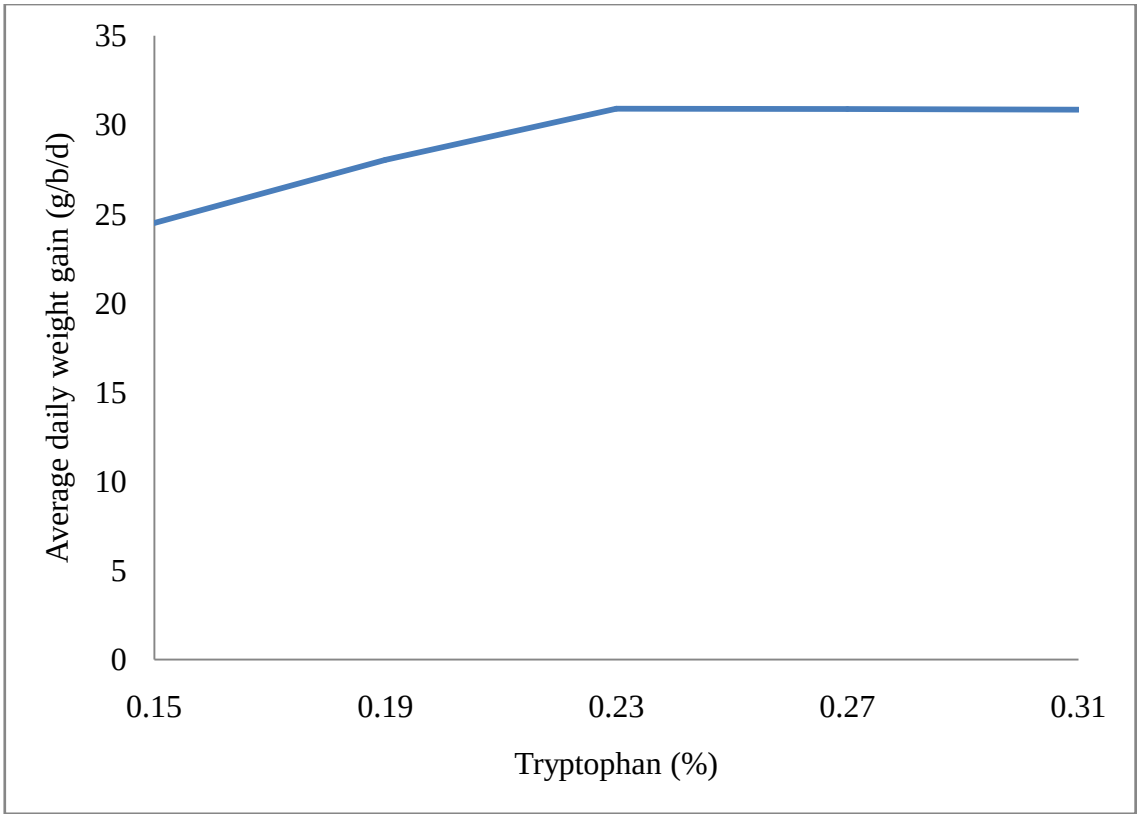


Figure 4.9: Optimum dietary tryptophan level to maximize average daily weight gain for broiler chicks during the hot season under tropical environment (0-4 weeks).

4.2.2 Performance of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (5 - 8Weeks)

The performance of broiler finisher chickens fed graded dietary levels of tryptophan during the hot season are shown in Table 4.10. Dietary treatments had significant ($P<0.05$) effect son final weight, weight gain, average daily weight gain, feed intake, average daily feed intake and feed cost per kg weight gain. No significant ($P>0.05$) effect of dietary treatments was observed on the feed conversion ratio and mortality. The performances of chickens fed diets supplemented with tryptophan were similar and significantly better than those on the control diet in terms of the final weight, weight gain and feed intake. There were trends of improved performance in final weights and weight gains observed as the levels of dietary tryptophan increased. Chickens fed 0.21% dietary tryptophan had the best results in terms of final weight, weight gain and average daily weight gain (Figures 4.10, 4.11 and 4.12). Chickens fed 0.21% dietary tryptophan level had the lowest value for feed cost per kg gain.

4.2.3 Haematological Parameters and Serum Biochemical Indices of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Hot Season (0-4 weeks)

The effects of graded levels of dietary tryptophan on blood parameters and blood serum biochemical indices of broiler chicks are presented in Table 4.11. Dietary levels of tryptophan had significant ($P>0.05$) effects on packed cell volume, haemoglobin count, red blood cells and mean cell haemoglobin count. Chicks fed 0.15% dietary tryptophan had the highest values in term of packed cell volume, red blood cell and haemoglobin count while chicks fed 0.31% dietary tryptophan had the lowest values for the packed cell volume, red blood cells and haemoglobin count.

Table 4.10: Performance of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (5-8weeks)

	Tryptophan Levels %					
Parameters	0.13	0.17	0.21	0.25	0.29	SEM
Initial weight (g)	1170.88	1170.61	1170.41	1171.67	1170.00	0.65
Final weight(g)	2453.12^{b}	2623.39^{a}	2776.14^{a}	2771.43^{a}	2763.22^{a}	49.27
Weight Gain (g)	1282.40^{b}	1452.80^{ab}	1605.70^{a}	1599.80^{a}	1434.90^{ab}	89.67
Ave daily gain (g)	61.06^{b}	69.18^{ab}	76.46^{a}	76.18^{a}	68.33^{ab}	4.27
Feed Intake (g)	3012.00^{b}	3183.80^{ab}	3627.60^{a}	3715.70^{a}	3390.30^{ab}	175.15
Feed Intake (g/b/d)	143.45^{b}	151.61^{ab}	172.75^{a}	176.94^{a}	161.44^{ab}	8.34
FCR	2.34	2.20	2.26	2.32	2.40	1.09
Feed cost/kg Gain(₦)	76.28^{b}	70.74^{ab}	67.73^{a}	71.80^{b}	86.83^{b}	3.73
Mortality (%)	8.33	0.00	0.00	0.00	0.00	5.80

a, b,= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
FCR = Feed conversion ratio

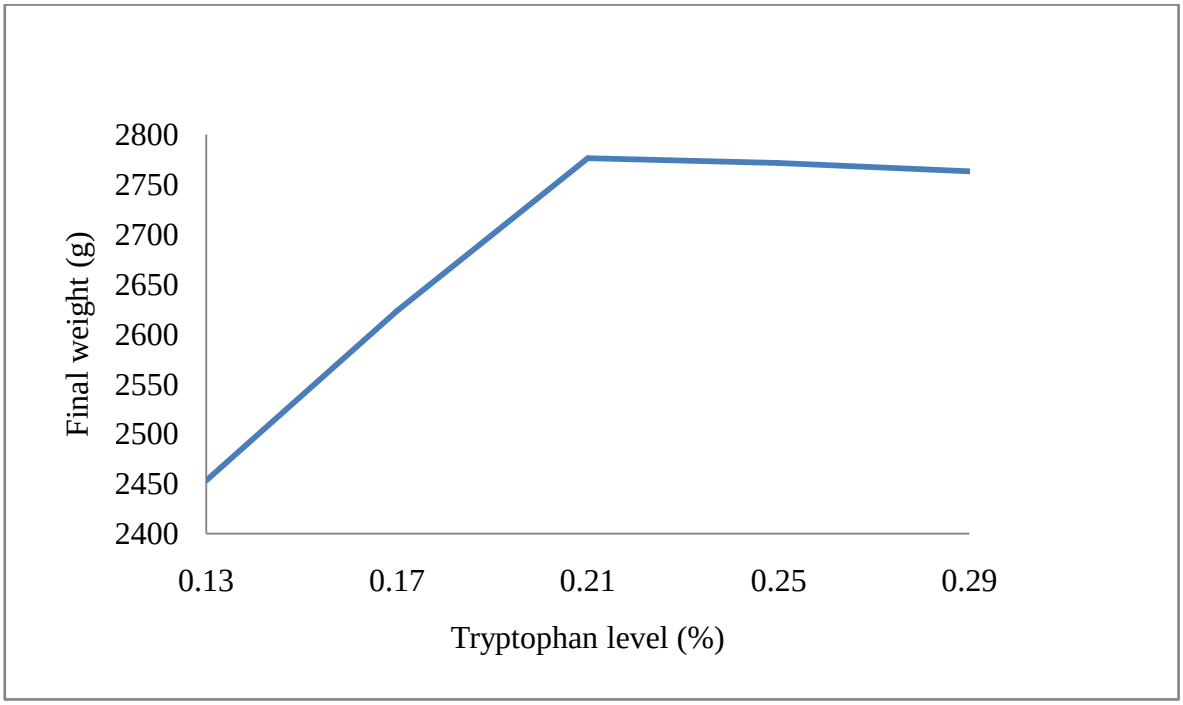


Figure 4.10: Optimum dietary tryptophan level to maximize final weight for broiler finisher chickens during the hot season under tropical environment. (5-8 weeks).

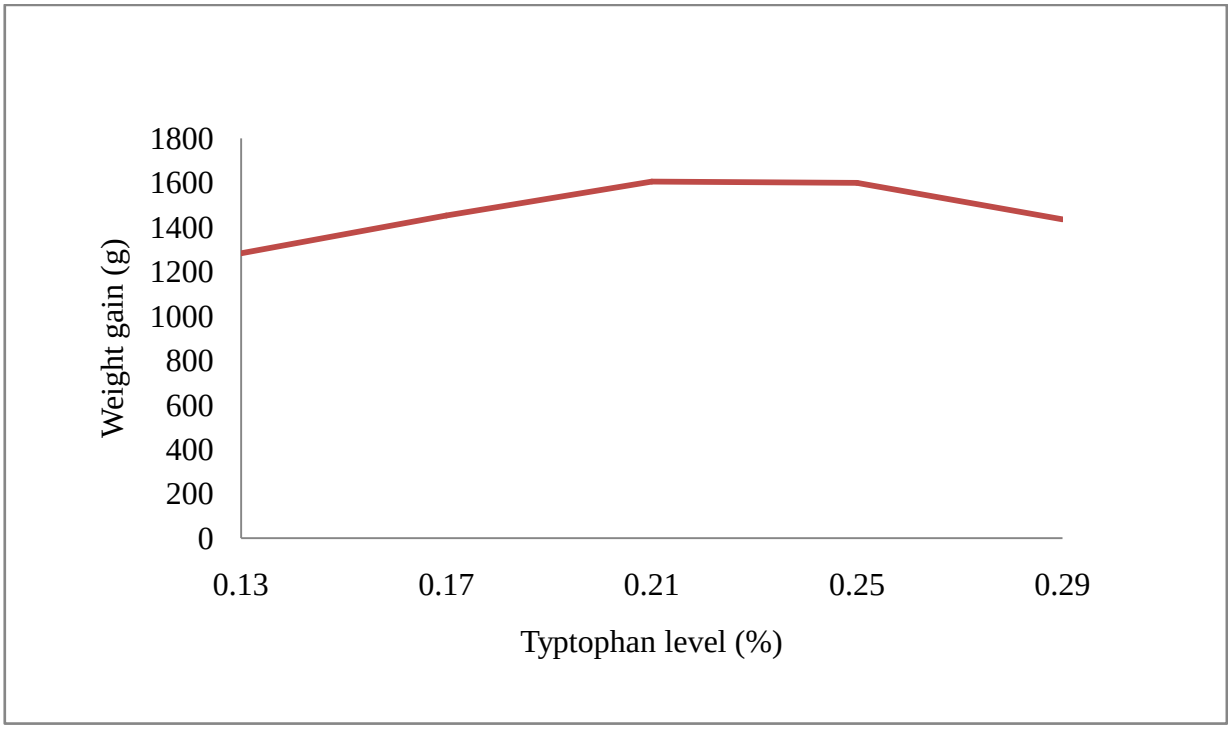


Figure 4.11: Optimum dietary tryptophan level to maximize weight gain for broiler finisher chickens during the hot season under tropical environment. (5-8 weeks).

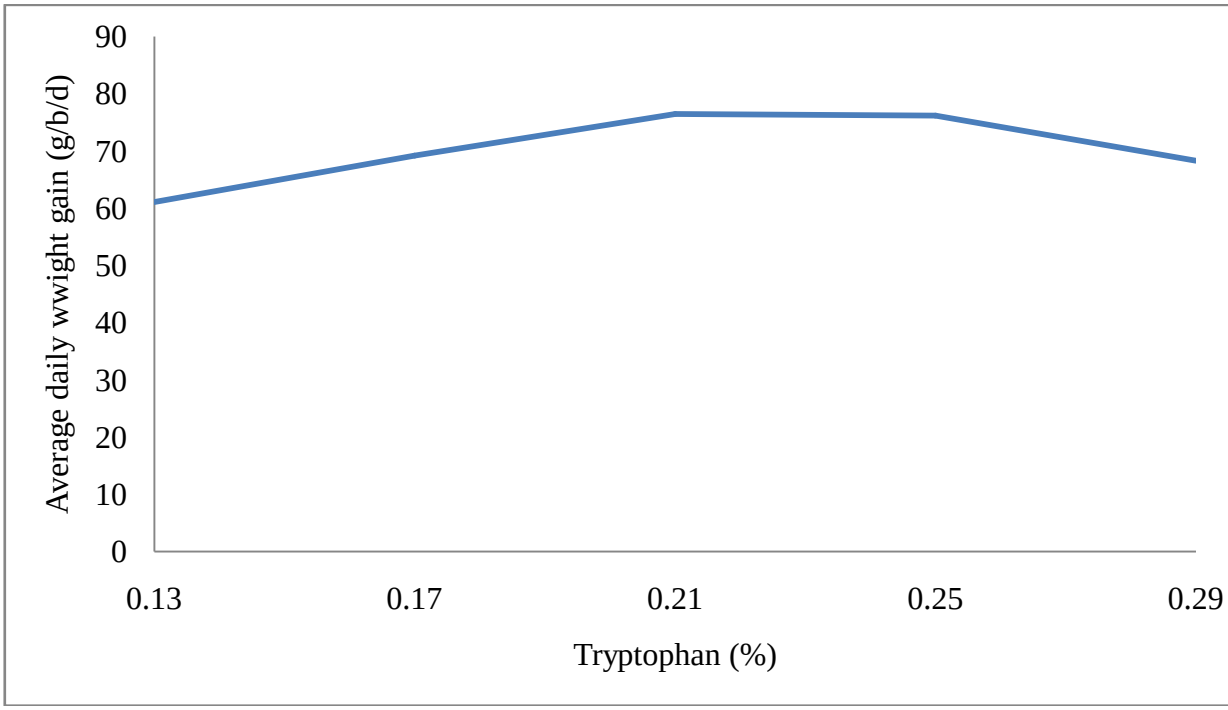


Figure 4.12: Optimum dietary tryptophan level to average daily weight gain for broiler finisher chickens during the hot season under tropical environment. (5 - 8 weeks).

Table 4.11: Effect of Graded Dietary Levels of Tryptophan on Haematological Parameters and Serum Biochemical Indices of Broiler Chick During the Hot Season (0–4 Weeks)

	Tryptophan Levels (%)					
Parameters	0.15	0.19	0.23	0.27	0.31	SEM
PCV (%)	28.33^{a}	25.33^{ab}	24.67^{ab}	27.00^{ab}	19.00^{b}	2.42
Hb (g/dl)	9.40^{a}	8.43^{ab}	8.20^{ab}	8.97^{ab}	6.27^{b}	0.81
RBC (x10^{6}/l)	4.70^{a}	4.27^{ab}	3.97^{ab}	4.40^{ab}	3.37^{b}	0.36
WBC(x10^{3}/l)	7.40	6.60	3.53	5.13	4.93	1.70
MCH(pg)	20.16	19.77	20.97	20.40	18.12	1.36
MCV(fl)	60.57	59.39	63.05	61.43	54.96	4.05
MCHC(g/dl)	33.29^{a}	33.29^{a}	33.25^{a}	33.94^{a}	32.94^{b}	0.08
Urea(mmol/l)	1.60	1.75	1.90	1.72	1.83	0.13
Uric acid(mg/dl)	490	503.30	420.00	390.00	454.50	92.08
Creatine (mg/dl)	32.00^{b}	43.00^{a}	30.00^{b}	30.67^{b}	24.33^{b}	2.80
Glucose(mg/dl)	2.67^{a}	2.75^{a}	2.51^{a}	1.83^{b}	2.23^{ab}	0.16
AST(i.u/l)	3.00^{b}	3.00^{b}	3.07^{b}	4.83^{a}	3.03^{b}	0.52
ALT(i.u/l)	4.00^{ab}	5.33^{a}	4.15^{ab}	4.48^{b}	4.27^{ab}	0.48
ALP(i.u/l)	65.00^{a}	52.50^{ab}	47.67^{b}	44.33^{b}	51.33^{ab}	4.32
Cholesterol(mg/dl)	4.25	4.60	4.37	3.87	4.53	0.58
Triglycerides(mg/dl)	1.55	1.30	1.45	1.20	1.90	0.35
Albumin(g/dl)	3.67	3.00	4.00	4.40	5.00	1.01
Total protein(g/dl)	2.73^{ab}	2.87^{a}	2.67^{ab}	2.47^{ab}	2.00^{b}	0.25

a, b, = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
PCV= Packed cell volume
Hb=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase

ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

Dietary tryptophan had no significant effect ($P<0.05$) on the white blood cells, mean cell haemoglobin and mean cell volume. Dietary levels of tryptophan significantly, ($P<0.05$) increased, Aspartate Amino-Transferase (AST) at 0.27% tryptophan level and thereafter declined, but ALP decreased as dietary level of tryptophan increased. Chicks fed 0.19% dietary tryptophan dietary level of tryptophan increased had the highest value for total protein and thereafter total protein decreased as tryptophan level increased. Serum concentration of glucose were similar for chicks fed 0.15%, 0.19%, 0.23% and 0.31% dietary tryptophan but were significantly ($P<0.05$) higher than chicks fed 0.27% dietary tryptophan.

Dietary tryptophan levels had no significant effect on serum albumin concentration. The serum concentration of ALT and creatine for chicks fed 0.19% dietary tryptophan significantly ($P<0.05$) increased above the value for the control and thereafter decreased at higher dietary levels of tryptophan. Dietary tryptophan had significant ($P<0.05$) effect on the ALP, it was observed that the ALP decreased for all tryptophan supplemented diets above the value for the control diet. The serum concentration of AST was observed to be higher for chicks fed 0.27% dietary tryptophan while chicks

fed other diets were similar but with lower values. Dietary tryptophan had no effect ($P<0.05$) on urea, uric acid, triglyceride, albumin and cholesterol levels.

4.2.4 Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens Fed Graded Dietary Levels of Tryptophan During the Hot Season. (5 – 8 Weeks).

The effect of graded levels of dietary tryptophan on haematological parameters and serum biochemical indices of broiler finisher chickens reared in the hot season were as presented in Table 4.12.

Table 4.12: Effect of Graded Dietary Levels of Tryptophan on Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens Reared in the Hot Season. (5- 8 Weeks).

	Tryptophan Levels (%)					
Parameters	0.13	0.17	0.21	0.25	0.29	SEM
PCV (%)	27.27	27.89	29.86	29.66	27.25	1.61
Hb (g/dl)	8.91^{b}	9.62^{ab}	9.76^{ab}	10.58^{a}	8.67^{b}	0.34
RBC ($x10^{6}$/l)	1.95	2.79	2.14	1.58	2.11	0.46
WBC($x10^{3}$/l)	14.89^{c}	16.89^{a}	16.95^{a}	16.17^{ab}	15.73^{bc}	0.30
MCH(pg)	45.91^{ab}	35.82^{b}	58.61^{ab}	67.05^{a}	42.66^{ab}	8.38
MCV(fl)	141.04	104.31	182.14	188.87	135.19	29.19
MCHC(g/dl)	33.18	34.52	32.72	35.86	31.95	1.97
Urea(mmol/l)	5.53^{b}	6.13^{b}	7.44^{a}	4.00^{c}	5.37^{b}	0.41
Uric acid(mg/dl)	174.67^{bc}	95.00^{bc}	85.00^{c}	190.00^{b}	320.00^{a}	29.83
Creatine(mg/dl)	71.67^{a}	57.67^{b}	80.33^{a}	36.67^{c}	49.67^{a}	4.05
Glucose(mg/dl)	5.43^{c}	11.43^{a}	9.07^{b}	3.90^{d}	5.40^{c}	0.40
AST(u.i/l)	46.33^{a}	29.67^{bc}	24.00^{c}	35.67^{b}	49.67^{a}	3.30
ALT(u.i/l)	17.00^{b}	24.67^{a}	25.00^{a}	26.33^{a}	27.33^{a}	2.37
ALP(u.i/l)	52.67^{b}	65.33^{b}	64.67^{b}	94.00^{a}	86.67^{a}	5.90
Cholesterol(mg/dl)	3.32^{ab}	2.16^{bc}	2.08^{bc}	1.83^{c}	3.87^{a}	0.23
Triglycerides(mg/dl)	2.53^{a}	2.23^{ab}	1.50^{bc}	1.27^{c}	0.90^{c}	0.25
Albumin(g/dl)	3.47^{b}	4.27^{a}	3.40^{b}	1.73^{d}	2.3^{c}	1.60
Total protein(g/dl)	3.77^{a}	2.37^{b}	2.67^{ab}	1.58^{ab}	3.05^{ab}	0.41

a, b, = Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means
PCV= Packed cell volume
HC=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

Dietary treatments had significant ($P<0.05$) effects on haemoglobin count, white blood cell, mean cell haemoglobin, urea, uric acid, creatine, glucose, AST, ALT, ALP, cholesterol, triglyceride, albumin and total protein. Chickens fed 0.25% dietary tryptophan had the highest value for haemoglobin count followed by birds fed 0.21%, 0.17%, 0.13% and 0.29 respectively. White blood cells were observed to increase significantly above the value for the control diet. The increase peaked at 0.21% dietary tryptophan level thereafter decreased significantly at 0.29% dietary level of tryptophan. Mean cell haemoglobin increased for the 0.21% and 0.25% dietary tryptophan levels and thereafter decreased 0.29% dietary tryptophan of level.

The serum concentration of urea increased first up to 0.21% dietary level tryptophan but significantly decreased thereafter. Uric acid value did not show any pattern. It decreased from 174.67g/dl to 95 and 85g/dl for tryptophan levels of 0.17% and 0.21% respectively and thereafter increased to 190 and 320g/dl respectively. Dietary treatment had no significant ($P>0.05$) effects on packed cell volume and red blood cells. The effect of dietary tryptophan levels on creatine did not follow any definite pattern as it decreased first at 0.17% tryptophan level, then went up to 80.33mg/dl at 0.21% dietary tryptophan and decreased thereafter. Increasing dietary tryptophan levels significantly

decreased triglycerides, albumin and total protein levels when compared with the value for the control diet. Cholesterol value also decreased with increasing dietary levels of tryptophan and then suddenly increased at the highest dietary level of tryptophan.

4.2.5 Nutrient Digestibility of Broiler Chicks fed Graded Dietary levels of Tryptophan During the Hot Season (0- 4 Weeks)

Table 4.13 shows the effect of dietary tryptophan levels on nutrient digestibility of broiler chicks in the hot season (0-4 weeks). It was observed that dietary treatments had significant ($P<0.05$) effects on the apparent crude protein (CP), ether extract (EE) and nitrogen free extract (NFE) digestibilities. The chicks fed 0.19, 0.23 and 0.27% dietary tryptophan had the highest value for apparent CP (83.19, 79.08, 83.11 and 81.49%) respectively. The lowest apparent CP digestibility was obtained for the 0.31% dietary tryptophan. Chicks fed 0.23% dietary tryptophan had the highest value of 87.07% for the apparent EE digestibility, although this value is similar to the value (81.33%) obtained for chicks fed 0.19% dietary tryptophan. It was observed that chicks fed diets supplemented with tryptophan had better NFE values compared to chickens without supplemental tryptophan. Chickens fed supplemented tryptophan diets had the higher apparent NFE digestibilities values than the control diet. Dietary treatments did not have any significant ($P>0.05$) effects on dry matter, crude fibre (CF) and ash digestibilities.

Table 4.13: Nutrient Digestibility of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Hot Season (0 – 4 Weeks)

	Tryptophan Levels (%)					
Parameters (%)	0.15	0.19	0.23	0.27	0.31	SEM
Dry matter	88.06	87.4	85.49	86.12	93.77	2.87
Crude protein	77.60^{ab}	83.19^{a}	83.11^{a}	81.49^{ab}	75.76^{b}	1.85
Crude fibre	70.77	76.05	76.20	74.86	74.41	2.54
Ether extract	76.61^{b}	81.33^{ab}	87.07^{a}	74.75^{bc}	71.91^{c}	1.84
Ash	63.21	58.49	53.12	50.76	51.87	5.32
NFE	60.04^{c}	66.10^{a}	62.23^{b}	62.11^{b}	64.87^{a}	1.42

a, b, c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
NFE = Nitrogen free extract

4.2.6 Nutrient Digestibility of Broiler Finisher Chickens fed Graded Dietary levels of Tryptophan During the Hot Season (5-8 Weeks)

The results of the nutrient digestibility of broiler finisher chickens fed graded dietary levels of tryptophan in their diets during the hot season shown Table 4.14. The result shows that dietary treatments had significant ($P<0.05$) effect on the apparent CP digestibility. Digestibilities for the tryptophan supplemented diets were generally better than that of the control diet. Chickens fed 0.21% dietary tryptophan had comparatively the highest value for CP. Dietary treatments had to significant effects on the apparent, dry matter, CF, EE, ash and NFE digestibility.

4.2.7 Carcass Analysis of Broiler Finisher Chickens fed Grade Dietary Levels of Tryptophan During the Hot Season (5-8 Weeks)

The results for the carcass study of chickens fed graded dietary levels of tryptophan during the hot season are shown in Table 4.15. The live weight, carcass weight, dressing percentage, breast, thigh, drumstick, heart, kidney, abdominal fat and spleen were significantly ($P<0.05$) affected by dietary treatments. It was observed that chickens fed 0.13, 0.17, 0.21, 0.25 and 0.29% dietary tryptophan had similar values and the best results in terms of the carcass weight, dressing percentage, breast, thigh and

drumstick. Chickens fed low concentration of tryptophan had the least carcass weight, dressing percentage and thigh. The wings, liver, gizzard, lung and back were not significantly (P>0.05) affected by dietary treatments. There was no consistent pattern in the result for the abdominal fat although the effect showed significance.

Table 4.14: Nutrient Digestibility of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (5- 8 Weeks)

	Tryptophan Levels					
Parameters (%)	0.13	0.17	0.21	0.25	0.29	SEM
Dry matter	87.40	88.46	87.72	85.05	85.18	0.87
Crude protein	70.00^{d}	75.88^{c}	84.67^{a}	80.36^{b}	76.09^{c}	1.20
Crude fibre	62.33	73.15	60.79	71.87	69.61	11.04
Ether extract	73.32	73.95	76.27	65.71	71.35	5.49
Ash	61.61	75.39	63.75	67.62	64.40	7.09
NFE	62.10	66.96	64.17	65.45	66.92	1.70

a, b, c, d= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
NFE = Nitrogen free extract

Table 4.15: Carcass Characteristics of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (5-8 Weeks)

	Tryptophan Levels (%)					
Parameters	0.13	0.17	0.21	4 0.25	5 0.29	SEM
Live weight (g)	2472[b]	2663[a]	2811[a]	2792[a]	2790[a]	0.05
Carcass weight (g)	1972[b]	2233[a]	2394[a]	2312[a]	2275[a]	0.06
Dressing (%)	79.71[b]	83.84[a]	85.13[a]	82.77[ab]	81.33[ab]	1.14
Prime cuts and organ weights expressed as % of dressed weights						
Breast	6.46[c]	14.05[ab]	15.14[a]	12.69[a]	14.05[ab]	0.54
Wings	3.20	3.46	4.21	3.81	3.74	0.46
Drumsticks	5.38[b]	7.81[a]	8.12[a]	8.29[a]	8.37[a]	0.54
Back	4.36	5.78	6.24	6.40	6.45	8.99
Liver	0.84	1.04	0.34	0.32	0.33	0.08
Heart	0.15[b]	0.21[ab]	0.32[a]	0.28[ab]	0.24[b]	0.04
Kidney	0.19[b]	0.24[ab]	0.31	0.29[ab]	0.33[a]	0.03
Gizzard	0.75	0.89	0.94	0.92	0.95	0.08

Abdominal fat	0.52[a]	1.15[b]	0.70[a]	0.53[a]	0.45[a]	0.11
Spleen	0.05[ab]	0.07[a]	0.06[ab]	0.06[ab]	0.05[b]	0.08
Thigh	4.69[c]	7.11[b]	7.69[b]	9.38[a]	7.99[b]	0.34
Lung	0.21	0.28	0.34	0.32	0.33	0.07

a, b, c= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means

4.2.8. Effect of Graded Dietary Levels of Tryptophan on Tissue Fat Deposition and Crude Protein of Broiler Finisher Chickens Fed During the Hot Season (5-8 weeks)

The results of the tissue fat and tissue crude protein analyses of broiler finisher chickens fed graded dietary levels of tryptophan during the hot season is presented in Table 4.16. This result showed that tissue fat was significantly (P<0.05) affected by dietary treatments. Dietary treatments however had no significant (P>0.05) effect on tissue protein. There were however no definite patterns for the effect of dietary tryptophan on tissue fat. The value first increased at 0.17% tryptophan then it significantly dropped at 0.21% level of tryptophan. Thereafter, it increased for the higher dietary tryptophan level of 0.25% and 0.29%.

Table 4.16: Effect of Graded Dietary Levels of Tryptophan on Tissue Fat Deposition and Tissue Crude Protein of Broiler Finisher Birds Reared During the Hot Season (5-8 Weeks)

	Tryptophan Levels %					
Parameters	0.13	0.17	0.21	0.25	0.29	SEM
Crude protein (%)	18.36	19.81	18.70	18.69	17.78	1.46
Ether extract (%)	2.67^{ab}	3.22^{ab}	1.94^{b}	2.77^{ab}	4.04^{a}	0.29

a, b,= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means

4.3 Experiment 3: Crude Protein Requirements and Performance of Broiler Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids

4.3.1 Crude Protein Requirements and Performance of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (0-4 Weeks)

Crude protein requirements and performance of broiler starter chicks fed diets formulated on the basis of four critical essential amino acids is shown on Table 4.17. Dietary treatments had significant effects on final weight, weight gain, average daily gain, feed intake, average daily feed intake and feed conversion ratio. It was generally observed that supplementation of the first four limiting amino acids (methionine, lysine, threonine and tryptophan) resulted in improved ($P<0.05$) final weight, weight gain, average daily gain and feed conversion ratio for chicks fed 21% CP diet. Chicks fed 22% crude protein diets had the highest feed intake followed by chicks fed 20% and 21% crude protein respectively. It was also observed that chicks fed 21 and 23% CP diets had significantly ($P<0.05$) better feed conversion ratio. The highest value (1.77) was observed for chicks fed 22% CP diet. No significant ($P<0.05$) difference was observed for feed cost per kg gain and for mortality rate.

4.3.2 Crude Protein Requirements and Performance of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (5-8Weeks)

The crude protein requirements and performance of broiler finisher chickens diets formulated on the basis of four critical essential amino acids is presented in Table 4.18. Final weight, weight gain, average daily weight gain, feed intake, average daily feed intake, feed conversion ratio and feed cost per kg weight gain were significantly ($P<0.05$) affected by dietary treatments.

Table 4.17: Crude Protein Requirements and Performance of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (0- 4 Weeks)

	Crude protein level (%)				
Parameters	20	21	22	23	SEM
Initial weight (g)	51.45	51.43	51.39	51.29	0.07
Final weight(g)	1061.88^{b}	1085.17^{a}	1079.17^{a}	1083.33^{a}	10.62
Weight Gain (g)	1010.43^{b}	1033.73^{a}	1027.78^{a}	1032.04^{a}	10.56
Ave daily gain (g)	36.09^{b}	36.92^{a}	36.71^{a}	36.92^{a}	0.02
Feed Intake (g)	1620.80^{ab}	1579.20^{ab}	1818.80^{a}	1500.00^{b}	76.73
Feed Intake (g/b/d)	57.88^{ab}	56.40^{ab}	64.96^{a}	53.57^{b}	2.74
FCR	1.60^{a}	1.52^{a}	1.77^{b}	1.45^{a}	0.05
Feed cost/kg Gain(₦)	103.19	102.27	103.22	101.26	0.15
Mortality (%)	0.00	0.00	0.00	0.00	0.00

a, b, = Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means
FCR = Feed conversion ratio

Table 4.18: Crude Protein Requirements and Performance of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (5 – 8 Weeks)

	Crude protein level (%)				
Parameters	18	19	20	21	SEM
Initial weight (g)	1401.83	1402.45	1402.81	1402.02	1.02
Final weight(g)	2857.33^{b}	3090.16^{a}	3248.81^{a}	3263.49^{a}	51.49
Weight Gain (g)	1455.50^{b}	1687.71^{a}	1846.00^{a}	1861.48^{a}	51.32
Ave daily gain (g)	69.31^{b}	80.37^{a}	87.91^{a}	88.64^{a}	2.44
Feed Intake (g)	4012.20^{b}	4012.90^{b}	4274.80^{a}	4212.50^{a}	77.75
Feed Intake (g/b/d)	191.08^{b}	191.90^{b}	203.56^{a}	200.60^{a}	3.70
FCR	2.77^{b}	2.38^{a}	2.32^{a}	2.27^{a}	0.09
Feed cost/kg Gain(₦)	75.82^{b}	64.87^{a}	59.29^{a}	58.61^{a}	2.49
Mortality (%)	0.00	0.00	0.00	1.75	0.88

a, b, = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
FCR = Feed conversion ratio

Feeding 18% CP diet resulted in significantly (P<0.05) lower final weight, weight gain and average daily weight gain than those fed diets higher crude protein diets. Chicks fed low crude protein (18% CP) showed also the poorest feed conversion ratio and feed cost per kg gain compared to those fed diets with higher levels of crude protein. FCR values improved as dietary protein levels increased. The performance of chickens fed 19% CP was similar to chickens fed 20 and 21% CP diets in terms of final weight, weight gain, feed conversion ratio and feed cost per kg weight gain. Dietary treatments had no significant (P<0.05) effects on mortality rate.

4.3.3 Haematological Parameters and Serum Biochemical Indices of Broiler Starter Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (0- 4 Weeks)

The effect of feeding diets on the basis of four critical essential amino acids along with graded levels of dietary crude protein on the haematological and serum biochemical parameters of starter chicks were as shown in Table 4.19. Dietary tryptophan levels significantly (P<0.05) affected the packed cell volume, haemoglobin count, red blood cells, white blood cells, uric acid, glucose, and cholesterol, ALT, ALP and cholesterol values. Chicks fed 22% CP had the highest value for PCV, haemoglobin count and ALT. Dietary tryptophan had significant (P<0.05) effects on uric acid, but values followed no definite pattern. ALP values increased as the percentage CP increased. The value for ALT increased up to the 22% CP diet and thereafter decreased. Dietary

treatments did not affect the mean cell volume, mean cell haemoglobin, mean cell haemoglobin count, urea, creatine, AST, triglyceride and total protein.

Table 4.19: Haematological Parameters and Serum Biochemical Indices of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (0 – 4 Weeks)

Parameters	Crude Protein Levels (%)				SEM
	20	21	22	23	
PCV (%)	28.33[a]	26.00[b]	29.67[a]	26.33[b]	1.80
Hb (g/dl)	9.43[ab]	8.04[b]	9.87[a]	8.73[b]	0.29
RBC ($x10^6$/l)	4.77[a]	3.90[b]	4.70[a]	4.03[b]	0.31
WBC ($x10^6$/l)	15.70[a]	12.93[ab]	10.43[b]	14.00[a]	0.83
MCH(Pg)	19.81	23.53	21.07	21.66	2.53
MCV(fl)	59.50	70.90	63.35	65.30	8.59
MCHC(g/dl)	33.27	33.25	33.26	34.27	1.08
Urea(mmol/l)	3.83	3.73	3.17	3.54	4.51
Uric acid(mg/dl)	246.70[a]	73.30[c]	80.00[c]	146.70[b]	71.50
Creatine(mg/dl)	51.00	51.00	47.67	47.33	11.90
Glucose(mg/dl)	4.83[b]	4.43[b]	5.00[b]	7.03[a]	0.90
AST(u.i/l)	32.33	28.77	21.00	22.00	9.83
ALT(u.i/l)	19.00[b]	20.33[b]	32.00[a]	22.33[b]	6.16
ALP(u.i/l)	43.33[c]	55.33[b]	60.33[b]	75.00[a]	9.33
Cholesterol(mg/dl)	2.90[b]	2.93[b]	3.73[a]	3.09[ab]	0.24
Triglycerides(mg/dl)	1.63	1.27	1.07	1.13	0.27
Albumin(g/dl)	4.23	4.10	4.40	3.70	3.20

Total protein(g/dl)	2.80	2.53	2.73	2.73	0.29

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
PCV= Packed cell volume
HC=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

4.3.4 Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5 – 8 Weeks)

Table 4.20 shows the effect of feeding diets formulated on the basis of four critical essential amino acids along with graded levels of dietary protein on haematological parameters and serum biochemical indices of broiler finisher chickens from 5-8 weeks. Dietary treatments had significant (P<0.05) effects on, packed cell volume, haemoglobin count, red blood cells, white blood cell, uric acid and albumin. Chickens fed 18% CP had significantly the highest values in terms of the packed cell volume, haemoglobin count, white blood cells and uric acid.

Chickens fed 0.19% CP had slightly lower values for packed cell volume and haemoglobin count. There was no significant (P>0.05) difference in the values for PCV

between 19, 20 and 21% crude protein. Uric acid significantly decreased for all diets above the 18% CP. The values for uric acid were similar for the 19, 20 and the 21% CP diets. The value for haemoglobin was observed to be higher for the 18 % CP and the 21%CP diet but slightly lower for the 19% and the 20% CP diets. Albumin content of the serum was significantly increased for chickens fed 19%CP and 20 CP% diets but decreased significantly again at the 21% CP diet. Dietary treatments had no significant ($P>0.05$) effects on the values forred blood cell, mean cell haemoglobin, mean cell volume, mean cell haemoglobin concentration, urea, glucose, creatine, ALT, AST, ALP, cholesterol, triglyceride the and total protein.

4.3.5 Nutrient Digestibility of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino acids At Each Protein Level (0 - 4Weeks)

Table 4.21 shows the effect nutrient digestibility of chicks fed diets formulated on the basis of four critical essential amino acids at each protein level.

Table 4.20: Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens fed Graded Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5 – 8 Weeks)

	Crude Protein Levels (%)				
Parameters	18	19	20	21	SEM
PCV (%)	32.33^{a}	30.00^{b}	30.00^{b}	29.67^{b}	0.84
Hb (g/dl)	9.67^{a}	8.80^{b}	8.67^{b}	9.37^{a}	0.45
RBC ($x10^{6}$/l)	3.53	3.57	3.73	3.83	0.53
WBC ($x10^{6}$/l)	16.07^{a}	13.02^{b}	10.70^{c}	14.67^{ab}	0.34
MCH(Pg)	27.46	26.42	23.33	24.47	2.87
MCV(fl)	91.75	88.92	80.56	77.75	7.89
MCHC(g/dl)	29.96	29.34	28.91	31.70	1.73
Urea(mmol/l)	4.03	3.43	3.53	3.37	0.31
Uric acid(mg/dl)	250.33^{a}	47.67^{b}	60.67^{b}	85.67^{b}	25.24

Creatine(mg/dl)	49.33	53.33	50.00	35.32	9.08
Glucose(mg/dl)	3.77	4.34	4.50	5.33	1.45
AST(u.i/l)	38.67	47.33	32.00	34.33	8.38
ALT(u.i/l)	23.67	21.00	25.33	29.33	5.64
ALP(u.i/l)	45.33	53.33	66.33	65.67	8.88
Cholesterol(mg/dl)	3.10	3.20	3.73	2.47	0.66
Triglycerides(mg/dl)	2.30	2.33	1.53	2.20	0.52
Albumin(g/dl)	3.20^{b}	4.23^{a}	4.20^{a}	3.12^{b}	3.04
Total protein(g/dl)	3.20	3.80	4.07	3.67	0.70

a, b,c = Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
PCV= Packed cell volume
HC=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean cell haemoglobin
MCV=Mean cell volume
MCHC=Mean cell haemoglobin count
AST=Aspartate Amino-Transferase
ALT=Alanine Amino-Transferase
ALP=Alkaline Phosphatase

Table 4.21: Nutrient Digestibility of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino acids At Each Protein Level (0 - 4 Weeks)

	Crude Protein Levels %				
Parameters (%)	20	21	22	23	SEM
Dry matter	85.93	84.53	86.83	86.52	2.95
Crude protein	73.29^{b}	83.60^{ab}	87.50^{a}	84.83^{ab}	2.72
Crude fibre	72.48	71.44	70.11	76.05	4.33

Ether extract	71.47^{b}	85.30^{a}	77.99^{b}	85.47^{ab}	2.12
Ash	73.98^{b}	85.62^{a}	77.78^{ab}	71.17^{b}	3.01
NFE	57.34^{c}	71.22^{a}	69.35^{b}	68.58^{b}	1.05

a, b= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
NFE = Nitrogen free extract

It was observed that dietary treatments had significant (P<0.05) effects on digestibilities of the apparent crude protein (CP), ether extract (EE), ash and nitrogen free extract (NFE) digestibility. The chicks fed 22, 23 and 21% CP had the highest the apparent CP digestibility (87.50, 84.83 and 83.60%) respectively followed by chicks fed 20 % CP (73.29%). The values obtained for apparent EE digestibility across the treatment groups shows that chicks fed 23% CP had comparatively the highest value of 85.47% followed by chicks fed 21, 22 and 20% CP respectively. Average ash values obtained

indicated that chicks fed 21% CP had the highest value of 85.62% followed by chicks fed 22, 20 and 203% CP with values of 77.78, 73.98 and 71.17. Dietary treatment did not have any significant (P>0.05) effect on dry matter. The highest apparent NFE digestibility value of 71.22% was observed in chicks fed 21% CP supplemented with essential amino acids followed by chicks fed 22, 23 and 20% CP supplemented with essential amino acids. It was generally observed that chicks fed higher levels of CP supplemented with essential amino acids had better overall apparent nutrient digestibility values compared to those with low CP supplemented with essential amino acids.

4.3.6 Nutrient Digestibility of Broiler Finisher Chickens fed Diets Formulated on the Basis of Critical Essential Amino Acids At Each Protein Level (5 – 8 Weeks)

The nutrient digestibilities of broiler finisher chickens fed diets formulated on the basis of four critical essential amino acids at each level is shown in Table 4.22. Dietary treatments had significant (P<0.05) effects on apparent crude protein, crude fibre, ash and NFE The results showed that chickens fed diet containing 21% CP supplemented with balanced essential amino acids had the best results in terms of the apparent crude protein, crude fibre and NFE digestibilities compared to chickens fed 18% CP fortified with balanced essential amino acids.

Table 4.22: Nutrient Digestibility of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5 – 8 Weeks)

	Crude Protein Levels %				
Parameters (%)	18	19	20	21	SEM
Dry matter	88.18	87.33	84.56	87.41	1.87
Crude protein	64.57^{d}	74.07^{b}	70.88^{c}	77.28^{a}	1.98
Crude fibre	59.29^{c}	67.93^{b}	67.34^{b}	75.31^{a}	3.27
Ether extract	85.58	90.20	88.07	88.08	1.38

Ash	75.19[c]	87.53[a]	56.90[d]	82.35[b]	0.98
NFE	59.16[d]	70.33[b]	66.80[c]	78.15[a]	0.62

a, b,c,d= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means
NFE = Nitrogen free extract

It was observed that chickens fed 19% CP fortified with balanced essential amino acids had the best apparent ash digestibility. Dietary treatment had no significant (P<0.05) effect on ether extract across the treatment means.

4.3.7 Carcass Characteristics of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5 – 8 Weeks)

The effect of diets formulated on the basis of four critical essential amino acids on carcass characteristics of broiler chickens were as shown in Table 4.23. Dietary

treatments had significant ($P<0.05$) effects on live weight, carcass weight, dressing percentage, breast, wings, thigh, drumsticks, heart, kidney, gizzard, lungs and back. Live weights and carcass weights increased as the graded levels of crude protein supplemented with essential amino acids increased. The carcass weight was comparatively higher in broiler chickens fed diet containing 20% CP when compared with the chickens fed 18 and 19% CP supplemented with essential amino acids. The lowest dressing percentage was recorded in broiler chickens fed 18% CP diet and maximum values from groups fed 19, 21 and 20% CP respectively ($P < 0.05$). Generally, it was observed that chickens fed 19, 20 and 21% CP supplemented with balanced essential amino acids were statistically similar in terms of the carcass weight, dressing percentage, thighs, drumsticks, heart, lung and back weights compared to the chickens fed 18% CP supplemented with balanced essential amino acids.

Table 4.23: Carcass Characteristics of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5–8 Weeks)

	Crude Protein Levels (%)				
Parameters	18	19	20	21	SEM
Live weight (g)	2916.67[c]	3150.00[b]	3296.67[a]	3283.33[a]	0.03
Carcass weight (g)	2280.00[b]	2626.67[a]	2740.00[a]	2736.67[a]	0.03
Dressing (%)	78.21[b]	83.37[a]	83.13[a]	83.34[a]	1.26
Prime cuts and organ weights expressed as % of dressed weight					
Breast	16.75[b]	20.92[ab]	20.74[ab]	23.05[a]	1.43
Wings	5.94[c]	7.50[b]	7.56[b]	8.81[a]	0.29
Thigh	8.69[b]	11.58[a]	12.19[a]	12.21[a]	0.36
Drumsticks	5.80[b]	7.84[a]	7.28[a]	8.49[a]	0.41
Back	9.73[b]	11.09[a]	11.30[a]	11.90[a]	0.41
Liver	1.05	1.29	1.52	1.60	0.20
Heart	0.25[b]	0.52[a]	0.41[a]	0.48[a]	0.05
Kidney	0.36[b]	0.48[a]	0.46[ab]	0.46[ab]	0.03
Gizzard	1.07[b]	1.27[ab]	1.51[a]	1.59[a]	0.12
Abdominal fat	0.70	0.68	0.56	0.69	0.11
Spleen	0.08	0.10	0.11	0.08	0.02
Lung	0.29[b]	0.44[a]	0.43[a]	0.53[a]	0.09

a, b, c= Means with different superscript on the same row differ significantly ($P<0.05$)
SEM = Standard Error of Means

4.3.8. Tissue Crude Protein and Tissue Fat Deposition of Broiler Finisher Chickens fed Graded Levels of Crude Protein Diets Formulated on the Basis of Essential Amino Acids At Each Protein Level (5-8 weeks)

The tissue fat and tissue crude protein deposition of broiler finisher chickens fed graded levels of crude protein diets formulated on the basis of essential amino acidsis presented in Table 4.24. The result showed that tissue protein and tissue fat were not significantly ($P>0.05$) affected by dietary treatments.

Table 4.24: Tissue Crude Protein and Tissue Fat Deposition of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5-8 Weeks)

	Crude Protein Levels %				
Parameters	18	19	20	21	SEM
Crude protein (%)	20.54	21.86	22.84	21.10	1.23
Ether extract (%)	2.15	1.72	1.66	3.15	1.95

a, b= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard Error of Means

CHAPTER FIVE

5.0 DISCUSSION

5.1 Performance of Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Cool Season (0- 4 Weeks)

In the study, dietary tryptophan had significant effects on final body weight, weight gain, average daily weight gain, total feed intake, average feed intake, feed conversion ratio and feed cost per kg gain. Chicks fed dietary tryptophan performed better in terms of the final weight, weight gain; average daily weight gain and feed conversion ratio compared with the chicks fed the control diet. Chicks fed dietary tryptophan at 0.23% had better performances in terms of weight gain and mortality rate compared to the chicks fed other tryptophan levels. This observation is contrary to the NRC (1994) and Rostagno *et al*. (2007) recommendation of 0.20% tryptophan for the starter phase period.

Weight gain increased as the levels of tryptophan increased and decrease at 0.27% dietary tryptophan. This result is in agreement with the report of Fatufe *et al*. (2005), Emadi *et al*. (2010), Yu *et al*. (2010), Harms and Russel (2000), Rosa *et al*. (2001) who reported an increase in body weight gain in broiler chickens which received the high concentrations of dietary tryptophan compared with those which received low concentrations. This may be due to the phenomenon of amino acid balance. Adequate tryptophan content brought about optimal amino acid balance while excess tryptophan supplementations disrupted the balance of amino acid. The increase in body weight gain observed may be partly due to the increased feed intake as the dietary tryptophan increased. The optimum value of 0.23% dietary tryptophan also disagreed with the

findings of Rosa *et al*. (2001) who reported that no more than 0.18% tryptophan is needed by starting broiler chickens, regardless of genotype.

Chicks fed diet without supplemental tryptophan but with in-situ dietary tryptophan level of 0.17% had higher feed consumption rate compared to those receiving higher dietary concentrations of tryptophan. This result is contrary to the findings of Harms and Russel (2000); Corzo et al.,2005a; Mozhdeh et al., 2010), who reported reduced feed intake of broiler chicks, fed the lowest concentration of tryptophan. This may be partially explained by the fact that chicks were searching for the missing nutrients which precisely is the tryptophan. Generally, it was observed that chicks fed supplemental tryptophan fortified diets had higher feed consumption (1569.20g – 1687.99g) than the feed consumption rates reported by NRC (1994) of 1085g for male and 965g for female). The reasons for this may be due to the function of tryptophan as a precursor of neurotransmitter serotonin which is formed in the brain and influence feed intake, energy levels of the diets, the fibre level, environmental temperature and breed of chickens. In this study, tryptophan deficient diet had the highest feed conversion ratio (1.79). This result was similar to the findings of Mozhdeh et al. (2010) and Rostagno et al. (2007) who reported increase in feed conversion ratio and a gradual decrease in feed conversion ratio as the levels of dietary tryptophan increased. However, this result is contrary to the findings of Karina et al. (2013), who reported that digestible tryptophan levels did not influence feed conversion ratio. The increase in feed conversion ratio observed in the chicks fed basal diet containing 0.15% dietary tryptophan might be considered to be due to the deficiency of some critical amino acids. Hence, the efficiency of protein utilization was decreased.

5.2 Performance of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Cool Season. (5- 8 Weeks)

Dietary treatments had significant effects on final body weights, weight gains, average daily gain, feed intake, feed conversion ratio and feed cost per kg gain. Dietary treatments however had no significant effects on mortality rate. Chicks fed 0.21% and 0.25% tryptophan diets had the best performance in terms of weight gain, daily weight gain, feed conversion ratio and feed cost per kg gain. A steady increase in weight gain was observed as the levels of dietary tryptophan increased from 0.13 – 0.21% after which weight gain decline were observed at 0.29% dietary tryptophan level. Chickens fed 0.21% dietary tryptophan showed improved final body weight; weight gain and average daily weight gain were optimal at that level. This result disagrees with the 0.18% suggested by NRC (1994) and Rostagno *et al.* (2007) for broilers between 22 to 42 days of age. Chickens fed 0.13% dietary tryptophan had the lowest weight gain and average daily weight gain. The observed low body weight for the chickens fed 0.13% dietary tryptophan could be related to possible stress-induced insufficiency of dietary tryptophan and amino acid imbalance.

Birds fed Diet1 without supplemental tryptophan but with insitu dietary tryptophan of 0.13% had significantly higher feed consumption compared to those receiving tryptophan supplemented diets. This result is contrary to the findings of Harms and Russel (2000), Mozhdeh et al.(2010) and Corzo et al. (2005a), who reported reduced feed intake for broiler chickens, fed the low concentration of dietary tryptophan. This may be due to the fact that birds fed diets deficient in any nutrient consume more to meet their requirements for gain. In this study, tryptophan deficient diet had the poorest feed conversion ratio of 2.90 which is similar to the findings of Mozhdeh et al. (2010), Rostagno et al. (2007). These authors reported increase high feed conversion ratio for

lower dietary tryptophan and a gradual decrease in feed conversion ratio as the levels of dietary tryptophan increased. The high feed conversion ratio observed in the chicks fed 0.13% dietary tryptophan might be the result of the fed low tryptophan or difference in amino acid digestibility of diets. This result is in contrast to the findings of Karina et al. (2013), who reported that digestible tryptophan levels did not influence feed conversion ratio.

5.3 Haematological Parameters and Serum Biochemical indices for Broiler Starter Chicks fed Graded Dietary Levels of Tryptophan During the Cool Season. (0- 4 Weeks)

It has been suggested that there is a relationship between of ingested feed and blood composition of broiler chickens. It was observed that chicken fed the highest level (0.31%) dietary tryptophan had the highest values in term of the packed cell volume, haemoglobin count, red blood cell, and mean cell haemoglobin count. This result is similar to the finding of Mirtuka and Rawnsley, (1977) who reported significant higher values of red blood cells, heaemoglobin count and packed cell volume in broiler birds fed high protein diets than those fed low protein diets. Chicks fed 0.31% dietary tryptophan had the highest value (34.50%) for packed cell volume. Although it was observed that values obtained from all the treatment groups (30.67 to 34.50%) fell within the normal range (22.00-35.00%) for healthy chickens. This result is in consonance with the result obtained from Jain (1993), Oladele and Ayo (1999). Both researchers reported values of 21-35% for PCV. This result showed that the chicks were immunologically balanced and were not anaemic. The high value (11.33g/dl) for haemoglobin count observed for Chicks fed 0.31% dietary tryptophan fell within the range value of values obtained by Mohamed *et al*. (2009) who reported 11.48-12.53g/dl for healthy birds but was contrary to the value (8.5g/dl) obtained by Simarak

et al. (2004).The differences in the values may be due to genotype and the age of the birds used. Chicks fed 0.31% dietary tryptophan also had the highest value of mean cell haemoglobin counts although all the values obtained across treatments (31.47-32.83%) in this study fell within the normal values of 33-47 g/dl for healthy chickens as reported by Jain (1996).

The values of 3.30-4.25 (x 10^6ul) obtained across treatments for the red blood cells were higher than the normal range 2.19 x10^6 (ul) as reported by Simarak et al. (2004) although no sign of illness was observed with this high values. The reason may be due to the inclusion of tryptophan in the diets, age, season of rearing and genotype. Chicks fed 0.27% and 0.31% dietary tryptophan levels had the highest value for total protein, although all the values obtained across the treatment groups fell within the normal range of 2.00 - 3.40g/dl reported by Ayo *et al*, (1996) and the values of 3.0-4.08g/dl reported by Diarra and Usman (2008). The low total protein values observed for chicks fed low tryptophan based diet is similar to the findings of Corzo *et al*.(2005b) who reported that feeding low protein diets have been shown to be associated with decreased total serum protein. Dietary treatments had no effect on the white blood cell; mean cell volume and mean cell haemoglobin, but it was observed that all numerical values fell within the normal range for healthy birds which signify that the inclusion of tryptophan pose no physiological threat to chicks.

The mean value for urea shows significant differences across the treatment means with values ranging between 1.45-3.40mg/dl. These values also fell within the normal values of 1.50- 6.30mg/dl as reported by Mirtuka and Rawnsley, (1977). ALT is more specific to the liver and it's a better parameter for detecting liver injury. Mean values for ALT shows no significant differences and it also indicated no injury to the liver since the

values were within the normal range of 9.50 to 37.20 as reported by Mirtuka and Rawnsley, (1977). The importance of the significantly higher levels of creatinine observed in this study could be attributed to the use of creatinine as an indicator of muscle metabolism but this remains to be confirmed. Mean values of 24.33 to 40.33 g/dlfor albumim also fell within normal values reported by Mirtuka and Rawnsley, (1977). It was also observed that dietary treatment had no significant effect on the plasma glucose. This result is similar to the finding of Swennen *et al*. (2006), who reported no significant effect of diets fortified with essential amino acids on plasma glucose concentration of broiler chickens. It indicated that carbohydrate metabolism was not affected by the diet as chickens used carbohydrates as energy source rather than free fatty acid.

5.4 Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Cool Season. (5 – 8 Weeks)

The mean value for packed cell volume (23.33- 30.33%) obtained in this study is similar to the normal values (21-45%) reported by Jain (1986), indicating that the diets were ideal and adequate for broiler finisher chicken. The mean values of 7.33-10.07 for heamoglogin count fell within the normal value of 8.50g/dl reported by Jain (1993). Furthermore, the values for white blood cells of 5.07-6.33 and red blood cells of 3.90-5.00 (x 10^6ul) also fell with the normal values of 1.58- 2.05 x 10^6ul for WBC and 3.82 for RBC reported by Oladele and Ayo (1999). The mean cell volume (MCV) of 59.77-60.74 (fl), mean cell heamoglobin count (MCHC) of 33.12-33.92 (g/dl) and mean cell heamoglogin (MCH) of 19.84-20.27 (pg) fell within the normal valuesas reported by Mitruka and Rawnsley(1977), Jain (1993) of 60-141.39fl for MCV,27.79g/dl for MCHC and 22.11pg for MCH respectively. This result indicated that dietary tryptophan levels up to 0.29% tested in this study had no adverse effects on the heamatological

parameters and it further explains that all dietary levels of tryptophan tested were satisfactory for broiler finisher chicken. The adequacy of the diets is also shown in the stability of the serum total protein, cholesterol, albumin, creatine, ALP, AST, ALT, glucose, urea, uric acid and triglyceride on different diets regardless of the levels of dietary tryptophan. Dietary treatments however significantly affected the serum urea, uric acid, creatine, glucose, cholesterol, triglyceride, albumin and total protein; although a trend was not observed as there are variations in the values amongst treatment means. Chickens fed 0.25% dietary tryptophan had the highest mean value for uric acid, this value corresponded with the value as reported by Mirtuka and Rawnsley, (1977).

Furthermore chickens fed 0.21% dietary tryptophan had the highest urea value of 3.00mg/dl. Although it was observed, that most of the values for urea across the treatment fell within the normal range (1.50-6.30mg/dl) for healthy birds as reported by Nworgo (2001). The values in the serum parameters of this study are at variance with those of Nworgo (2001) with albumin being higher and cholesterol being lower. Creatine, albumin, total protein and glucose values were almost similar as they all fell with the normal range as reported by Mirtuka and Rawnsley, (1977).

5.5 Nutrient Digestibility of Chicks fed Graded Dietary Levels of Tryptophan During the Cool Season (0- 4 Weeks)

Dietary tryptophan levels had significant influence on the apparent dry matter, crude protein, crude protein, ether extract, ash and NFE digestibilities of chicks fed graded levels of dietary tryptophan. Chicks fed 0.23% dietary tryptophan had the best value for the apparent dry matter digestibility. This result disagreed with the report of Faria Filho *et al*.(2007), who reported that the apparent dry matter digestibility was not affect by inclusion of tryptophan in chick's diet. Chicks fed high concentrations of dietary

tryptophan were observed to have higher values for apparent crude protein digestibility compared to chicks fed the control diet without supplemental tryptophan. This result is in congruence with the findings of Ratriyanto *et al*. (2012), who reported that chickens fed high concentration of tryptophan, recorded the highest value for apparent CP digestibility indicating more efficient nutrient degradation and absorption as evident in the increased weight observed in this study.

The high value for apparent EE digestibility of Chicks fed 0.23% dietary tryptophan is similar to the findings of Dean *et al*. (2006), who reported that chickens fed high concentration of tryptophan recorded the highest value for apparent EE digestibility indicating more efficient nutrient degradation and absorption of ether extract. Dietary treatment had effect on apparent NFE digestibility, the highest value was observed in chicks fed 0.27. This result is similar to the study conducted by El-Husseiny *et al*. (2007), who reported an increase in the apparent NFE digestibility as the levels of tryptophan increased. It was observed generally in this study that chicks fed diets with high concentrations of supplemental tryptophan had the best apparent nutrient digestibility values. This indicated an efficient nutrient utilization by the chicks across the treatments.

5.6 Nutrient Digestibility of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Cool Season (5-8 weeks).

Dietary treatments had significant effect on the apparent NFE digestibility of chickens. Dietary treatments did not have any significant effects on the apparent CP, CF, EE and ash. This result disagreed with the findings of Faria Filho *et al*. (2007), who reported a significant effect of levels of tryptophan on apparent digestibilities of CP, CF, EE and ash for broiler chickens. Chickens fed 0.17% dietary tryptophan had the highest value

for apparent NFE digestibility, although it was observed that the values obtained across all the treatments were not high. The low values obtained might be as a result of natural variability of these ingredients due to ingredient quality and processing that can affect digestibility.

5.7 Effect of Graded Dietary Levels of Tryptophan on Carcass Characteristics of Broiler Chickens Reared During the Cool Season. (5-8 Weeks)

In this present study dietary tryptophan had significant effects on live weights, carcass weights, dressing percentage, breast, wings, thigh, drum sticks, liver, heart, gizzard, abdominal fat, lung and back expressed as percentage of the carcass weights. The spleen and kidney expressed as percentage of carcass weight were however not significantly ($P>0.05$) affected by dietary treatments. Birds fed 0.21% dietary tryptophan had comparatively the highest live weight, carcass weight, dressing percentage, breast and the lowest abdominal fat. This result is similar to the findings of Rezaei *et al*. (2004), who reported that carcass yield, carcass quality and breast yield were significantly decreased with low supplementation of amino acids in both the starter and grower phases. However, this result disagreed with the findings of Kerr and Kidd (1999a) who reported no significant difference in the carcass yield and breast yield of chickens fed diet fortified of essential amino acids.

Generally birds fed diets supplemented with synthetic tryptophan had better carcass characteristic compared to birds fed without supplemental tryptophan. This result is contrary to the findings of Kerr and Kidd (1999a) who reported no consistent effects of tryptophan supplementation on any carcass parameters. Kerr and Kidd (1999b) further reported that addition of 0.15% crystalline L- tryptophan to a withdrawal feed containing 0.17% total tryptophan in broilers from 46 to 49 d of age had no discernable

impact on carcass quality. The increased breast weight, thighs and drumsticks might be due to better utilization of protein and amino acids.

One of the most consistent complains made by broiler chicken consumers is the high percentage of abdominal fat pads and tissue fat observed in broiler chickens meat. It was observed that abdominal fat pads were reduced in all tryptophan supplemented diets compared to the chickens fed the control diet without supplemental tryptophan. This result was similar to the findings of Neto *et al*. (2000), who observed that abdominal fat pad weight increased by 104% when birds were fed diets without tryptophan as compared to the fed 24% crude protein diet with tryptophan. This may be explained partially due to the protein and amino acid status of the diets and also the ability of protein to act as a lipotrophic agent. This result also disagreed with the result of Rosa *et al*. (2001), who reported that fat pad weight observed in broiler chickens were significantly increased with the elevated tryptophan level.

The significantly low abdominal fat observed across the treatment groups in this study is similar to the results of the research conducted by Hsai *et al*. (2005). They observed that abdominal fat pad weight content was high in diet low in tryptophan concentration. The increased fat pad observed chickens fed diet without supplemental tryptophan might be due to the fact that birds fed diets deficient in any nutrient consume more feed to meet their requirements for gain, resulting in increased fat deposition.

5.8 Effect of Graded Dietary Levels of Tryptophan on Tissue Protein and Tissue Fat Deposition of Broiler Finisher Chickens During the Cool Season. (5 – 8 Weeks).

Dietary treatments had significant effect on tissue fat deposition of birds reared in the cold season. Chickens fed 0.27% dietary tryptophan had the least tissue fat deposition.

A decrease in the level of fat in the tissue was observed as the levels of dietary tryptophan increased up to 0.27% and thereafter increased. This result is similar to the findings of Mukhtar *et al*. (2007) and Ghari *et al*. (2010). They reported that the higher the levels of methionine, tryptophan and lysine in the diets, the lower the body fat. The low fat observed in the tissue meat may be due to the effect of crude protein and amino acids status of the diets and the ability of protein to act as lipotrophic agent as reported by Neto *et al*. (2000) and Si *et al*. (2001). Dietary treatment did not have any significant effect on tissue protein. This result is similar to the result of Hai and Blaha (2000), who reported no significant differences for tissue crude protein and fat for chickens fed diets fortified with essential amino acids.

5.9 Performance of Broiler Starter Chicks Fed Graded Dietary Levels of Tryptophan During the Hot Season. (0 - 4 Weeks).

Dietary treatments had significant effects on final weight, weight gain, average daily weight gain, feed intake and feed cost per kg gain. An increased trend in weight gain was observed as the levels of tryptophan increased up to 0.23% and began to decline at 0.27%. This result is in agreement with the results obtained by Fatufe *et al*. (2005), Emadi *et al*. (2010b) and Yu *et al*. (2010), Harms and Russel (2000), and Rosa *et al*. (2001) who reported an increase in body weight gain in broiler chickens which received the high concentrations of dietary tryptophan compared with those which received low concentration . This increase in body weight gain may be partly due to the increased feed intake and dietary essential amino acid balance.

Chicks fed 0.23%, 0.27% and 0.31% had similar results in term of the final weight, weight gain and average daily weight gain. In this experiment, it was observed that the level of 0.23% dietary tryptophan improved final weight, weight gain and average

weight daily weight gain, and this value disagreed with the 0.20% suggested by NRC (1994). Chicks fed diet without supplemental tryptophan (basal diet) of 0.15% had the least performance in terms of the body weight gain and feed conversion ratio compared to those receiving higher concentrations of tryptophan in their diets. These results are in agreement with the findings of Mozhdeh et al. (2010) and Rosa et al. (2001) who reported depressed body weight and high feed conversion ratio of broiler chicks fed the lowest concentration of tryptophan. This may be due to imbalance amino acid interactions and inefficient nutrient utilization.

A steady increase in feed consumption was observed as the dietary level of tryptophan increased. The increase increased for dietary tryptophan levels of between 0.15 and 0.27% and thereafter decreased. This result is similar to the findings of Harms and Russel (2000), Mozhdeh et al. (2010) and Corzo et al. (2005b), who reported reduced feed intake of broiler chickens, fed the low concentration tryptophan diets. The increased feed intake observed may partially be the function of tryptophan as a precursor of neurotransmitter of serotonin which is responsible for increased appetite. In this study, tryptophan deficient diet had the poorest feed conversion ratio of 1.55 compared with chicks fed supplemental tryptophan. This result is similar to the findings of Rostagno et al. (1995), Mozhdeh et al. (2010) and Karina et al. (2013). They reported increase in feed conversion ratio and a gradual decrease as the levels of dietary tryptophan increased. This may be due to dietary amino acids imbalance.

5.10 Performance of Broiler Finisher Chickens Fed Graded Dietary Levels of Tryptophan During the Hot Season. (5 – 8 Weeks).

Dietary treatments had significant effects on final weight, weight gain, average daily weight gain, feed intake, average daily feed intake and feed cost per kg weight gain. No

significant effect of dietary treatments was observed on the feed conversion ratio and mortality. A steady increase in weight gain was observed as the levels of dietary tryptophan increased from 0.13 – 0.21%. A sudden decline was observed at 0.29% dietary tryptophan. This result also agreed with the report of Rostagno et al. (1995), who stated that supplemental tryptophan alleviated body weight decline. Chickens fed tryptophan deficient diets (0.13%) had the least weight gain. This result is similar to the findings of Corzo *et al*. (2005a), Rosa *et al*.(2001) who reported depressed body weight gain, feed intake and feed conversion ration. The observed body weight decline could be related to possible stress-induced insufficiency of dietary tryptophan, amino acid imbalance and the season of rearing as the chickens were raised during the peak of hot season. However since a partial trend was observed for the weight gain, polynomial regression was used to ascertain the optimum requirement of tryptophan.

Improved the final weight, weight gain and average daily weight gain was observed at 0.21% dietary tryptophan. This result disagreed with the 0.18% suggested by NRC (1994). Birds fed diet supplemented with tryptophan consumed more feed compared to those receiving lower dietary concentrations of tryptophan. This result is similar to the findings of Harms and Russel (2000), Mozhdeh et al.(2010) and Corzo et al. (2005a), who reported reduced feed intake of broiler chickens, fed the low concentration of tryptophan. The increased feed intake observed may partially be the function of tryptophan as a precursor of neurotransmitter of serotonin which is responsible for increased appetite. The feed conversion ratio was observed to be best for chickens fed higher concentrations of dietary tryptophan. This result was similar to the findings of Mozhdeh et al. (2010) and Rostagno et al. (1995) who reported increase in feed conversion ratio and a gradual decrease in feed conversion ratio as the levels of dietary tryptophan increased. Although there was no significant differences observed in terms

of the feed conversion ratio. This result is similar to the findings of Corzo et al. (2005b), who reported that digestible tryptophan levels did not influence feed conversion ratio.

5.11 Haematological Parameters and Serum Biochemical Indices of Broiler Starter Chicks Fed Graded Dietary Levels of Tryptophan During the Hot Season. (0 – 4 Weeks).

Dietary tryptophan had significant effects on packed cell volume, haemoglobin counts, red blood cells, mean cell haemoglobin counts, creatine, glucose, AST, ALT, ALP and total protein. Chicks fed 0.15% dietary tryptophan had the highest values for packed cell volume (PCV), haemoglobin count, ALP, and MCHC respectively. There were no significant differences between the mean values of white blood cells, mean cell haemoglobin, mean cell volume, urea, uric acid, cholesterol triglycerides and albumin, though the values obtained all fell within the normal range as reported by (Diarra and Usman 2008). The PCV values of (19.00%-28.33%) fell within the normal range (21-45%) for healthy chicks as reported by Mitruka ans Rawnslay (1977). The oversupply of tryptophan in the diets of chicks fed 0.31% could have contributed to the lower PCV observed for the chicks. Coles (1986) reported that PCV is the quickest indirect way of assessing values of red blood cells in circulation and is often used as a simple screening test for anaemia.

The haemoglogin count value of 6.27-9.40% fell below the normal value (11.30 ±1.82 g/dl) as reported by Oladele and Ayo (1999) but fell within the normal value of 8.5g/dl reported by Mitruka and Rawnslay (1977). The discrepancies in the values may be due to the season of rearing, species, sex and age of birds. All the RBC mean values (3.37-4.70) across the treatments fell within the normal range (4.21-4.88) reported for broilers by Mitruka and Rawnsely (1977). This means that chicks were not anaemic nor show

sign of polycythemia. This may probably be as a result of the beneficial effects conferred on the inclusion of tryptophan in chick's diet which favours the haematological indices of the birds.

Mean values of AST shows significant difference with values ranging between 3.00 and 4.83 which fell between the normal values (3.04 to 6.30) reported by Mitruka and Rawnsley (1977). ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Mean values for ALT shows significant difference but shows no injury to the liver since the value fell within the normal value for health chicks as reported by Mitruka and Rawnsley (1977). Majority of the haematological parameters and blood serum biochemical indices were similar to values reported in literature as normal for chickens (Awoniyi *et al*., 2000; Faniyi, 2002).This indicated nutritional adequacy of all the diets, since blood profile and serum biochemical indices offers a valuable investigation and explanatory tool in nutritional assessment and health implications.Diarra and Usman (2008) reported serum protein as a source of replacement of tissue proteins, buffer in acid-base balance and as transporter of constituents of blood such as vitamins, iron, copper, hormones, lipids and enzymes.

Chicks fed 0.19% dietary tryptophan had the highest value of total protein compared to other treatments. Total protein across the treatments fell below the value recommended for healthy chickens of 3.0 and 4.08 by Diarra and Usman (2008). This might be due to amino imbalance and natural variability of the feedstuffs used. The oversupply of tryptophan in the diets of chicks fed 0.31% could have contributed to the lower total protein observed for the chicks, because retained proteins furnish the body with amino acids that are used for the synthesis of body proteins including blood proteins.

5.12 HaematologIcal Parameters and Serum Biochemical Indices of Broiler Finisher Chickens Fed Graded Dietary Levels of Tryptophan During the Hot Season. (5 – 8 Weeks).

Dietary treatments had significant effects on haemoglobin count, white blood cells, mean cell haemoglobin, urea, uric acid, creatine, glucose, AST, ALT, ALP, cholesterol, triglycerides, albumin and total protein. Dietary tryptophan had no significant effects on PCV, RBC, MCV and MCHC. This is similar with the findings of Awoniyi *et al*. (2000); Faniyi, (2002). They reported no significant effect of broiler chickens dietary levels of thy first four limiting amino acids.

The haemoblobin count values obtained (8.67- 10.58) in this study however fell below the range for healthy chickens' values reported by Oladele and Ayo (1999) and Abeke *et al*., (2008). Lower levels of haemoglobin in the chicken's blood during the hot season can be related to the effect of high environmental temperature on changing the distribution of iron in the organisms of broilers. These results conform to the report of Jamadar and Jalnapurkar (1995) who reported a drop in haemoglobin of broiler chickens reared during the hot season. The decrease in haemoglobin concentration is in accordance with that reported by Vecerek *et al*. (2002); Khan *et al*. (2002); Comito *et al*. (2007) who reported that the exposure of birds to high temperature cause significant decrease in heamoglobin concentration. The values obtained also for the white blood cells (14.89 – 16.95) in this study fell below the range obtained by Bashar *et al*. (2010) of 23.07- 24.70. The values obtained also for the Total protein 1.58 – 4.27g/dl in this study fell within the range for healthy chicken's values of (3.08 – 4.08g/dl) as reported by Oladele and Ayo (1999). The mean values of cholesterol, triglycerides, AST, ASP, ALT, creatine, urea and uric acid fell within the normal range for healthy birds as

reported by Diarra and Usman (2008). This means that the diet fed were not harmful to the chickens across the treatment groups.

5.13 Nutrient Digestibility of Broiler Chicks Fed Graded Dietary Levels of Tryptophan During the Hot Season (0- 4 Weeks)

The inclusion of tryptophan in the diet of broiler chicks had significant effects on the apparent crude protein, ether extract and NFE digestibilities. The high apparent crude protein digestibility observed across the treatment groups indicated the efficient utilization of the level of crude protein and amino acids fed. This disagreed with the findings of Noy and Sklan, (1995) and Noy and Sklan, (2001). They reported no significant effect of chicks fed essential amino acids on the apparent crude protein. They based their arguments on the fact that the chicks had reduced proventricular acid secretions, reduced pancreatic and intestinal mucosa secretion such as bile and proteolytic enzyme.

It was also observed the chicks fed 0.31% dietary tryptophan which is far above the NRC (1994) requirement of 0.20%. This may be due to the oversupply of tryptophan. This result is similar to the findings of Zarate *et al*. (2003), who found out that the oversupply of lysine, methionine, threonine, tryptophan and arginine supplementation did not improve weight gain as evident in the low value recorded for apparent CP digestibility, this finding can be supported by the result of Ratriyanto *et al*. (2012) who reported no improvement in the apparent crude protein digestibility as the levels of essential amino acids were oversupplied to the diets of chicks. The increase in the values for the apparent ether extract digestibility across the treatment groups is an indication the fat content in the diets were efficiently utilized, although chicks fed 0.23% dietary tryptophan had the highest value. The low values obtained for NFE could

be due to the reduced proventicular acid secretions, reduced pancreatic and intestinal mucosa secretion. These factors could limit both the digestion and absorption of nutrients especially in chicks as reported by Geyra *et al*., (2001), Parsons, (2004) and Sklan (2001). Dietary tryptophan did not affect the apparent, dry matter, CF and ash.

5.14 Nutrient Digestibility of Broiler Finisher Chickens fed Graded Dietary Levels of Tryptophan During the Hot Season (5- 8 Weeks).

Dietary treatments had significant effect on the apparent crude protein (CP) digestibility of broiler chickens reared in the hot season. Chicks fed 0.23% dietary tryptophan had the highest value for apparent CP digestibility. This result is similar to the report of Noy and Sklan, (2001) and Adedokun *et al*.(2007). They reported that finisher broiler chicken were able to digest crude protein more efficient compared to broiler chick. The reason could be the age of the birds, as age could have significant effect on the pro-ventrucular acid secretions and intestinal mucosa secretions. Dietary tryptophan had no significant effects of apparent CF, EE, NFE and ash.

5.15 Effect of Graded Dietary Levels of Tryptophan on Carcass Characteristics of Broiler Chickens Reared During the Hot Season (5 - 8 Weeks)

Dietary treatment had significant effects on live weight, carcass weight, dressing percentage, breast, thighs, drumstick, heart, kidney, abdominal fat and spleen as expressed as percentage of carcass weight. The live weights, carcass weights and dressing percentage increased as the levels of tryptophan increased from 0.13% to 0.21% and began to drop at 0.25% dietary tryptophan. This result is similar to the findings of Si et al. (2001) and Baker et al. (2002) who reported a significant increase in the carcass weight and dressing percentage of broiler chickens fed high concentrations of tryptophan. This might be due to better utilization of protein and amino acids in the diets. Although this result disagreed with the findings of Sterling, (2006) who reported

no significant differences in diets with balanced amino and those without a balanced amino acid profile.

Increasing the dietary tryptophan positively affected the dressing percentage, breast yield, wing, drumstick weights and thigh of birds. Chickens fed dietary tryptophan had the best results in terms of the aforementioned cut parts compared to the chickens fed the control diet without supplemental tryptophan. This result disagrees with the findings of Rezaei et al. (2004), Sterling et al. (2006) who found no differences in breast yield and most prime cut parts of chickens fed different levels of low levels of dietary CP with amino acid supplementation. The most consistent problem of broiler meat is the tendency for high abdominal fat which deteriorates the quality of carcass. It was observed in this study that abdominal fat pad was reduced across the treatment groups. This result disagreed with the finding of Rosa *et al*. (2001) who reported that the responses of fat pad weights observed in broiler chickens were significant increased with the elevated tryptophan levels.

5.16 Effect of Graded Dietary Levels of Tryptophan on Tissue Protein and Tissue Fat Deposition of Broiler Finisher Chickens During the Hot Season. (5–8 Weeks).

Tissue fat was significantly affected by dietary treatments. Chickens fed 0.21% dietary tryptophan was observed to have the least value for tissue fat. This result is similar to the report of Begendahl *et al*. (2002), who reported a low tissue fat deposition as the levels of essential amino acids of the diets were increased. The increase in the dietary amino acids to metabolizable energy ratio may be responsible for the reduction in the percent body fat observed in this experiment. Dietary treatment had no significant effect on carcass crude protein. This result was similar to the report of Hai and Blaha (2000),

who reported that diets supplemented with essential amino acids had no significant effect on carcass protein.

5.17 Crude Protein Requirements and Performance of Broiler Starter Chicks Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (0-4 Weeks)

Many studies have tried to determine the minimum level of dietary crude protein below which even with sufficient amino acid levels, performance will be negatively affected. In this present experiment which was conducted to evaluate the performance of broiler chicks fed diets formulated on the basis of four critical essential amino acids on the crude protein requirement. It was observed that dietary treatment had significant effects on the final weight, weight gain and average daily weight gain of broiler chicks.

Chicks fed 21% CP had similar results in terms of the final weight and average daily gain as those fed 22% to 23% CP. This result is similar to the findings of Parr and Summer, (1991), Temim *et al*. (2000) and Zarate *et al*. (2003), who reported that reducing dietary crude protein by 2% did not affect body weight gain. It was also reported by Fatufe and Rodehutscord, (2005) and Dean *et al*. (2006) that broiler performance was not affected even when crude protein of amino acid supplemented diet was decreased from 25-21%. The similar final weight, weight gain and average daily weight gain observed across the treatments might be due to the fact that the balanced dietary crude protein and amino acids according to the requirement were attained even at 20% CP therefore giving higher that requirement is unnecessary. However, some researchers have reported reduced growth performance with low crude protein diets (Bregendahl *et al*., 2002; Aletor *et al*., 2000). The great discrepancies, in result of feeding broiler chicks, low protein diets supplemented with essential amino acid may be due to among other factors, the extent of reduction of crude protein contents, the

inclusion or otherwise the crude protein and metabolizable energy contributions of the amino acid supplements, the class and age of the chickens and whether or not the intact protein sources are kept at constant ratios to minimize amino acid imbalances which was clearly seen in chicks fed 20% crude protein. The issue of feed intake on low crude protein diets is critical for growth performance. In this study dietary treatment had significant effects on feed intake, feed intake bird per day and feed conversion ratio.

Chicks fed 22% crude protein supplemented with the four essential amino acids had the highest feed intake. This result disagreed with the findings of Han *et al*.(1992), Bregendahl *et al*. (2002), Araujo *et al*. (2004), Fatufe and Rodehutscord, (2005) who reported no significant on chicks fed graded levels of crude protein with essential amino acids. Although the feed intake (1620.80g) for chicks fed the lowest crude level (20%) was similar to the feed intake (1579.20g- 1818.20g) for chicks fed high crude protein levels. This result is similar to the reports of Parr and Summer, (1991); Hussein *et al*. (2001), who reported equal feed intakes when comparing high crude protein versus low crude protein diets supplemented with amino acid during 7 to 21 days period. The reason for the similarities in the feed intake may be due to the fact that feed intake depends on the calorific value of the feed because birds primarily eat to maintain their energy intake. Dietary treatments had significant effect on feed conversion ratio. Chicks fed 22% crude protein supplemented with essential amino acids had the highest value. This result disagreed with the findings of Araujo *et al*. (2004) and Razaei *et al*. (2004) who reported no differences in feed conversion ratio of the birds fed low protein diets supplemented with amino acid according to an ideal amino acid ratio.

Chicks fed 23% crude protein with essential amino acids had the lowest feed conversion ratio (FCR). This result is similar with the finding of Moran and Bushong,

(1992) who reported an increase in feed conversion ratio with decrease in crude protein (23 to 20% and 20 to 17% in starter and grower periods, respectively).The increased FCR might be the result of the chicks consuming more feed and growing more slowly. Furthermore, there may also be differences in amino acids digestibility of diets because there were slight variations in the amount of corn, soybean and synthetic amino acids resulting in different availability of amino acids and increased feed conversion ratio.

5.18 Crude Protein Requirements and Performance of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acid. (5 - 8 Weeks).

Dietary treatment had effects on final body weight, weight gain, average daily weight gain, feed intake, average daily feed intake, feed conversion ratio and feed cost per kg weight gain. Broiler chickens fed 19%CP, 20%CP and 21%CP had similar weight gains. This result is similar to the research conducted by Han *et al*. (1992), Moran and Bushong (1992); Moran and Stilborn (1996). They reported that there were no adverse effects on weight gain with low crude protein diets supplemented with essential amino acids during the first six weeks of age.

Chickens fed 18% crude protein with essential amino acids had the least weight gain. This result is similar to the report of Colnago *et al*. (1991), who reported a significant depression in body weight when crude protein diets supplemented with essential amino acids were reduced from 23 to 18%. The increase in weight gain observed for chickens fed diets high in crude protein with the four most critical essential amino acids may be due to the efficient protein and amino acid utilizations. The poor performance with low

crude protein (18%) supplemented with the four critical essential amino acids observed in this study could be associated with the differences in amino acids digestibility of diets because of the slight variation in the levels of the feed ingredients. The significant effect observed for feed intake disagreed with the findings of Bregendahl *et al*.(2002) and Elmutaz *et al*. (2014), who reported no significant differences across the treatments fed low crude protein versus high crude protein but similar to the findings of Kidds *et al*. (2001) who reported an increase in feed intake of chickens fed diets containing 19%CP as compared to those fed diets containing 22.5% CP with essential amino acids. The reason may be because of the changes in amino acid contents of low crude protein diets. Although it was reported by Han *et al*. (1992) that equal feed intakes may be expected if low crude protein diets with the same metabolizable energy are supplemented with limiting amino acid.

Broiler finisher chickens fed the lowest crude protein supplemented with amino acid (18% CP) had the poorest value for feed conversion ratio. This result is similar to the finding of Neto *et al*. (2000), who reported 13% increase in FCR of birds fed 17%CP diets as compared to those fed 24% CP diets. The poor feed conversion ratio might be the result of the birds fed low CP diets consuming more feed and growing more slowly. A linear decrease in feed cost per kg weight gain was observed as the levels of crude protein supplemented with amino acids increased. It was observed that chickens fed 19, 20 and 21% CP with essential amino acids were similar compared to chickens fed 18% CP diet. This means that the overall cost of producing 1kg of lean meat was minimal for birds fed diets high in crude protein supplemented diets as compared to birds fed low crude protein supplemented with amino acid. Dietary treatments had no significant effect on mortality.

5.19 Haematological Parameters and Serum Biochemical Indices of Broiler Starter Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (0 – 4 Weeks)

Packed cell volume, haemoglobin count, red blood cells, white blood cells, uric acid, glucose, ALT, ALP, and cholesterol were significantly affected by dietary treatments. Dietary treatments had no significant effects on MCH, MCV, MCHC, urea, creatine, AST, triglycerides, albumin and total protein, though all the values obtained fell within the values for normal healthy chickens as reported by Suchy, (2000). The mean values of PCV 26.00-29.67% obtained fell within the normal value (22-35%) for healthy chickens reported by Jain (1993), these value were also similar to the values reported by Oladaele and Ayo (1999); Mitruka and Rawnsley (1977). This implies that the chickens were not anaemic and were immunologically balanced.

The haemoglobin count for all the dietary treatments (8.73-9.85g/dl) were slightly lower than the value for normal healthy chickens reported by Oladele and Ayo (1999). This discordant submission could be attributed to environmental and seasonal factors. Chickens fed 20% CP fortified with balanced essential amino acids had the highest values of red blood cells (4.70) and white blood cells (15.70). The values obtained for red blood cells were in accordance with the values reported by Jain (2000), Suchy (2000); Mitruka and Rawnsley (1977) for healthy chickens while the mean values of WBC obtained in this present study were above the values for normal healthy chickens as reported by Mitruka and Rawnsley (1977). This implies that the birds were slightly polycythemic, although the chicks were not adversely affected as no sign of illness was observed.This discordant submission could be attributed to environmental and seasonal factors, age, genotype and physiological status of the chicks.

The mean values (73-246.70mg/dl) of uric acid in this experiment was not in accordance with the report of Victoria *et al*. (1989) who reported 245±49 - 639 ±175, however the lower mean values of uric acid in this experiment showed that there was no incidence of kidney damage as the high mean values reported by Victoria *et al.* (1998) was attributed to kidney damage. Chickens fed 22% CP had the highest mean value of cholesterol (3.73), but generally the mean values of cholesterol in this experiment were similar to the values (2.93±0.51 – 4.23 ±0.39) reported by Ayo *et al*. (1996). The mean values for AST and ALT obtained in this present study are in accordance with the values for normal healthy chickens as reported by (Jain 1993).

5.20 Haematological Parameters and Serum Biochemical Indices of Broiler Finisher Chickens fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level (5–8 Weeks)

Dietary treatments had significant effects on packed cell volume, haemoglobin count, white blood cells, uric acid and albumin. The evaluations of red blood cells, mean cell haemoglobin, mean cell volume, mean cell haemoglobin counts, urea, creatine, glucose, AST, ALT, ALP, cholesterol, triglycerides and total protein did not reveal any significant differences across the treatment groups, although the observed means for all the parameters fell within the normal values for healthy chickens as reported by Jain (1993); Suchy (2000).

Chickens fed 18% CP with balanced essential amino acids had the highest value for PCV compared to those fed higher levels of CP. The reasons could as a result of amino acid imbalance from the variations in amount of the feed ingredient used and physiological status of chickens. The mean values for haemoglobin count of 8.67-9.67g/dl obtained in this experiment is similar to the values of 11.30 ± 1.82g/dl reported by Oladele and Ayo (1999). This means that the chickens in all the treatment groups

were not anemic. The high values of 10.70-16.07 X^6/l for red blood cells in this study are contrary to the values of 1.58- 3.82 X^6/l reported by Mitruka and Rawnsley (1977). This implies that chickens were polycytheamic. Chickens fed diets containing 18% CP had the highest uric acid value of 250.33 compared to other treatments with higher levels of CP. This result is contrary to the findings of Collins *et al*. (2003), Malheiros *et al.,* (2003) who reported considerably lower uric acid levels are measured in the plasma of low crude protein. The mean values of 3.12 - 4.23 g/dl of albumin are similar to the value reported by Jain (1993).

5.21 Nutrient Digestibility of Broiler Chicks Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level. (0 – 4 Weeks).

Dietary treatment improved the apparent crude protein (CP), ether extract, ash and NFE digestibilities. Chicks fed 22, 23 and 21% CP supplemented with essential amino acids respectively had the highest values for the digestible CP. This result conforms to the result of Faira Filho*et al*. (2007), who reported that digestibility of CP increased with increase in the levels of crude protein. This might be due to the efficient protein degradation and absorption. The chicks fed low crude protein supplemented with the essential amino acids recorded the lowest value for digestible CP; this may be due to amino acid imbalance which might be associated with low protein utilization. The high values for digestible ether extract observed in chicks fed 21 and 23% CP supplemented with essential amino acids confirms the report of El-Husseiny *et al*. (2007). They noted that digestibility of ether extract was improved with increase levels of dietary CP supplemented with lysine, methionine and tryptophan. Ash digestibility however, was influenced by dietary treatment.

It was observed that chicks fed 21 and 22 % CP supplemented with essential amino acids had the highest values for ash digestibility. The elevated high values recorded across the treatment suggest that more minerals were available to the birds as none of the chicks showed any deficiency symptoms throughout the experimental period. Digestibility of NFE was significantly affected by dietary treatment. This result is disagrees with the report of Fario Filho *et al*., (2007), who reported no significant difference for chicks fed varying levels of crude proteins supplemented with amino acids.

5.22 Nutrient Digestibility of Broiler Finisher Chickens Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level. (5–8Weeks).

Dietary crude protein supplemented with essential amino acids had influence on the digestibility of crude protein, crude fibre, ash and NFE. Chickens fed 21% CP fortified with balanced essential amino acids had the best value for apparent digestible crude protein, crude fibre and NFE compared with the chickens fed 18% CP with balanced essential amino acid. This result agreed with the findings of Neto *et al*. (2000) and Ratriyanto *et al*. (2012) who reported that efficiency of nutrient utilization was better for chickens fed higher level of CP diets supplemented with essential amino acids. The reasons may be as a result of increased proventricular acid secretions, increased pancreatic and intestinal mucosa secretions at this phase of rearing. Digestible ash however, was influenced by dietary treatment, the high value suggest that more minerals were available to the birds as none of the birds showed any mineral deficiency symptoms throughout the experiment period.

5.23 Carcass Characteristics of Broiler Finisher Chickens Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level. (5–8weeks).

Dietary treatments had significant effects on live weight, carcass weight, dressing percentage, back, gizzard, lungs, kidney, heart, breast weight, wings, thigh and drumstick. Chickens fed 19, 20 and 21%CP with the supplementation of amino acids had the best results in most of the parameters measured for carcass characteristics. The results disagreed with the findings of Si *et al*., (2001), Baker *et al*. (2002) who reported that lowering the dietary CP level may not affect carcass characteristics yield, breast meat and thigh yield of birds. Moran and Stillborn (1996) found no effect on the carcass yield of broiler fed low CP diets adequate in essential amino acids (EAA). Chickens fed 18%CP with diets adequate in EAA had the least carcass yield; this report is similar to the findings of Kerr and Kidd (1999a) who also reported a significant decrease in the carcass yield of the birds fed low CP diets supplemented with EAA. Abdominal fat pad was observed to be higher in birds fed 18% CP diet with adequate EAA. This result is similar to the findings of Sterlings *et al*. (2002), Hai and Blaha (2000) and Neto *et al*. (2000) who observed that abdominal fat pad weight increased with low CP diets.

5.24 Tissue protein and Tissue Fat of Broiler Finisher Chickens Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids At Each Protein Level. (5 – 8Weeks).

Dietary treatment had no significant effects on the tissue fat and tissue protein. This result was similar to the findings of Hia and Blaha (2000) who conducted experiments to study the effects of isocaloric low CP diets adequate in the levels of essential amino acids on broiler chickens performance. They concluded that decrease in dietary CP level had no negative effects on carcass protein and tissue fat. Another experiment conducted by Bregendahl *et al*. (2002), found that chickens fed low CP diets retained more body fat than the chickens fed control diet. Their reason was based on the fact that

chicken fed low CP consumed more to meet their requirements for weight gain, resulting in increased fat deposition.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

It has been admitted that there has been considerable progress over the years in the productivity of broiler chickens by continuous genetic improvement and by the advancement in the aspect of nutrition. Improved productivity needs balanced nutrients to sustain the optimum growth for fast growing animals like the broiler chickens. However, a great deal of production costs of broiler chicken feeds depends on the prices of protein sources. Therefore, maximizing the efficiency of protein utilization through optimum amino acids balance is very critical, in view of this, three experiments were conducted. Experiment 1, was conducted to determine the optimum tryptophan requirements for broiler chickens reared during the cool season in the tropical environment. Furthermore, to investigate the effect of dietary tryptophan on carcass characteristics, nutrient digestibility, tissue crude protein, tissue fat, blood haematological and serum biochemical indices of broiler chickens reared during the cool season under tropical environment. Broiler chicks were reared in the cool season (November- January) and were fed graded dietary levels of tryptophan 0.15, 0.19, 0.23, 0.27and 0.31% respectively. Chicks fed 0.23% dietary tryptophan had better results in term of the final weight, weight gain and average daily weight gain as compared to those fed other tryptophan levels. For broiler finisher chickens reared in the cool season and fed graded dietary levels of tryptophan at 0.13, 0.17 0.21, 0.25 and 0.29% respectively. Birds fed 0.21% and 0.25% dietary tryptophan had similar results in terms of final body weight, weight gain; average daily weight gain, feed conversion ratio and feed cost per kg gain. However, a trend line graph showed that at 0.21% a slightly

better final body weight and weight gain was attained. It was observed both at the starter and finisher periods that chickens fed higher percentage (0.21% vs 0.13%) of tryptophan had better apparent nutrient digestibility values, tissue crude protein, tissue fat, compared to chickens fed lower tryptophan. Furthermore, chickens fed diets supplemented with tryptophan had better carcass characteristics compared with those without tryptophan supplementation.

Experiment 2 was conducted during the hot season (March- May) to determine the optimum tryptophan requirements for broiler chickens and to further evaluate the effect of dietary tryptophan on growth performance, carcass characteristics, nutrient digestibility, blood haematological parameters and serum biochemical indices of broiler chickens. For this study, broiler chicks (0 - 4 weeks) were fed diets containing graded dietary levels of tryptophan at 0.15, 0.19, 0.23, 0.27 and 0.31% respectively. Broiler finisher chickens were fed graded dietary levels of tryptophan at 0.13, 0.17, 0.21, 0.25 and 0.29% dietary tryptophan respectively. A trend in growth performance as the level of dietary tryptophan increased was observed at both the starter and finisher phases, therefore a trend analysis revealed that at 0.23% dietary tryptophan broiler chicks performed better in terms of the final weight, weight gain and average daily weight gain compared to the chicks fed other tryptophan levels. During the finisher phase (5–8weeks), the birds fed diets supplemented with tryptophan had the best result in terms of final weight, weight gain, feed conversion ratio, cost per kg gain and carcass yield when compared with the treatment without tryptophan. A trend analysis indicated that at 0.21% dietary tryptophan, the best final weight, weight gain and average daily weight gain were attained for the finisher phase of chickens reared in the hot season. Generally, it was observed that tryptophan supplementation resulted in reduced tissue fat deposition. It was also observed that at the starter and finisher phases, diets fed higher

concentration of tryptophan had better apparent nutrient digestibility values. Significantly higher values obtained in all the, apparent digestibility values, haematological and blood serum biochemical indices in this study revealed the adequate utilization of all the nutrients in the diets. It was noted that synthetic tryptophan in the diets of broiler chicks and broiler finisher chickens up to the highest level tested did not have any adverse effect on the haematological and blood serum indices of chickens as most values obtained fell within the normal range.

The third experiment was conducted to evaluate the effect of diet formulated on the basis of four critical essential amino acids on the crude proteins requirements of broiler chicken. Furthermore, to investigate the effect of graded levels of crude protein diets formulated on the basis of four critical essential amino acids at each protein level on growth performance, nutrient digestibilities, haematological parameters and serum biochemical indices of broiler chickens. For this study, broiler chicks (0 - 4 weeks) were fed diets containing graded levels of dietary crude protein of, 20, 21, 22 and 23% respectively, formulated on the basis of four critical essential amino acids and broiler finishers chickens were fed diets containing, 18, 19, 20 and 21% crude protein, formulated on the basis of four critical essential amino acids. At the starter phase, chicks fed 21, 22 and 23% crude protein were similar in terms of final weight, weight gain and average daily weight gain. The results of the starter phase of experiment 3 revealed that crude protein levels of broiler chicks can be reduced from 23 to 21% crude protein if the essential amino acids, were balanced. It was observed that chicks fed 21% CP of had the best values for the apparent nutrient digestibility compared to those fed other crude protein levels. For the finisher phase of experiment 3, chickens fed 19, 20 and 21% crude protein were similar in terms the final weight, weight gain, average daily weight gain, feed conversion ratio and feed cost per kg gain. The 21% CP

diet was however regarded to be the optimum crude protein requirement due to slightly better results for FCR and feed cost per kg gain.

6.2 Conclusion

For the experiments conducted during the cool and hot seasons, it was concluded that the tryptophan requirements for the starter phases was 0.23% and the finisher phases was 0.21%. For experiment 3, it was concluded that the crude protein requirement for broiler starter chicks can be reduced from 23% to 21% CP while the crude protein requirement for the finisher phase stood at 21% without having any adverse effects on growth performance, nutrient digestibilities, carcass characteristics, blood haematological parameters and serum biochemical indices of broiler chickens.

6.3 Recommendations

More research needs to be done on the requirements for individual amino acids other than methionine, lysine, threonine and tryptophan in the tropical environment. Consequently further studies will be necessary to alleviate contradictory findings and inconsistent results as the inclusion of tryptophan in broiler and poultry rations in general on growth and other production indices are speculative at this time in the tropical environment.

6.4 Contributions to Knowledge

- Dietary tryptophan at 0.23% improved the growth performance of broiler chicks by 13.97% and 20.72% during the cool and hot seasons respectively.
- Dietary tryptophan at 0.21% improved the growth performance of broiler finisher chickens by 24.29% and 25.21% during the cool and hot seasons.
- Formulating diets on the basis of four critical essential amino acids (lysine, methionine, tryptophan and threonine) reduced dietary protein requirement of broiler starter chicks from 23% CP and above down to 21% CP.
- Formulating diets on the basis of four critical essential amino acids (lysine, methionine, tryptophan and threonine) resulted in 21% CP as the crude protein requirement of broiler finisher birds.
- Dietary tryptophan at 0.23% reduced feed cost per kg gain of broiler starter by 11.40% and 11.20% for the finisher phase during the hot season.

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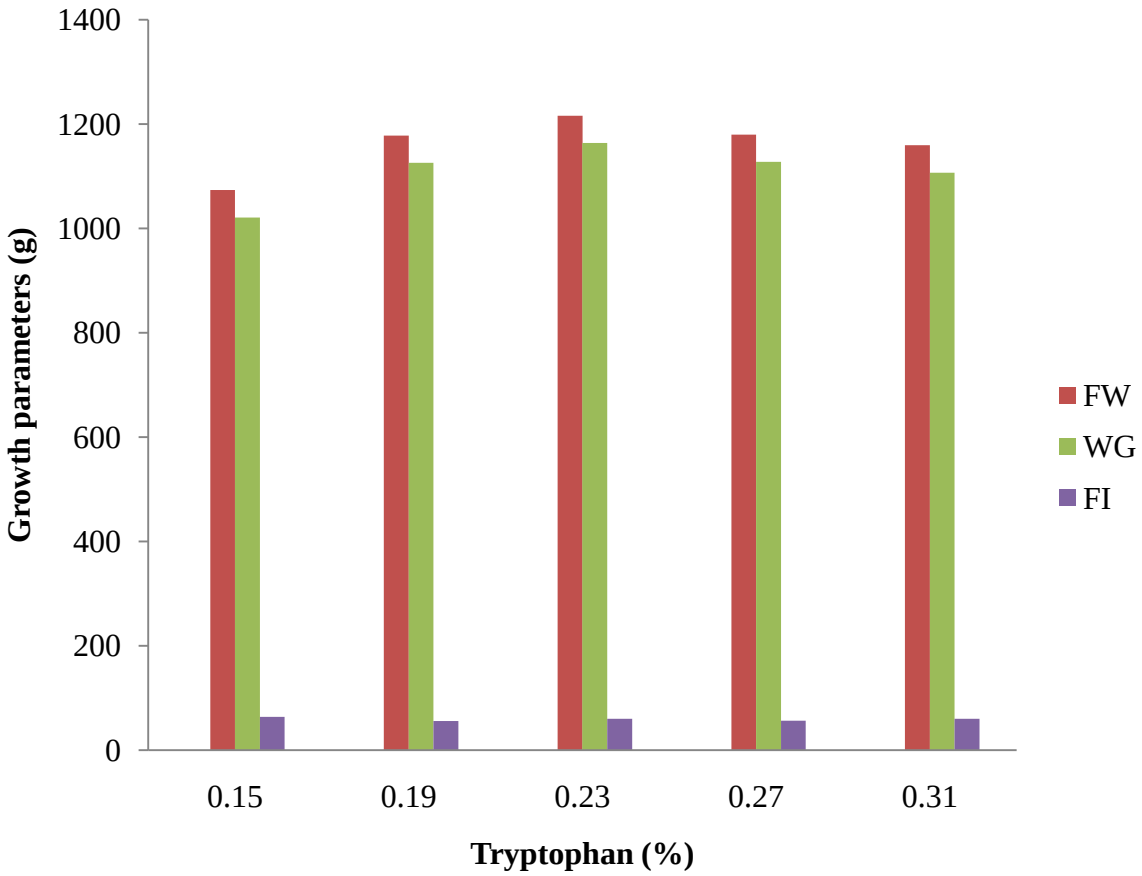
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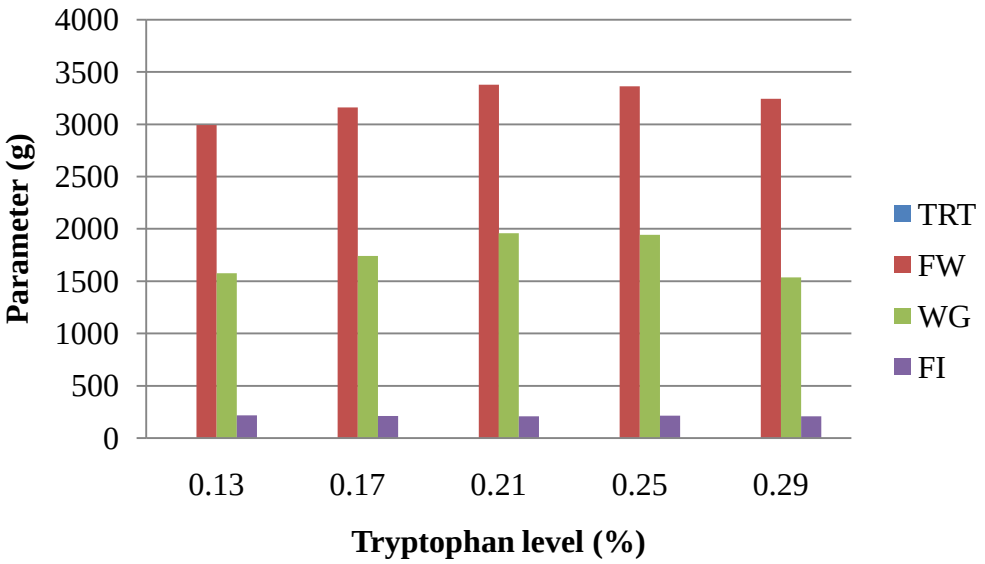
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APPENDICES



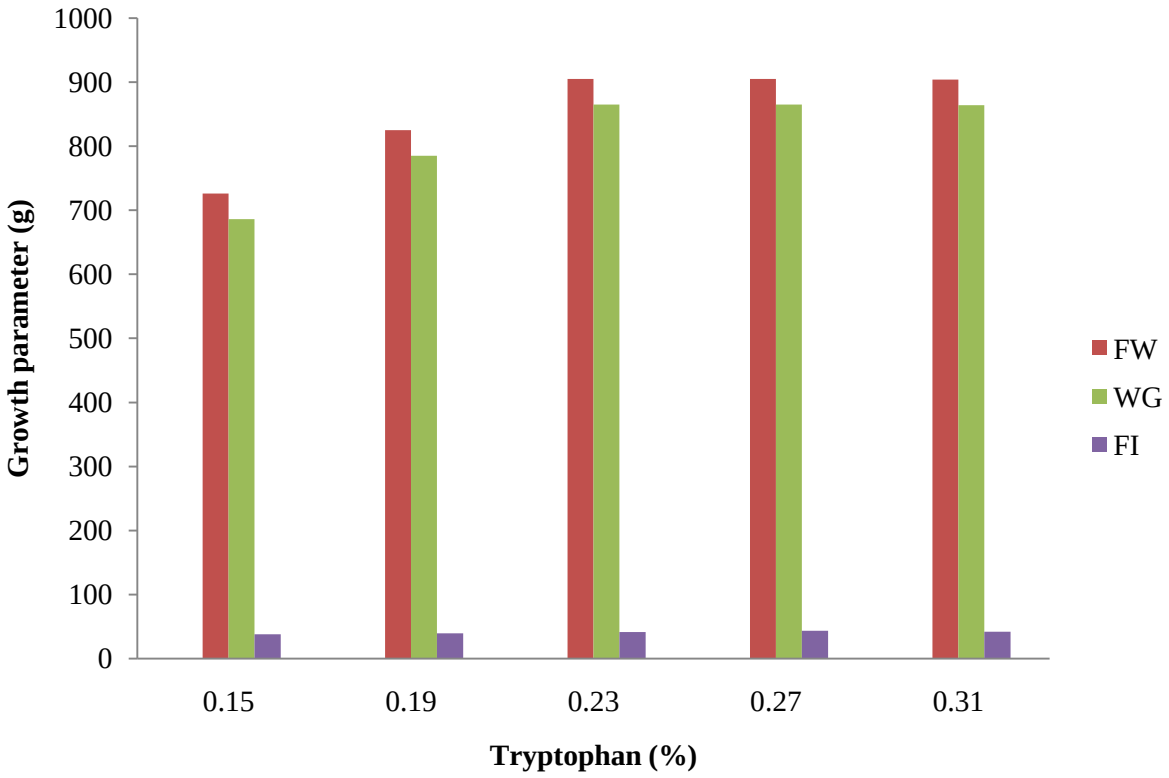
Appendix 1: A graph showing the performance of broiler chicks fed dietary levels of tryptophan in the cool season. (0 - 4 weeks).

FW=Final weight
WG=Weight gain
FI=Feed intake (g/b/d)



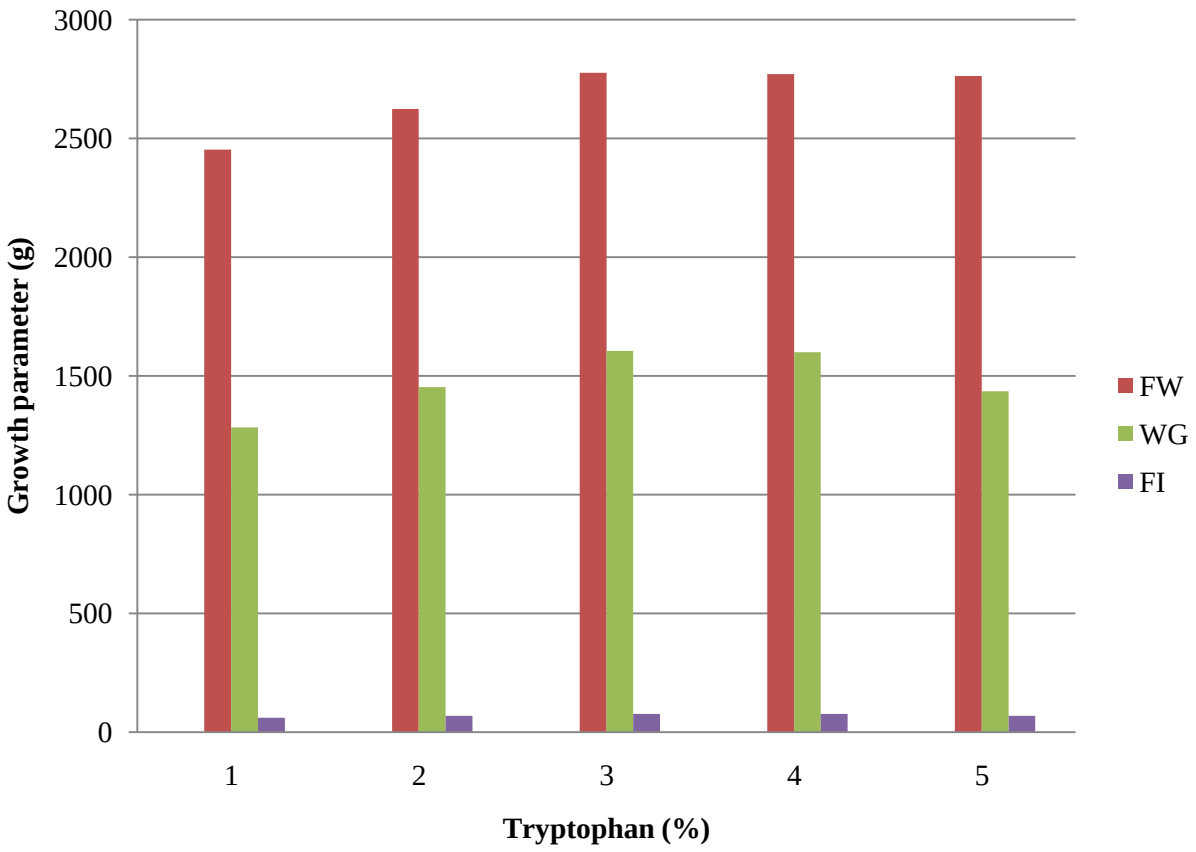
Appendix 2: A graph showing the performance of broiler finisher chickens fed dietary levels of tryptophan in the cool season. (5 - 8 weeks).

FW=Final weight
WG=Weight gain
FI=Feed intake (g/b/d)



Appendix 3: A graph showing the performance of broiler chicks fed dietary levels of tryptophan in the hot season. (0 - 4 weeks).

FW=Final weight
WG=Weight gain
FI=Feed intake (g/b/d)



Appendix 4: A graph showing the performance of broiler finisher chickens fed dietary levels of tryptophan in the hot season. (5 - 8 weeks).

FW=Final weight
WG=Weight gain
FI=Feed intake (g/b/d)