GROWTH AND AMINO ACID UTILISATION IN BROILER CHICKENS FED SUPPLEMENTAL GLYCINE IN LOW CRUDE PROTEIN DIETS WITH VARIED METHIONINE AND THREONINE

BY

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CERTIFICATION

This is to certify that this study "Growth and amino acid utilization in broiler chickens fed supplemental glycine in low crude protein diets with varied methionine and threonine"was carried out by **PASCHAL CHUKWUDI AGUIHE** under my supervision in the Department of Animal Science, University of Ibadan, Ibadan, Nigeria and Department of Animal Science, Universidade Estadual de Maringa, Maringa, Brasil.

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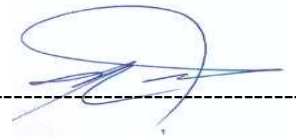

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DEDICATION

I dedicate this thesis to the Holy Trinity, my adorable wife - Esther Omenogo AGUIHE, amazing daughter - Munachimso Emmanuella AGUIHE and also my amiable parents – Mr and Mrs Pius Irechukwu AGUIHE.

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ABSTRACT

Limiting Amino Acid (AA) fortification such as methionine and threonine of vegetable based low Crude Protein (CP) diets for broiler chicks is often adopted for decreasing production cost and nitrogen emission but could impact negatively on growth of broiler chickens. Supplemental glycine could improve broiler chicken performance when low CP diets are fed. However, information on the quantification of the sparing effect of glycine for methionine and threonine in low CP diets is scanty. Therefore, the impact of supplemental glycine on performance and AA utilisation of broiler chicken (21 days) offered low CP diets with varying methionine and threonine concentrations were investigated.

Nine diets were formulated to contain 22, 20 and 18% CP levels each in combination with supplemental glycine at 0.0, 0.2 and 0.4% inclusion. One-day old Arbor Acre chicks (n=288) were randomly allotted to the diets in a 3×3 factorial arrangement with four replicates of eight birds/replicate. Fifteen diets containing 0.3, 0.5 and 0.7% methionine levels each having 0.2, 0.4, 0.6, 0.8 and 1.0% glycine were formulated. Another 15 diets were formulated containing 0.7, 0.8, and 0.9% threonine, each in combination with 0.2, 0.4, 0.6, 0.8 and 1.0% supplemental glycine. One-day old Cobb-vantress chicks (n=1275) were randomly distributed to the 15 diets each in a 3×5 factorial arrangements with five replications of 17 birds/replicate. Average Daily Gain-AWG (g), Feed Conversion Ratio-FCR, Average Breast Weight-ABW (g/kg) and Abdominal Fat-AF (g/kg) were measured; Apparent Ileal AA Digestibility-AIAAD, Serum Uric Acid-SUA (mg/dL) and Pectoral Muscle Creatine-PMC (mg/g) were assayed. Data were analysed using descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

A significant interaction between CP and glycine levels was recorded on AWG, FCR, AIAAD and AF. Reducing CP levels from 22 to 18% with 0.4% glycine addition significantly improved AWG (31.64±0.97), FCR (1.51±0.12), AIAAD (77.67±4.62%) and reduced AF (13.67±2.87). FCR and ABW of the birds showed a significant interaction between methionine and glycine levels. Linear effect (R^2=0.88) of increasing glycine levels was recorded for 0.30% methionine, whereas a quadratic effect was observed for 0.5% (R^2=0.92) and 0.7% (R^2=0.90) methionine diet on FCR and ABW. Increasing levels of glycine resulted in quadratic response on FCR (1.33±0.06; R^2=0.88), ABW (150.30±7.83; R^2=0.89) and PMC (3.43±0.38; R^2=0.85) with an estimated optimum point at 0.80% glycine. Significant interaction was observed between the glycine and threonine levels on FCR and SUA. With increased glycine levels, FCR improved linearly for 0.7% (R^2=0.96) and 0.8% (R^2=0.93) threonine diets but quadratically for 0.9% (R^2=0.97) threonine diets, with optimum point estimated at 0.8% glycine (1.43±0.03). Decreased linear response of SUA with increasing levels of glycine was observed and lowest concentration recorded for the 0.7% threonine and 1.0% glycine diet (3.43±0.67mg/dL). Also, improved linear (R^2=0.88) response of glycine levels were observed for ABW while a quadratic effect was recorded for AWG (842.12±43.13; R^2=0.96), FCR (1.43±0.06; R^2=0.98) and PMC (3.79±0.41mg/g, R^2=0.74) at optimum values of 0.8, 0.8 and 0.6% glycine, respectively.

Low dietary crude protein with varying methionine and threonine concentration resulted in optimum performance of broiler chicks at 0.8% glycine level.

K**eywords**: Supplemental amino acid, Growth, Amino acid digestibility, Serum uric acid, Broiler

Word count: 500

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LIST OF ABBREVIATIONS

AA – Amino acid
AFI – Average feed intake
AGT – Alanine:glyoxylate aminotransferase
AWG – Average weight gain
CP – Crude protein
Cre - Creatine
Cys – Cysteine
DM – Dry matter
DNA – Deoxyribonucleic acid
EAA – Essential amino acid
FCR – Feed conversion ratio
GAA – Guanidino acetic acid
GCS – Glycine cleavage enzyme system
Gly – Glycine
Gly+Ser – Glycine+Serine
GPx – Glutathione peroxidase
GSH – Free Glutathione
GSSG – Oxidized Glutathione
HMTBA – Hydroxy analogue of methionine,
IP – Ideal protein
LCPD – Low crude protein diet
ME – Metabolizable energy
Met – Methionine
NEAA – Non essential amino acid
NRC – Nutrient requirement council
PUFA - Polyunsaturated fatty acids
RNA – Ribo nucleic acid
ROS – Reactive oxygen species
SA – Serum ammonia
SAA – Sulphur amino acid
SAM – S-adenosylmethionine
Ser - Serine
SGly – Supplemental Glycine
SHMT – Serine hydroxymethyltransferase
SID – Standardized ileal digestibility
SUA – Serum uric acid
TBARS - Thiobarbituric acid reactive substance
Thr – Threonine
TSAA – Total sulfur amino acid
UA – Uric acid

CHAPTER ONE

1.0 INTRODUCTION

The growing need for animal protein and the associated global scarcity of cropland especially in developing countries, results in the anticipated deficit of feed ingredients rich in proteins. Minimal cropland for food and feed processing limits availability, and this in turn affects the supplies of feedstuffs and animal product prices. The consequent implications of shortage and higher prices of feed ingredients have been shown to have direct effect on sustainability of feed ingredient, thereby leading to increased cost of poultry production (Fazeni and Steinmüller 2011). In addition, intensive animal production has beenseen as one of thesignificantsources of environmental pollution. Production of greater quantities of excreted nitrogenous compounds from broiler chickens and other farm animals has the potential to cause detrimental impacts on the environment such as water pollution (eutrophication), soil acidification and nitrous oxide pollution, which may harm wildlife and humans (Bouwman *et al*., 2002; Martínes-Lagos *et al*. 2013). According to Lilly *et al*. (2011), over 70% of ingested N are excreted by the animal through metabolic processes due to oxidation of amino acids (AA) provided in excess of their requirement. Excess N in the excreta arisng from the oversupply of CP/AAs may increase the emission of ammonia by degradation of undigested protein and uric acid from the poultry manure. Elevated ammonia concentrations in the poultry pens can negatively affect human and animal health due to its offensive odors. Thus, accretion of feed ingredients cost and environmental problems with N runoff due to excessive protein intake from confined livestock such as poultry bird are two significant challenges confronting poultry scientist. Thus, this has caused increased research to reduce production cost and environmental N pollution.

Lowering CP concentration in the diet with adequate supplemental limiting AAs for broiler chickens has been successfully adopted as a technique to decrease the production cost and N excretion (Bregendahl *et al*., 2002; Yuan *et al*., 2012, Belloir *et al.,* 2017; Hofmann *et al*., 2019). This is obtainable by decreasing the incorporation of high crude protein feed

ingredients in poultry diets especially soybean meal, which translate to the reduction of dietary crude protein level. Consequently, provision of low CP levels in the diets of poultry birds has been observed to decrease the excretion of nitrogen (Waldroup *et al*., 2005; Namroud *et al*., 2008; Hernandez *et al*., 2012) and emissions of ammonia (Ferguson *et al*., 1998), matched to those offered greater diets CP diets. Nevertheless, previous research has indicated that lowering CP in the diet can impair the growth output of broilers even with the addition of supplemental limiting AAs such as DL-Methionine, L-lysine and L-Threonine ((Namroud *et al*., 2008; Hernandez *et al*., 2012; Belloir *et al*., 2017). Several possible reasons have been examined for the poor growth rate encountered in broiler chickens served feeds of smaller CP volume, such as insufficient dietary potassium, essential amino acid (EAA) deficiencies, lack of non-specific amino-N, inadequate non-essential amino acids (NEAA) to EAAratio, inadequateprovision of specific non-essential AAs and decrease voluntary feed consumption (Hussein *et al*., 2001; Aftab *et al*., 2006).

Although, one potential conceivable explanation behind the poor performance encountered with low CP diets is insufficient provision of the specific non-essential AAs (Alertor *et al*., 2000). Between several NEAAs, a shortage of glutamic acid and glycine (Gly) is perhaps the veryendearing explanation of decreased growth of birds supplied with feeds of smaller CP volume (Waguespack *et al*., 2009; Siegert *et al*., 2016; Wang *et al*., 2020). Nonetheless, in several cases, the glutamic acid failed toenhance the productive parameters of the broilerchickens(Waldroup *et al*., 2005); whereas, addition of crystalline glycine in reducedprotein diets has shown remarkable results (Berres *et al*., 2010; Ospina-Rojas *et al.*, 2012; Awad *et al*., 2015, Hofmann *et al*., 2019). Whenever there is a decreased in dietaty CP, dietary Gly levels drop substantially as low CP diet entails a great reduction in intact protein sources like soybean meal, which contain reasonably higher Gly content compared to other ingredients. Hence,Gly inadequacy was observed to inhibit the growth efficiency in broiler chickens fed low CP diets, developed without animal by-products, since the amount of endogenous production could not sustain optimal performance at the early stage (Yuan *et al*., 2012; Belloir *et al*., 2017, Wang *et al.,* 2020).

In formulation of broiler diet, dietary Gly is generally considered together with serine (Ser), due to the continuous reversible conversion of Gly into Ser by the enzyme Ser hydroxymethyl transferase (Velíšek and Cejpek, 2011). Various experiments have demonstrated that dietary Ser performs similar roles as dietary Gly provided it is supply in equimolar quantities (Sugahara and Kandatsu, 1976). Moreover, Gly plus Ser are typically

measured jointly(commonly known as 'Gly+Ser' (NRC, 1994)) when assessing the nutritional value of diets, as a result of their supposed unrestricted metabolic interconversion (Dean *et al.,* 2006).

1.1 Justification of the study

To counter the increasing global demand for poultry, exceptionally broiler chickens, and to increase profitability within the enterprise, it is necessary to create new approaches to remain viable in reducing production cost and environmental N contamination, while achieving optimum growth for broiler chickens. Formulation of low CP is important to tackle surging feed cost and environmental risks in broiler production. However, providing diets with reduced CP concentration have resulted in broilers with impaired performance not equalvent to birds consuming diets with sufficient CP levels, despite satisfying the requirements of all EAA (Waguespack *et al*., 2009; Ospina-Rojas *et al*., 2012; Awad *et al*., 2015). Research evidences have proved that reducing CP concentrations in the diet to the range of 17 – 18% relative to diets containing adequate CP levels of 22 – 23% resulted to improved growth output of broiler chickens at 21 days of age when augmented with NEAA Gly (Ospina-Rojas *et al.,* 2013a and 2014; Siegert *et al*., 2016; Awad *et al*., 2018).A large amount of data has accrued over the years for AA Gly, which shows that this AA is essential to poultry feeding, especially at early phase of productionwhen low protein diet is adopted. Since N-related compounds are abundant in a broiler diet, there seems to be consensus that addition of supplemental Gly is essential to produce uric acid to remove surplus N (Dari *et al*., 2005). Most of the prevailing willingness to continue to explore the possibilities of reducing CP levels with supplemental Gly in broiler diets centers around the need to optimize the utilization of limiting AAs such as methionine (Met) and threonine (Thr) for increased synthesis of protein.

Moreover, sincelowering CP concentrations in the diet of broilers may result to a situation where dietary Gly slightly reduces and become insufficient, especially when formulating with all-plant ingredients, Glysupplementation is necessary to satisfy the metabolic needs of limiting AAs especially methionine and threonine in a low CP diet. Therefore, adequate supplemental Glymay spares some methionine as a methyl donor by acting as a readily available precursor for dietary supply of cysteine via trans-sulfuration pathway for protein synthesis, and dietary Gly level above requirement may equally exert a sparing effect on

dietary threonine in low CP diet, thereby increasing the amount of AAs available for the physiological processes of growth and maintenance.

1.2 General objective of the study

To establish the optimal concentration of supplemental Gly inLCP diets of chickens (1 - 21 d) containing different levels of standardized ileal digestible (SID) methionine and threonine on performance and AA utilization.

1.3 Specific aims of the study

1) To evalaute theimpact of varied concentrations of supplemental Glyto reduced CP, corn-soybean based diet.
2) To ascertain the optimum level of supplemental Gly in low CP, corn-soybean based diets containing varying dietary concentrations of methionine.
3) To ascertain the optimum level of supplemental Gly in low CP, corn-soybean based diet containing varying dietary concentrations of threonine.

1.4 Research Hypothesis

H_O 1: Dietary concentrations of crude protein and supplemental glycine levels has no effect ($p > 0.05$) on performance and AA utilisation of broiler chicks.

H_O 2: Concentrations of SID methionine and supplemental glycine has no effect ($p > 0.05$) on growth and amino acid utilization of broiler birds offered diets with reduced CP concentration.

H_O 3: Concentrations of SID threonine and supplemental glycine has no effect ($p > 0.05$) on growth and amino acid utilization of broiler birds offered diets reduced CP concentration.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition of protein in poultry

Protein as avital dietary component of poultry is necessary for life together with other major nutrient groups such as carbohydrate, fat, vitamin, mineral and water. Proteins are organic compounds consisting of α-amino acids covalently connected through peptide chains, and the composition of single α-amino acids involves unit of amino and carboxyl group attached to α-carbon (Perry *et al*., 2003). During digestive process, proteins are often degraded through a process of hydrolysis to produce these AAs, that are further used in the body to perform range of roles in birds, including as structural parts of the skin, feathers and muscles;along with performing essential physiological responsibilities such as enzymes,hormones, serum proteins and immune antibodies, most of them being actively engaged independently in particular body activities (Pond *et al*., 1995).Though it has been established earlier that some specific AAs played a significant function in animal growth, a large number of investigation in poultry has been carried out to determine sufficient protein levels that would help performance. There has been a substantial reduction in the productivity of birds givenreducedCPration, significantly reduced protein efficiency and total feed use as birds gets older. The discrepancy in recorded protein concentrations for optimum growth may be partly due to variations in the levels of crude protein evaluated, and variables like protein source or other nutrients and ingredientslevels which might have varied between studies.

Protein is universally regarded as one of the largest cost drivers of poultry rations. During feed formulation for broiler chickens, the primary focus is on CP, since the amount of CP contained in the diet greatly influences efficiency and ration costs, and thus the gross margin in the production poultry birds (Eits *et al*., 2004). Broilers have large dietary CP

requirements, since they need reasonable amount of protein in their diet to maximize feed conversion and other performance features. Hence, concentrations of dietary CP have a huge impact on production, carcass structure and complete charges of the end product (Aletor *et al*., 2000). There has been evidence showing that providing adequate ration for optimumgrowth of broilers could minimize the profit of a conventional broiler production instead of the best gain (Eits *et al*., 2004). It is therefore necessary to provide a sufficient CP in the diet according to the needs, but it is unnecessary to provide excess of their need. The benefit of using sufficient quantities of adequateprotein and AA for broilers is thus a top priority concern for many purposes such as feed quality, economies and degradation of the environment. The principle of compounding diet for broilers on the premise of adequatecrude protein eliminates major nutritional issues related toprotein/AA.

2.2 Metabolic significance of amino acid in broiler nutrition

The AA provides a significant influence on regulation of protein metabolism in poultry.Protein metabolism refers to the various biochemical processes that are responsible for the formation (anabolism) and breakdown (catabolism) of proteins and AAs in the animal body (Figure 2.1). The AAs are considered as anabolic agents promoting protein profit by facilitating protein production while impairing protein catabolism, and are essential substrates for the synthesis of diverse nitrogen metabolites. Also, there has been some growing consensus that amino acids serve as main metabolic moderators in different tissues and organs by regulating the major metabolic pathways (Wu 2013, 2014; Belloir *et al*., 2017). The description of the processes involved in this regulation can provide a justification for maximizing the demands for amino acids and regulating protein density, muscle strength and meat value while maintaining higher productivity. These are constituents of tissue proteins, and an inadequate availability of them contributes to decreased muscle growth and tissue repairs. Consequently, the availability of the multiple EAAs is a requirement for preserving maximalCP production levels, an undeniably important principle in protein nutrition. Maximizing AA intake, however, involves taking into account the various capabilities of AAs that are mostly identified as having many important impacts. The AAs are the forerunners of large molecules of protein. Example, AA like cysteine is needed in creating molecules of taurine andglutathione, being important ingredients for animal protection against oxidative damage while Methionine is the substrate of the methyl groups necessary in enhancing biochemical methylation reactions, particularly DNA and histones pattern of methylation involved in epigenetic modification (Waterland and Michels, 2007).

Amino acids have also been used to intensively monitor the nutritional modulation of protein metabolism in muscle tissue because these nutrients are considered as anabolic agents that promote protein benefit. Therefore, it is necessary to comprehend the pathways by which AAs regulate protein metabolism in order to boost regulation of nutrient utilisation and maximize the supply of dietary AA.The AAs in the feed are key determining factors of broiler carcass constituents, as their level affects abdominal fat content and breast meat yield (Leclercq, 1995). Apparently, raising the lysine concentration in broiler diets above its need for growth decreased the degradation of the breast muscle in preservation by improving its total pH (Berri *et al*., 2008). The supply of amino acid is shown to exhibit an influence on the tissue chemical properties, with a beneficial impact on the quality of the meat. Specifically, the deficit of Met and/or Cys alters the composition of lipids and proteins, and amino acid structure of tissue proteins in animals (Bunchasak *et al*., 1997; Pacheco *et al*., 2018). It has also been stated that the availability of sulfur amino acid affects meat vulnerability to deterioration.It has indeed been shown that the complete or partial substitution of DL-Met by its hydroxyl-analog HMTBA decreases breast meat vulnerability to deterioration and also improves its total pH, with beneficial influence on preservation and processing capacity (Mercier *et al*., 2009).

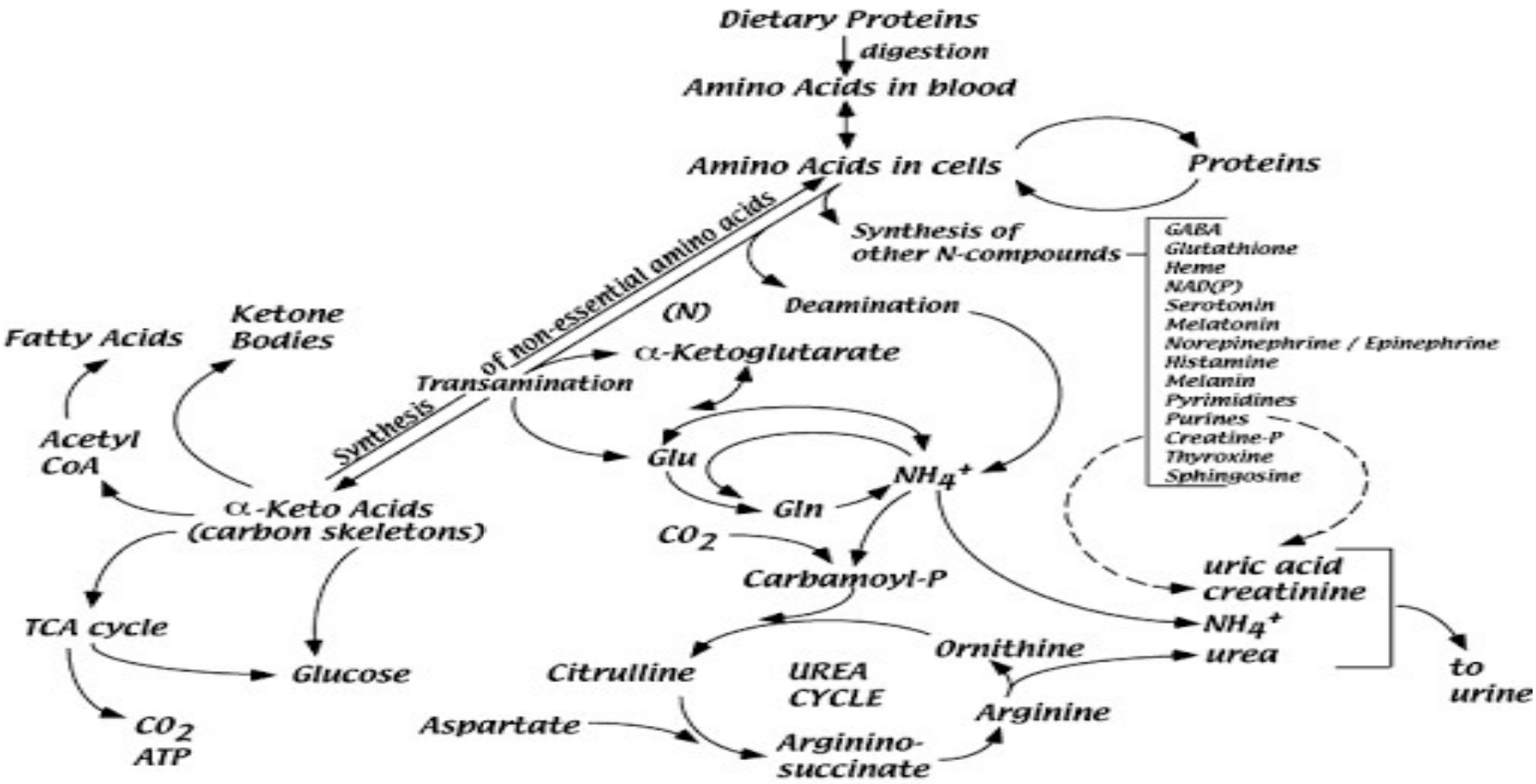

Figure 2.1: Overview of the pathways of amino acid metabolism

Source: Wu(2013)

2.3 Factors influencingthe need of protein and amino acid

The protein and amino acid needs in poultry are determined by several variables. The needs of birds vary with variations in their age, sex, development status, scale, species and strain, variations in CP content and digestibility(NRC, 1994; Kidd *et al*., 2005;Samadi and Liebert, 2006). The amino acid needs may also be influenced by temperature, as consumption is usually reduced in excess hot period and decreased during times of winter (Furlan *et al*., 2004). Consequently, changes to the concentration of dietary AAcan be needed during times of hot and cold stress, though there are inconsistencies in the literature regarding this method.According to Cheng *et al*. (1997), high temperatures reduced significantly the body weight gain, feed consumption, and feed:gain ratio, and also causes increased protein and AAs intake. When the impact of raising indispenableAA levels to excess requirement, while ensuring assessment of steady CP concentration, study showed thatthere were no changes in growth variables; but, an increased abdominal fat was obtained when AAs concentrations were increased indicating an enhanced recovery of usable energy stored more as fat instead of muscle (Zarate *et al*., 2003). Further investigation showed that no improvement in providing poor CPrations with supplemental EAAs on weight gain of broilers under heat stress and affected negativelythe feed conversion and fat accumulation in the body, indicating that severalAAs were limiting (Cheng *et al*., 1997). Moreover, using decreased level of CP in the diet with sufficient AA supplementation has the capacity to improving body weight gain and FCRof chicks under thermal stressed condition (Waldroup *et al*., 1976). Certain factors affecting the intake of feed and therefore the use of amino acids include bird's state of health, feed type (such as mash versus pellets), and a number of pressures from the environment (Maiorka *et al*., 2005).

2.4 Update on amino acid feeding strategies in broilers

Dietary amino acids have to be supplied in appropriate amounts in poultry, as in other farm animals, to boost performance and dietary utilisation and this includes accurate assessment of the AA needs, which can be affected by factors such assex, strain and age of an animal, and also environmental and dietary influences. In fact, the standards for amino acids in the ration have been defined to differ according to the growth parameters specified for improvement.For instance, it was demonstrated thatthe greater demand of lysine in the diet is usually achieved for feed:gain ratio and breast meat yield relative to weight gain (Mack*et al*., 1999; Cemin *et al.,* 2017). These studies jointly include strengthening investigations into the

responses to the supply of amino acids in terms of growth, muscle mass and product value.In addition, recent broiler genotypes, that have been massively enhanced with respect to weight gain and ration quality, can demand greater levels of amino acids in the ration to maximize production and carcass yield of chicks (Bernal *et al*., 2014). Indeed, there is an indication that genetic variation modifies the exposure to the supply of amino acids in the feed (Jlali *et al*., 2012).

Discovering feeding principlesof AA emerges out of the demand to lower the amount of protein in feed, which seems to be a key concern for cost, social and environmental perspective for sustainable production of broiler chickens. For instance, preparing ration that restricts excess amounts of CP leads to greater soybean meal inclusion.Due to cost fluctuations, its ecological effect such as deforestation or transport, and the issue of genetically modified organisms (GMO), this soybean is contentious as a feed ingredient (Belloir *et al*., 2017). Reducing crude protein levels in the feed can decrease soybean use, however resulting to the inclusion of greater amounts of supplemental AAs to maintain the balance of the dietary AAs.Moreover, it decreases the nitrogen pollution caused by higher quantities of indigestible nitrogen or surplus amino acid supply. However, preparing poor quality protein ration demands good knowledge of the nutritional properties of the feed stuffs and regulating the availability of digestible EAAs, the requirement for the most limiting EAAs is fulfilled through adding supplemental AAs. Practical approaches to balance the ration are generally based on a perfect composition of EAAs, for example optimizing growth efficiency.

2.5 Ideal Protein (IP) Concept

The ideal protein signifies a perfectAA balance which can satisfies the exact requirements of the birds for lean body development. In ideal protein, the EAA and NEAA concentrations provide the exact requirement to remove no additional AA-N. Balances of essential AA in broiler ration are significant. Adverse effects on the overall performance of birds may result through increasing a particular AA concentration if an inadequate amount of some AAs are supplied.The definition of ideal protein is focus on maintaining a complete dietary AA available in proportions that precisely satisfy the needs of the animals. As a result, excesses of AA are removed and thus synthesis of protein would proceed with optimum effectiveness (Chung and Baker, 1992).An alternative use of digestible values of AAs is focus on the compounding of diet based on ideal protein concept that is seen as the most current moves

towards truly correct amino acid specifications.The primary objective in the creation of an optimal protein framework or ideal protein has to do with the supply ofessential AAs mixturewhich specifically met the protein accretion and maintenance needs of an animal while preventing deficits or surpluses (Emmert and Baker, 1997).This form of strategy can cause all AAs to be similarly limiting, contributing to lower amount of extra AAs that need to be oxidized (Fuller *et al*., 1989). Also, it can be beneficial for assessing the consistency of other dietary proteins as a normal reference protein (Wang and Fuller, 1989).Adopting the style of ideal protein in formulating ration, all essential AAs are represented as appropriate proportions of lysine. Baker *et al*. (1993) document a range of benefits over other criteria on this method. Firstly, more has been established relative to lysine levels in feedstuffdcoupled with the requirement of lysine in different ages of birds, than any other AA. For commercial vegetable based diets, lysine is the second limiting AA, and plays the sole functions in the synthesis of protein.Furthermore, variables such as age, genetics, environment, density of energy and protein intake that influence the requirements of amino acids, and these factors may be taken into account by the IP method providing for more precise diet preparation.

Finally, adoption of IP modelassists to avoidexcessive formulation that greatly reduces the excretion of nitrogen. When using the IP concept for formulating feeds one should be aware of certain considerations.Clearly, the lysine requirement for the broiler chickens being fed must be very precise, and this reflects the foundation for the specifications of other EAA, thus, any flaw in the lysine needs would result in losses for other AAs (Baker *et al*., 1993; Emmert and Baker, 1997). Optimal AA sequence hasfocussed on amounts of digestible AAs in the diet; thus, reduces variations observed indigestionplus usage from specific CP sources, or when crystallineAAs are used (Emmert and Baker, 1997).Using the IP principle will allow for the assessment of birds ' digestible amino acid needs for any phase (Baker, 2003) and formulations of ration on digestibles basis. The IP definition can also be used with reintroduction of crystalline amino acids to formulate low CP diets.This has also revealed to be helpful in measuring the sequence of AA limitation, which may vary in rations where the CP or AA composition of the ingredient varies. (Han *et al*., 1992; Baker *et al*., 1993).

2.6 The Concept of Low Protein Diets

A crucial aspect of the IP principle involves the development of low CP diets, ensuring similar performance as opposed to high CP diets that are unbalanced. In reality, nevertheless,

it seems hard if possible to use natural feed ingredients tocompound diets that supply the complete AA required by birds in appropriate concentrations and try to sustain an adequate mixture of AA with limited surpluses (Han *et al*., 1992). Nonetheless, the incorporation of widely accessiblecrystalline EAA in diets of broiler chickenscould meet EAA requirements for birds (Moran and Stilborn, 1996). Using this approach, excess AA in the feed can be minimized, rendering the CP in the feed to become more desirable (Moran *et al*., 1992; Bregendahl *et al*., 2002).There has been evidence which showed broilers supplied withpoorCPfeedswith limiting AA fortificationhad performance similar to those diets provided with excess protein level (Moran *et al*., 1992). This might lower ration expensesobtainable in starter and grower broiler chickens (Noble *et al*., 1996). In recent times, limiting AAs (such as lysine, methionine, threonine and sometimes tryptophan) are cost-effectively incorporated in the preparation of chicken diets. Waldroup (2000) claimed that this would result in the production of greater number ofsynthetic AA for incorporation in compounding feed, contributing to further decrease in dietary CP levels.

Obviously the simultaneous combination of digestible values of AA, the principle of ideal protein, as well as greater accessibility and sustainability of certain additional AAs has caused crude protein to reduce to some degree, but the magnitude of reduction of CP is still remain uncertain even when maximum growth is being maintained. Report has indicated that reducing dietary CP without supplementing EAA is counterproductive to bird'sgrowth (Kerr and Kidd, 1999a), however, CP can be reduced efficiently to a leveltogether with incorporation of crystallineAAs and manifest in the same performance to conventional ration with greater crude protein concentration (Dean *et al*., 2006; Namroud *et al*., 2008).Nevertheless, a growing body of studies have reported reduced performance in low protein-fed animals, augmented by amino acid diets with minimal protein cuts of only 3 to 4 per cent in some cases (Corzo *et al*., 2005; Ospina-Rojas *et al*., 2012; Wang *et al.,* 2020). Series of reasons have been provided for the inconsistencies in performance between the studies.Discrepancies in CP concentrations, AA fortification, dietary ingredients, selected requirements for AA, age and strain of the bird may have made a contribution to most growth variations (Aletor *et al*., 2000; Dean *et al*., 2006).

2.7 Impact of dietry crude protein reduction on growth variables of broilers

2.7.1 Feed intake

Problem of ration consumption on reduced protein ration is crucial to growth rate of birds. There has been no impact or even improved consumption of feed in some studies,but in some trials; feed intake reduced with minimal dietary protein levels(Jiang *et al*., 2005). Obviously, in some scenarios, diminished growth success with lower dietary protein can be as a result of reduced feed consumption. Several results showed that feed consumption increased in early age because of shortage of any NEAA or poor NEAA to EAA ratio as the needs for protein during this time are high. Some authors found, however, no variations in consumption of feed with minimal dietary protein level(Araujo *et al*., 2004; Dean *et al*., 2006). Importantly,diet consumption relys on the feed's energy value, since birds consume mainly to sustain their calorieneeds (Leeson *et al*. 1993). The impact of energy in the ration on growth is reliant on the chick's ability to change rationconsumption with the difference in energy content of the feed.Similar feed consumption can therefore be anticipated if minimal CP feed with similarenergycontent are combined with adequate AA.

Several explanations that could change the consumption of feed from broilers given low CP diets are as follows:

1) Balance of electrolyte and potassium of the diet can affect consumption of feed through stimulation of water consumption especially during thermal distress (Aftab *et al*., 2006);

2) Net energy (NE) of poorproteinrations: The ratio of NE:ME is discovered to rise in diets with minimal protein levels.Due to lower heat gain, this increased ratio may result in increased feed consumption;

3) Variation in AA levels of reduced dietary protein: The amount of non-essential AA in low CP diets is decreased with diminished surplusEAA. Lack of several AAs can lead to higher consumption of feed;

4) Feed consumption that contains free AA may induce an accelerated / unsynchronized AA flow into the bloodstream. Feed consumption can decrease by constraining the composition of serum AA. Supplying minimal protein ration with steady energy:proteincan improve feed consumption of birds (Hidalgo *et al*., 2004).

2.7.2 Body weight gain

Early studies found that a 2 percent decrease in dietary proteinyielded no influence on weight gain (Parr and Summers, 1991; Moran and Stilborn, 1996). Providing broilers with minimal protein rations during the first 42 days did not have any deleterious effects on weight gain (Moran *et al*., 1992). It has been demonstrated that protein utilisation might be best with rations containing minimal protein levels(Temim *et al*., 2000; Zarate *et al*., 2003). Nonetheless, with low CP diets, some authors found decreased growth performance (Kerr and Kidd, 1999a; Aletor *et al*., 2000; Bregendahl *et al*., 2002). Therefore, it can be inferred that the use of synthetic AA will reduce dietary CP from NRC (1994) levels by 13-22 per cent.There has been evidence that when the amount of EAA is balanced as a result of the broiler needs, protein in the ration could be reduced drastically (Awad *et al*., 2017; Wang *et al*., 2020). Decreaseddietary CP levels might be higher as applied to the structure of the body when only the weight gain is considered (Srilatha *et al*., 2018).

2.7.3 Feed:gain

Previous findings observed lack of variations in feed:gain when birds fed reduced CPrationwere incorporated with adequate AA (Araujo *et al*., 2004;Rezaei *et al*., 2004). Higher feed consumption is believed to be attributed to some EAA deficits. Therefore, effectiveness of crude protein utilization is reduced. Digestibility of diets with AA may also vary due to different quantities of maize, soyabean and supplemental AA leading to varying availableamino acid and decreased consumption of ration and feed:gain.

2.7.4 Efficiency of nutrient utilization

With increase in nutrient costs, the quality of nutrient consumption for protein synthesis is just as critical as the growth or carcass quality in deciding best amounts in the feed. While efficacy of energy use was discovered to increase with reduced CP in the ration, studies on effective use of proteinwere less adequate.The effective use of protein has been reported to improve with rations with minimal protein level(Cheng *et al*., 1997). A decline in consumption capacity of rising quantities of usable protein seems to justify this maximum threshold of accretion of protein.On this basis, it is enticing to promote decreased dietary protein content, but negative effects on abdominal and carcass fat from low CP levels require taking into accountration with greaterCP levels, given the ratio is cost

beneficial.Nevertheless, lower CP diets should be addressed in cases where there is low calory ration, or where optimum feed protein returns are preferred over feed protein.

2.7.5 Carcass yield

Reducing the amount of dietary CP may not influence carcass attributes such as carcass quality, relative weight of breast and thigh. This might ba as a result of improved use of CP in well-balanced low protein ration, since protein efficiency can improve in low CP diets with supplemental AA (Si *et al*., 2001; Baker *et al*., 2002). Previous investigations confirmed that concentration of protein in the rationyieldeddid nothave influence on carcass attributes (Sterling *et al*., 2002, 2006). On the same note, it was proven that no variation has been observed on the quality of carcass in chicks givensmaller concentrations of protein in ration withsufficient supplementation of EAA (Moran and Stilborn, 1996). Nonetheless, in starting and growing phases of broiler chickens, carcass yield was reported to decrease considerably with minimal protein ration (Rezaei *et al*., 2004). Also, Kerr and Kidd (1999a) have demonstarted that the carcass yield of birds fed diet with lower CP concentrations combined with EAA and glutamic acid decrease substantially.

2.7.6 Breast meat quality

Early studies observed no variations in the breast meat quality when the concentrations of CP were decreased and adjusted to fulfil the demands of EAA in birds (Rezaei *et al*., 2004; Sterling *et al*., 2006). Early studies claimed that lowering the proteinlevels in the ration with AA supplementation did not influence breast meat quality of broilers (Kerr and Kidd, 1999a; b). It can be inferred that provided there is good balance of essential AA, particularly lysine and methionine, then reducing the dietary proteinhave no negeative impact on breast yield since it is extremely sensitive to amount of dietary limiting AAs(Si *et al*., 2001). Although, even when the EAA was at required concentrations, a marked reduction in breast yield trait with decrease in proteinconcentration was found (Moran *et al*., 1992).

2.7.7 Deposition of abdominal fat

The most negative impact of diets with minimal protein concentration is increased deposited fat at the abdominal region, which has the tendency to cause poor carcass content. It is anticipated that broilers supplied with minimal protein rations to store higher abdominal fat (AF) as a result of improved lipogenesis. Several authors found that the deposition of fatin birds consuming smaller volume of protein in their feed increased significantly (Smith *et al*.,

1998).The higher ME:CP is one of the major drivers causing elevated AF with reduced protein ration (Dozier and Moran, 2001).This higher energy availability in excess to that required for production of proteinresults to greater synthesis of lipid in the body of broiler chickens provided with minimal quantites of CP in the feed, thus, giving rise to higher abdominal fat deposition. On the contrary, previous studies confirmed that the quantity and yield of carcass sections were adversely affected in birds provided with rations compounded with reduced levels of protein and energy(Dozier and Moran, 2001).

2.7.8 Liver weight

In poultry, liver has been seen as the largest lipogenic organ. As a result of increased synthesis of lipid taking place in the liver of birds consuming diets with reduced CP concentrations, liver weights are anticipated to rise as a result of increase in caloric:protein ratio (Swennen *et al*., 2006). Conversely, some investigations have demonstarted that CP concentrations in rationsdid not affectrelative weights of liver in birds (Cheng *et al*., 1997; Sterling *et al*., 2006).

2.7.9 Nitrogen excretion

Several poultry scientists found a strong passion in decreasinglitter nitrogen ouptut and the quantities of ammonia released in recent times (Namroud *et al*., 2008; Belloir *et al*., 2017; Dietary protein above requirements induces an elevated heat and water consumption resulting in a high level of moisture in the litter (Moran *et al*., 1992; Namroud *et al*., 2008). During the process of removing supplus N a great deal of metabolic energy is waste, which is derived from energy required for development and synthesis of eggs which may lead to decrease in growth. Dietary manipulations are necessary to be adopted as techniques used to regulate ammonia emission (Ferguson *et al*., 1998). The best strategy and probably the cost effective way of regulating emission of ammonia is to decrease the quantity of excretedN from birds and reducing the concentration of dietary CP is beneficial in preventing N pollution (Ferguson *et al*., 1998; Namroud *et al*., 2008). High N losses in the excreta are primarily caused as a result of lack of protein ration to fulfil the demandsof AA inbroilers, especially inequalities arising from various AAs (Morris *et al*., 1999). As thepresence of EAA in the diet increases to reach the growth needs of the broilers, the less occurance of AA oxidation and excretion (Ferguson *et al*., 1998). In a study conducted by Ferguson *et al*. (1998), it was revealed that the amount of dietary CP can be decreased by almost 2% before adversely affecting the production process, provided EAA needs are met.This decrease in CP is

intended to cut down the concentrationexcreta and litter nitrogen, thereby minimizing the challenge of contamination and the ability of the resultant manure to contaminate the land and river bodies(Moran *et al*., 1992).

2.7.10 Serum Glucose

Previous experiment failed to find any dietary effect on serum glucose concentration (Swennen *et al*., 2006). In addition, dietary composition did not affect serum glucose levels, given the 33% higher intake of carbohydrates per physiological weight of chickens fed minimal protein diet (Swennen *et al*., 2006). Chickens providedration with minimal CP level typically utilised carbohydrates as energy source instead of fats leading to greater levels of free fatty acid.Nevertheless, the higher serum-free fatty acid concentrations in broilers fed minimal protein ration may be related to improved lipolysis for energetic needs, under fasting conditions. Consequently, the supply of carbohydrates would not control catabolism of lipid in chickens fed ration with minimal CP level (Rosebrough *et al*., 1999). The degree of dietary protein and in vitro lipid production had an inverse relationship and also, greater amount of protein in the feed reduced the absorption of glucose and elevated its production from metabolites initially required for production of fat (Yeh and Leveille, 1969).

2.7.11 Serum uric acid (UA)

The (UA) is the final metabolic product of proteins degradation in poultry animals. In higher protein-fed broilers, significantly lower serum uric acid concentrations are recorded and this has been validated by earlier research for broiler chickens fed ad libitum (Collins *et al*., 2003). There was a negative correlation between levels of serum uric acid and protein retention capacity and the concentration of uric acid in poultry suggests the extent of protein catabolism.Swennen *et al*. (2006) have stated that broiler chickens supplied with low CP diet, in addition to their improved protein retention ability, the oxidation rate of AA is decreased to save more protein.Therefore, it is believed that decreased protein degradation/AA oxidation results in more effective CP retention as a corrective factor for a diminished CP consumption. Under fasting conditions, variations under serum concentration of UA between chickens fed reducedprotein and high protein diets still exist, which suggests a saving impact on the body proteins of birds fed these ration with minimal CP level.Thus, earlier investigation found that birdsn fed on minimal protein ration greatly increased serum triglycerides, free fatty acids

and reduced levels of serum UA, while concentration of serum glucose were not influenced by composition of the diet (Swennen *et al*., 2006).

2.8 Strategies to reduce the dietary crude protein levels

2.8.1. Different optimum levels of essential amino acids

It is frequently debated that optimum concentration of EAA may vary between adequate and reduced protein ration, and this has been one possible explanation for decreased performance of broilers when minimal protein concentration is provided. Providing low CP ration with supplemental AA to increase the concentration of all EAA above recommendations did not achieve growth performance as attained with standard CP feed (Jiang *et al*., 2005). Previousfinding observed that combinations of EAA have been differed with the effect of higher growth efficiency partly but without fully resolving the hampered performance caused by minimlprotein content (Hussein *et al*., 2001). Impacts of EAA interactions with other feed nutrients may also have influenced growth which reduced along with CP (Namroud *et al*., 2008).

2.8.2Assessment of nonspecific non-essential amino acids

Impacts of non-specific NEAA were studied using various combinations of NEAA amounts in the feed.Some authors discovered that growth indice was not influenced when low protein ration was incorporated with thecombination of supplemental glutamic and aspartic acid,and also combination of supplementalalanine, aspartic and glutamic acid (Bregendahl *et al*., 2002; Nieb*et al*., 2003). Nonetheless, improvement in growth performance was observed with supplementation of a mixture of crystalline glutamic acid plus glycine (Deschepper and deGroote, 1995). Findings from the above research show that sufficient provision of unique NEAA is critical in broiers provided with ration low in protein quantities.

2.8.3. Specific nonessential amino acids

Experiments aimed at increasing NEAA-N by the addition of free glutamic acid could not restore the negative impact of growth encountered by feeding reduced protein feed (Kerr and Kidd, 1999a, b, c; Hussein *et al*., 2001). Previous investigations found that diets with 16 to 18% CP with Gly fortification to the rate of approximately 22% CP standard diet did not cause growth performance differences compared to standard feed, and supplementation of other individual NEAA could not improved performance (Dean *et al*., 2006; Awad *et al*.,

2015). Parr and Summers (1991) have documented no variation in growth rate of broiler chicks fed diets containing 20% protein combined with supplemental Gly and 23% protein diet. Several results showed increased performance when free glycine was applied to low CP feed(Yuan *et al*., 2012; Ospina-Rojas *et al*., 2013a, b, 2014; Awad *et al*., 2015; Hohman *et al*., 2019).

Glycine has been known for decades as a potential for growth promoter andsince the research of Dean *et al*. (2006) was conducted, it is now widely known that a glycine deficit in feed is one essential influence preventing the decrease of protein in chicken feed.According to Ospina-Rojas *et al*.(2012), Gly was indicated to become the first non-essential limiting AA as well as the 4th proteingenic limiting AA for broiler chickens (1 to 18 d) in corn and soybean based ration. Likewise, Val and Gly has been identified as being similarly limiting for broilers from 1 to 21 days after methionine, lysine, and threonine in diet formulated with vegetable ingredients (Ospina- Rojas*et al*., 2014).

2.9 Structure of Glycine

The word "glycine" came from the Greek word "glykys" signifying “sweet”, since they discovered that it was equivalent to glucose as per in sweetness(Wang *et al*., 2013). The structure was first established in 1857 (Figure 2.2).Glycine is a low-bulk protein-genic amino acid (AA) and has been regarded as theeasiest AA and lacks D- or L-configuration since an atome of hydrogen is bound to the alpha C-atom where most other AA are connected to a side chain (Wu, 2013). It's special in that there are no isomeric forms.Gly is the foundamentalAA in existence, and is an important component ofcollagen and elastin known as extracellular structural proteins (Wu 2013). Glyis typically considered as a nutritionally NEAA for humans, pigs and rodents because it’s endogenous production (Darling *et al*. 1999). Though mild inadaquacy of Glyis non-threatening to the animal life, a severe deficiency would lead to development of suboptimal body tissues, compromised immunity and several undesirable impact on nutrient utilisation(Lewis *et al*. 2005).

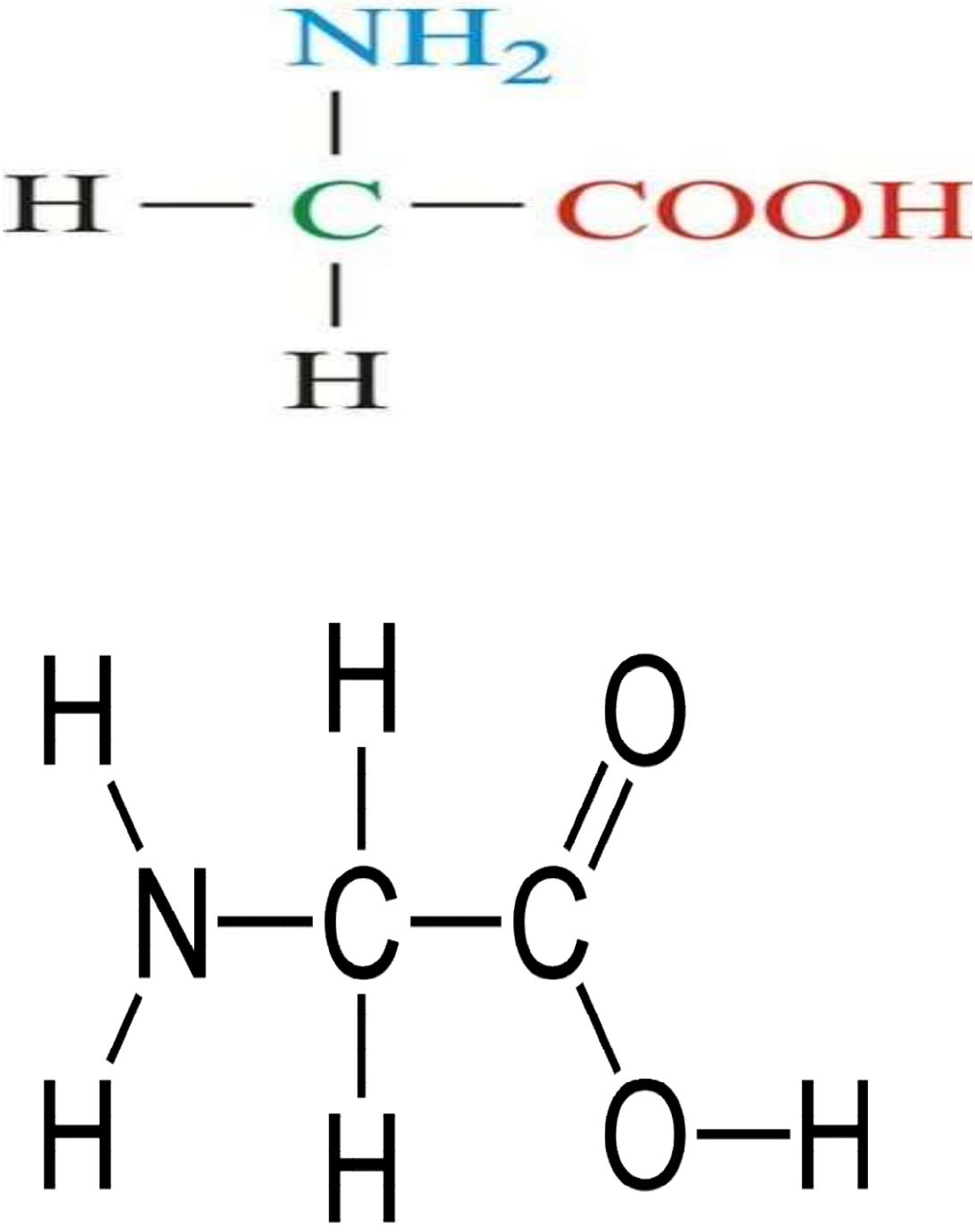

Figure 2.2: Structure of glycine

Source: en.wikipedia.org

2.10 Metabolic importance in utilization of amino acid Glycine

Glycine is needed as a precursor in many AAs and non-protein N pathways, given its primitive structure. Glycine is used for uric acid production in non-protein pathways to remove nitrogenous wastes and form blood haems. Glycine activity involves the production of collagen, keratin, elastin, and gut mucin in protein/AA anabolism. Both products contribute to the quality of food and gut interity. In fact, glycine is active in the synthesis of sulphur AAs (SAAs)-cysteine and methionine, and also metabolism of threonine and arginine.Structurally, only a single atom separates serine from cysteine, its hydroxyl oxygen is replaced to generate a thiol in the cycteine by sulphur.Glycine deficient birds exhibit muscle and nervous disorders at all stages of the body, suffer severe deficiencies in body weight and feather growth and these animals have reduced synthesis of hepatic protein, DNA and RNA as well as weak bone structure(Yuan *et al*., 2012).

Practical diets of corn-soya are considered to respond to additional inclusion of glycine. Previous study documented increased growth rate in impaired endotoxin broiler chickens suppiled with higher glycine levels (Takahashi *et al*., 2008). The involvement of glycine metabolic contributions and cellular participation suggest that it's a physiologically important AA, and potentially paticipate in controlling AA output, animal performance, and safety. A collection of Gly metabolic functions is shown in Table 2.1 which describes in part its nutritional necessity.

Table 2.1:Multi-functionalities of glycine in amino acid metabolism (Akinde, 2014a)

Final Product	Precursors
1. Metabolism of Nitrogen/Energy	
Production of steroids	Glycine
Traffication methyl group	Methionine+serine+glycine
Heme	Glycine
Sarcosine	Glycine
Uric acid	Glycine+glutamine+aspartate
Glucogenic AA, extrance into the	
TCAthroughPyruvate and serine	Glycine
Creatine	Glycine+ arginine+methionine
Bile salts	Glycine+taurine
Glutathione molecule	Glycine+cysteine+glutamate
DNA, RNAand purines	Glycine+glutamate+aspartate
2. Metabolism of Amino Acids	
Keratin, Collagen and elastin	Gly
Serine	Gly
Catabolism of Threonine	Gly
Total sulphur amino acid synthesis	Gly+Ser
Mucin proteins	Glycine+serine+threonine

2.11Synthesis of glycine in animals

Dietary and isotopic experiments have led to the discovery that animals synthesize glycine. Interestingly, these studies found that even though the diet lacks glycine, young animals can still grow.Meanwhile, rat-based biochemical studies found that glycine could be produced from:

(1) serine via serine hydroxymethyltransferase (SHMT);

(2) choline through sarcosine formation and

(3) threonine through threonine dehydrogenase

The identification ofthe three glycine synthetic route in other animals, including pigs, was identified in subsequent studies (Walsh and Sallach 1966; Balle ' vre *et al*. 1990). Studies have found that the precursors for production of glycine in animal are glyoxylate and hydroxyproline (Melendez-Hevia *et al*. 2009; Wu, 2014).

2.11.1 Production of glycine from serine

The SHMT is an enzyme that is pyridoxal phosphate- and tetrahydrofolate-dependent. It is primarily responsible for catalyzing the synthesis of Gly from Ser, which is obtained from the feed or biosynthesis from glucose and glutamate (Figure 2.3). It is readily available in the mammalian cells especially cytoplasm and mitochondria. The mitochondrial serine-hydroxymethyltransferase displays a key impactin the formation of the large quantities of Gly in several cells in the body, while cytosolic serine-hydroxymethyltransferaseis predominantly present in the kidneys and liver, and is not effective in the production of Gly from Sercompared to mitochondrial serine-hydroxymethyltransferase(Narkewicz *et al*. 1996; Stover *et al*. 1997; Girgis *et al*. 1998). In SHMT metabolised reaction, a C1 component is transformed to tetrahydrofolatefrom Ser C-3, generating Gly and tetrahydrofolate N_5-N_{10}-methylene (Stover *et al*. 1997). The tetrahydrofolate N_5-N_{10}-methylene is a methyl group source for certain reactions to methylation (Mudd *et al*. 2001). Most of these reactions lead to tetrahydrofolate being regenerated to assure its supply for synthesis of glycine from serine.

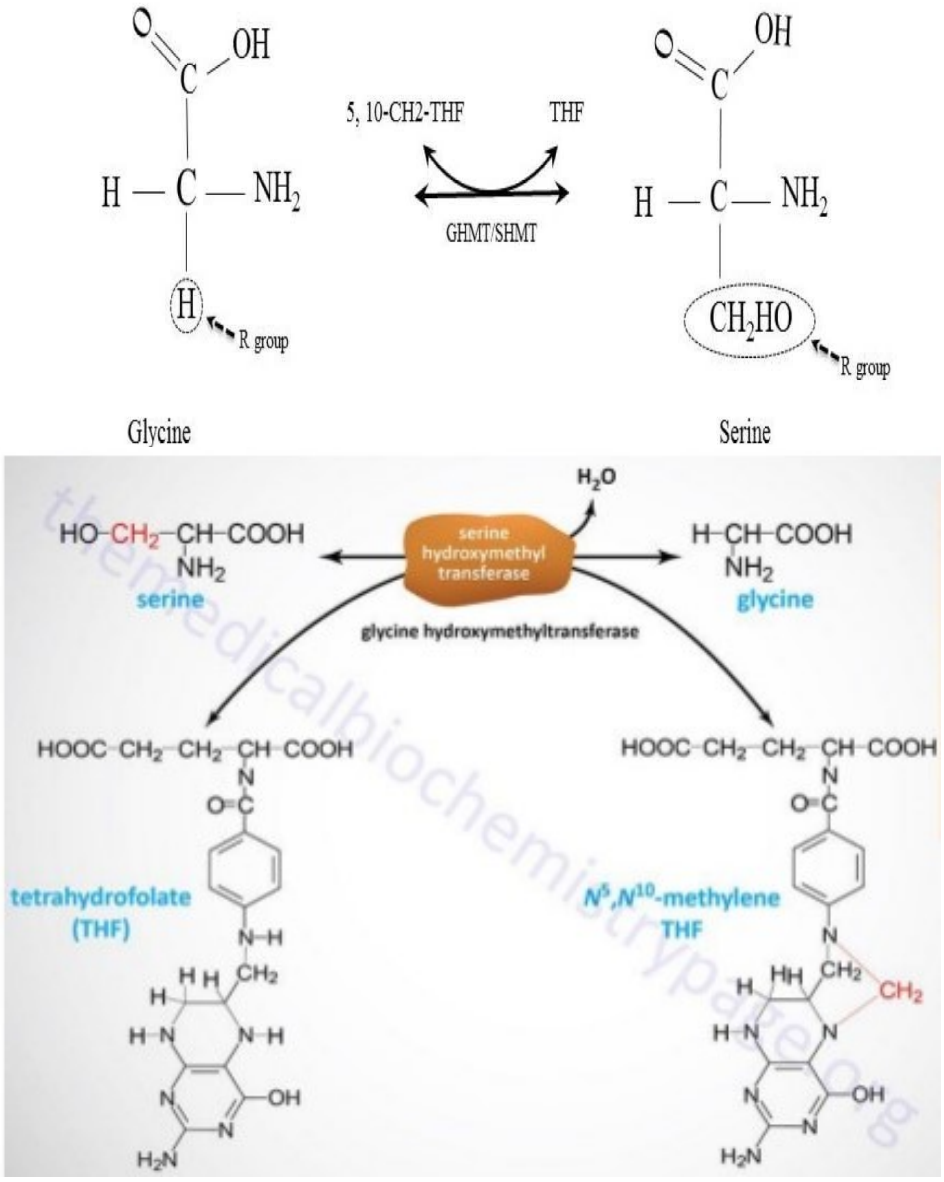

Figure 2.3: Gly - Ser bioconversion (5, 10-CH_2-THF, 5,10-methylenetetrahydrofolate; GHMT, Glycine hydroxymethyltransferase; SHMT, Serine hydroxymethyltransferase; THF, Tetrahydrofolate).

Source: https://themedicalbiochemistrypage.org/amino-acid-biosynthesis-and-catabolism/

2.11.2 Synthesis of glycine from threonine

It is claimed that SHMT degrades threonine to generate acetaldehyde and glycine (Lehninger *et al*., 1993). Nevertheless, it was explained that even when some species have low threonine aldolase activity in SHMT from the liver, both enzymes are unique in spite of their biochemistry and immunochemistry. Threonine dehydrogenase is the main enzyme in rats, pigs, cats and chickens to induce more than 80% threonine oxidation (Balle ' vre *et al*. 1990; Wu, 2014), having a by-product of Gly (Figure 2.4). Obviously, the measurable significance of glycine production from threonine may depend on the animal, the phase of development and the accessibility of diet. When threonine consumption is reduced in the diet, it becomes not a metabolic precursor of Gly in the animal (Le Floc'h *et al*., 1995; Wu, 2014).

2.11.3 Production of glycine from choline (Cho)

It has been observed that 40–45 percent of absorbedcholine can be oxidized to produce Gly, and efficacy can rise to 70% if the choline levels in the diet are poor (Soloway and Stetten, 1953). Metabolic breakdown of Cho into Gly induces methyl components for animal tissues (Wu, 2013). According to Zhang *et al*.(1992), following the Cho production into betaine by betaine aldehyde dehydrogenase and Cho dehydrogenase, the 3 groups of methyl-choline were accessible for processing:

(1) Homocysteine to Metto produce dimethylglycine via betaine

(2) Sarcosine (N-methylglycine) from dimethylglycine via dimethylglycine dehydrogenase,

(3) glycine from sarcosine via sarcosine dehydrogenase.

The last two enzymes are widely dispersed flavoenzymes in the mitochondrial [e.g., pancreas, lungs, heart, thymus, liver and oviduct (Bergeron *et al*. 1998)]. Glycine and sarcosin are interchangeable by conversion of methyl groups(Fig. 2.4). It should be remembered that since the dietary choline concentration is remarkably poor and participation to generate Gly in the body of animals is measurably small.

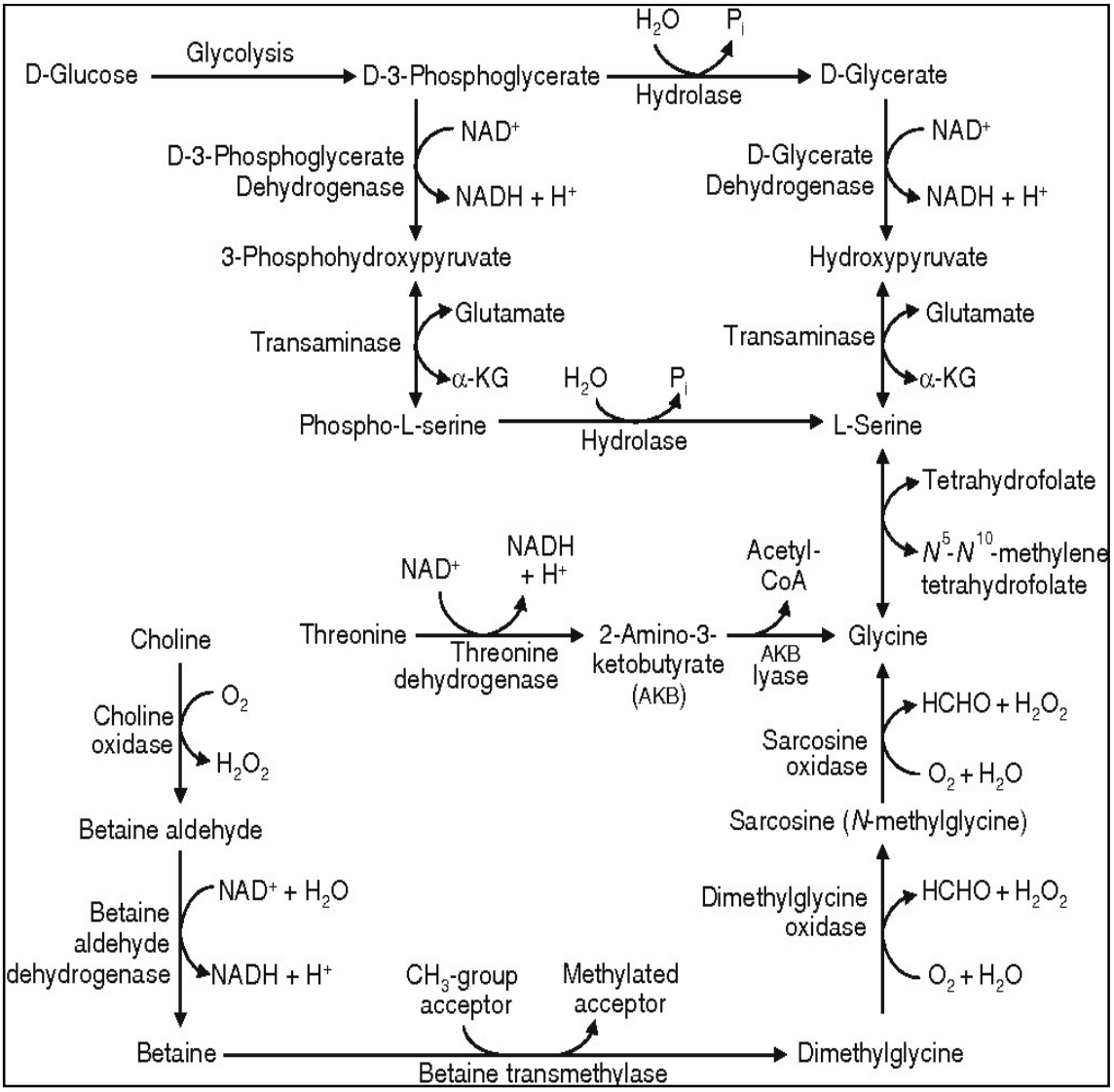

Figure 2.4: Glycine synthesis in animals

Source: Wang *et al.* (2013)

2.11.4 Biosynthesis of glycine from glyoxylate

An alternative precursor of Gly by transamination is glyoxylate which mainly uses alanine as a donor of amino group. Alanine:glyoxylate aminotransferase (AGT) as the key essential enzyme accountable for the almost permanent transition of the amino group arising from the from alanine to glyoxylate, producing Gly and pyruvate. Theyexhibit various proteins dependent on distinct metabolic features such as molar mass, isoelectric point, composition, kinetics andimmune system activities), however, possess simillar capacity to catalyze glyoxylate to yield glycine synthesis (Lee *et al*. 1995). The amounts of hepatic protein AGT1 are comparatively greater in primates, humans, dogs and cats, and lagomorphs such as rabbits, but quite minimal in lower animals such as guinea pigs, rats, hamsters, pigs, mice, goats, and sheep (Takada and Noguchi 1982). AGT1 is intracellularly expressed depending on animal species. In the mitochondrial, is the main hepatic AGT isoform enzyme present in rodents (guinea pigs, hamsters, mice and rats), cattle, pigs and sheep, is AGT2 (Takada and Noguchi 1982), and is present mainly in animal kidneys (Danpure1997).AGT2 is found at greaterquantities in the liver and kidneys of rabbits, sheep and pigs, but serves a small function in catabolismof glyoxylate in carnivorous animals (Noguchi *et al*., 1978).

2.12 Glycine Degradation in Animals

In the small intestine, a considerable amount of glycine is degraded (Wu, 2013). In animals,Glycatabolism takes place in three ways:

2.12.1Through the glycine cleavage enzyme system (GCS) via decarboxylation and deamination

Glycine is primarily catabolized by the mitochondrial GCS present in animals, plants, and bacteria through decarboxylation and deamination (Kikuchi *et al*., 2008). The mitochondrial GCS is the primary enzyme essential for body glycine catabolism and prevalent in the cell of the animal (Kikuchi *et al*. 2008). The GCS needs tetrahydrofolate whichis responsible for the spontaneous transformation of Gly into Ser (Figure 2.5).

The GCS catalyzes a totally reversible process, starting with the decarboxylation of Gly by P-proteinwith the involvement of H-protein being its first operation. Secondly, a T-protein deamines the decarboxylated moiety of glycine, creating dihydro-lipoamide. Thirdly, dihydrolipoamide is decreased by the dihydrolipoamide dehydrogenase (L protein) that is

nicotinamide adenine dinucleotide(NAD)-dependent and flavin adenine dinucleotide(FAD)-required (Figure 2.5). In fact, the GCS is known to be a very significant quantitative pathway for Ser degradationmostly inmammals(Kikuchi *et al*., 2008). The GCS provides single-carbon compounds from Gly oxidation for purine production in uricotelic animals such as chicken (Kikuchi *et al*., 2008). In the presence of serine dehydratase, Ser ismetabolised to pyruvate and ammonia (Berg *et al*., 2007).

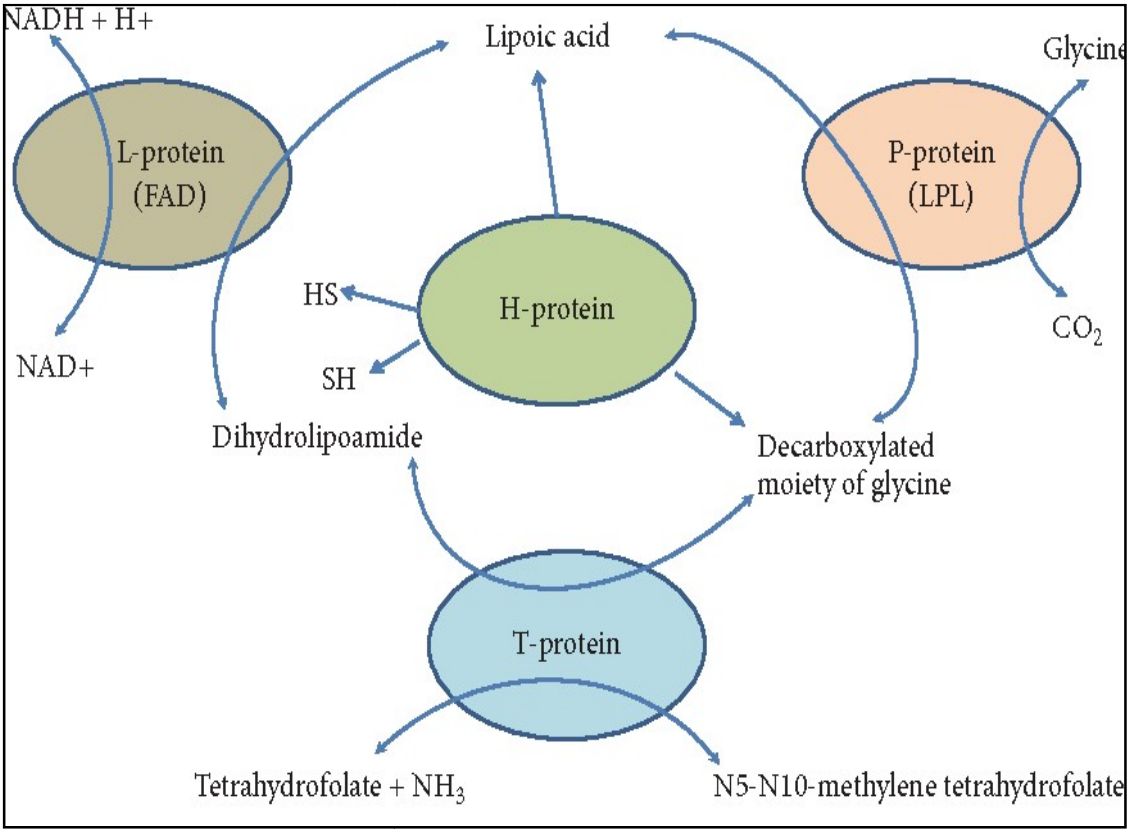

Figure2.5: Concurrent enzyme events in mammalian cells via glycine cleavage mechanism

Source: Wang *et al*. (2013)

2.12.2 Metabolism through conversion into serine by SHMT

SHMT is responsible for activating the reversible catalysis of glycine to serine synthesis and a single carbon unit provided by N5-N10-methylene tetrahydrofolate, as stated earlier. Almost 50% of GCS generated N5-N10-methylene tetrahydrofolate led to serine glycine synthesis (Lamers *et al*. 2007). Different pathways struggle for glycine and provide a diverse description of its usage by inter-organ cooperation (Wu, 2013). It could be assessed that because of the recovery of tetrahydrofolate, which is an important cofactor for SHMT, glycine is either transformed by SHMT into serine or reduced by GCS to ammonia and carbondioxide. Thus, GCS is paired with SHMT for the degradation of glycine (Figure 2.6). The GCS supports glycine degradation instead of glycine production from ammonia together withh CO_2 on the basis of substrates concentrations in the cell and products (Kikuchi *et al*., 2008).

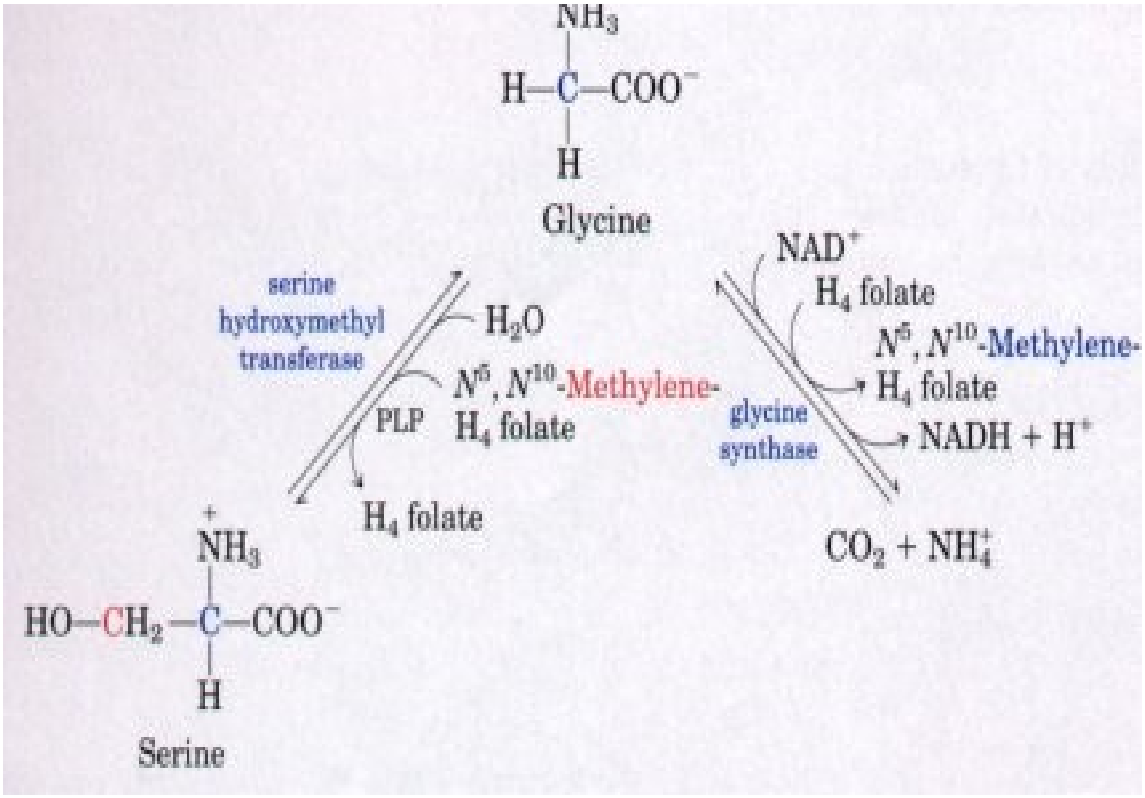

Figure2.6:GCS (Glycine Synthase) binding to SHMT for catabolism of glycine in animals

Source: Wang *et al*. (2013)

2.13 Physiological significance of glycine

Glycine plays vital functions in metabolism and nutrition of animals(Table 2.2).

1) Glycine in body proteins comprises 11.5 per cent of overall AAs and 20 per cent of AA-N (Wu, 2013). In growing animals, protein biosynthetic pathway provides for 80% of requirement of the total body glycine e.g. piglets (Brosnan *et al*., 2009). Glycine is necessary in a gelatinous composite protein (collagen) at each C-3 position, so that the helical structure arrangement of the protein would have the glycine fragment within the helix. Glycine offers the enzyme-binding sites flexibility(Yan and Sun, 1997).

2)Glycine use complex pathways for the production of creatine,hem, glutathione, serine, RNA and DNA (Hall, 1998). Creatine is often used in metabolizing systems of the muscle and nerve, while glutathione being considered as an effective antioxidant is found in cells. (Wu, 2014). Moreover, the production of DNA and protein as well as the multiplication of cells depends on purines, while for the oxygen and mitochondrial electron transport systems, heme containing proteins are essential(Dai *et al*., 2013).

3) Glycine is the primary AA used to conjugate mammalian bile acids (e.g., pigs and humans) and thus plays asignificant functionwith respect to degradation and uptake of fas and fat-soluble vitamins.

4)Glycine attenuates calcium levels via glycin-gated chloride channels in cells of leukocytes and macrophages, thus further controlling cytokine synthesis, superoxide production and immune responsibility.

5) Glycineas a neurotransducerin the central nervous system, regulates behaviour, feed cosumption and homeostasis of the whole body (Rajendra *et al*. 1997). In animal and human, glycine jointly performs a significant function in growth, immunity, development, metabolism, survival and cytoprotection.

Table 2.2: Glycine metabolic roles in animals and humans (Wu *et al*., 2013)

	Direct action of glycine or the functions of its metabolites
Direct action of glycine	Protein synthesis (particularly accounting for 1/3 of amino acids in collagen and elastin); inhibition of calcium influx through activation of the glycine-gated channel in the cell membrane; inhibitory neurotransmitter in the central nervous system; co-agonist with glutamate for *N*-methyl-D-aspartate receptor receptors; anti-oxidant; anti-inflammation; one-carbon-unit metabolism; conjugation with bile acids
Function of glycine metabolites Serine	Protein synthesis, one-carbon-unit metabolism, and gluconeogenesis; conversion into choline via a series of reactions requiring methionine; conversion into ethanolamine through formation of phosphatidylserine; synthesis of D-serine (a neurotransmitter) in the brain
Porphyrins and heme	Hemoproteins (e.g., hemoglobin, myoglobin, catalase, and cytochrome c); production of carbon monoxide (a signaling molecule); storage of iron in the body
Bilirubin	Natural ligand of aryl hydrocarbon receptor in the cytoplasm
Creatine[a]	Antioxidant; anti-viral; anti-tumor; energy metabolism in heart, skeletal muscle, and brain; neurological and muscular development and function
Glutathione[b]	Free radical scavenger; anti-oxidant; cell metabolism (e.g., formation of leukotrienes, mercapturate, glutathionylspermidine, glutathione-nitric oxide adduct and glutathionylproteins); signal transduction; regulation of gene expression; apoptosis; cellular redox; immune response
Nucleic acids[c]	Coding for genetic information; gene expression; cell cycle and function; protein and uric acid synthesis; lymphocyte proliferation; facilitation of wound healing
Uric acid	Antioxidant; the major end product of amino acid oxidation in avian species

Adapted from Wu et al. (2013)

[a] Requiring arginine, methionine, and glycine as substrates

[b] Requiring cysteine, glutamate, and glycine as substrates

[c] Requiring glutamine, aspartate, and glycine as substrates

2.14 Mechanisms utilizing products of Glycine metabolization (Figure 2.7)

1) In uricotelic species especially poultry,ammonia is rendered inert and metabolized as uric acid, as the key component of the N cycle for excretion (Kikuchi *et al*., 2008). When phosphoribosylamine synthesizes glycinamide ribotide, the production of a Gly molecule within each moiety of uric acid involves the creation of a purine ring. Furthermore, protein production and cell growth depend heavily on the proliferation of DNA that Gly needs to produce purines(Wang *et al*., 2013).

2) Gly and Arg form the main component of creatine. Creatine can be provided either directly by feed based on by-products from animal origin or by endogenous production that happens through a double-step reaction. The enzyme L-arginine: glycin amidinotransferase catalyzes the first step involving the reaction of Arg binding andglycine producing ornithine and GAA. It occurs principally in the liver, kidney and pancreas. The second step occurs in the liver and involves the methylation of guanidino acetic acid by S-adenosyl-L-methionine into the amidino group to generate creatine. Experiments conducted by Ospina-Rojas *et al*.(2013a) demonstrated that production of creatine rises in the muscle as glycine is supplied to the feed.

3) Majority of animals can not produce Cys in the body, but can synthesize Met (Berg *et al*., 2007). As intermediate steps, methionine is metabolized with S-adenosyl Met and S-adenosyl homocysteine to homocysteine. Ser is needed when cystathionine is produced by cystathionine β-synthase action from homocysteine.

4) Cholesterol in the liver produces the major bile salts which help to dissolve and absorb lipids and fat-soluble nutrients (Berg *et al*., 2007). The level of Gly- or taurine-conjugated bile salts varies between organisms. Incorporation of Gly in the feed has been proven to improve apparent digestibility of fat in poultry (Alzawqari *et al*., 2010), and as a result, increased the apparent concentration of metabolizable energy in diet of broilers (Ospina-Rojas *et al*., 2013a).

5) A molecule ofheme is generated from Gly andevery heme group is formed by dissipating eight Gly molecules (Meléndez-Hevia *et al*., 2009). The production of heme-

relatedcompounds such as myoglobin, hemoglobin or cytochromes, requires the active participation of Gly(Meléndez-Hevia *et al*., 2009).

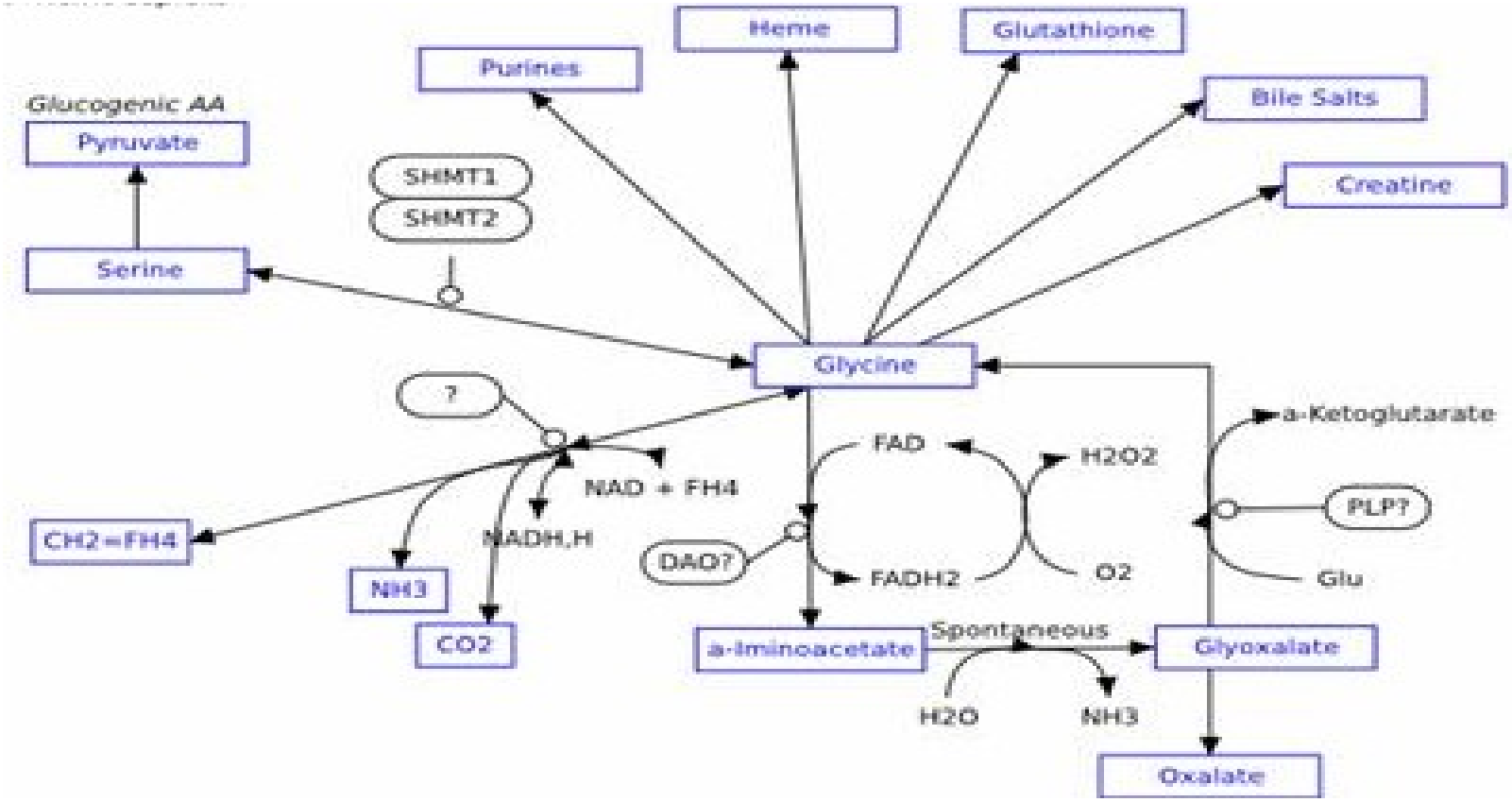

Figure 2.7: Pathways utilizing products of Glycine metabolization

Source: https://www.ergo-log.com/anticatabolic-effect-of-glycine.html

2.15 Main pathways involving glycine in health benefits.

Metabolic benefits mediated by glycine include the prevention of oxidative stress through increased formation of glutathione, an inhibitory effect on gluconeogenesis and food intake via activation of the N-methyl-D-aspartate (NMDA) receptor, curbing the overload. Glycine also shows positive influences on mitochondrial activity through synthesis of heme, detoxification processes via urinary excretion of glycine conjugates, and regulation of hormonal (enhanced secretion of key hormones in glucose homeostasis) and cytokine (reduced synthesis of pro-inflammatory cytokines) responses via activation of glycine receptors (GlyRs). Finally, glycine impinges the SAM biosynthetic process, lowerimg the of methyl-donoravailability, and thus regulating methylation. Favorable pathways induced by glycine are green; the harmful pathways inhibited by glycine are red (Figure 2.8).

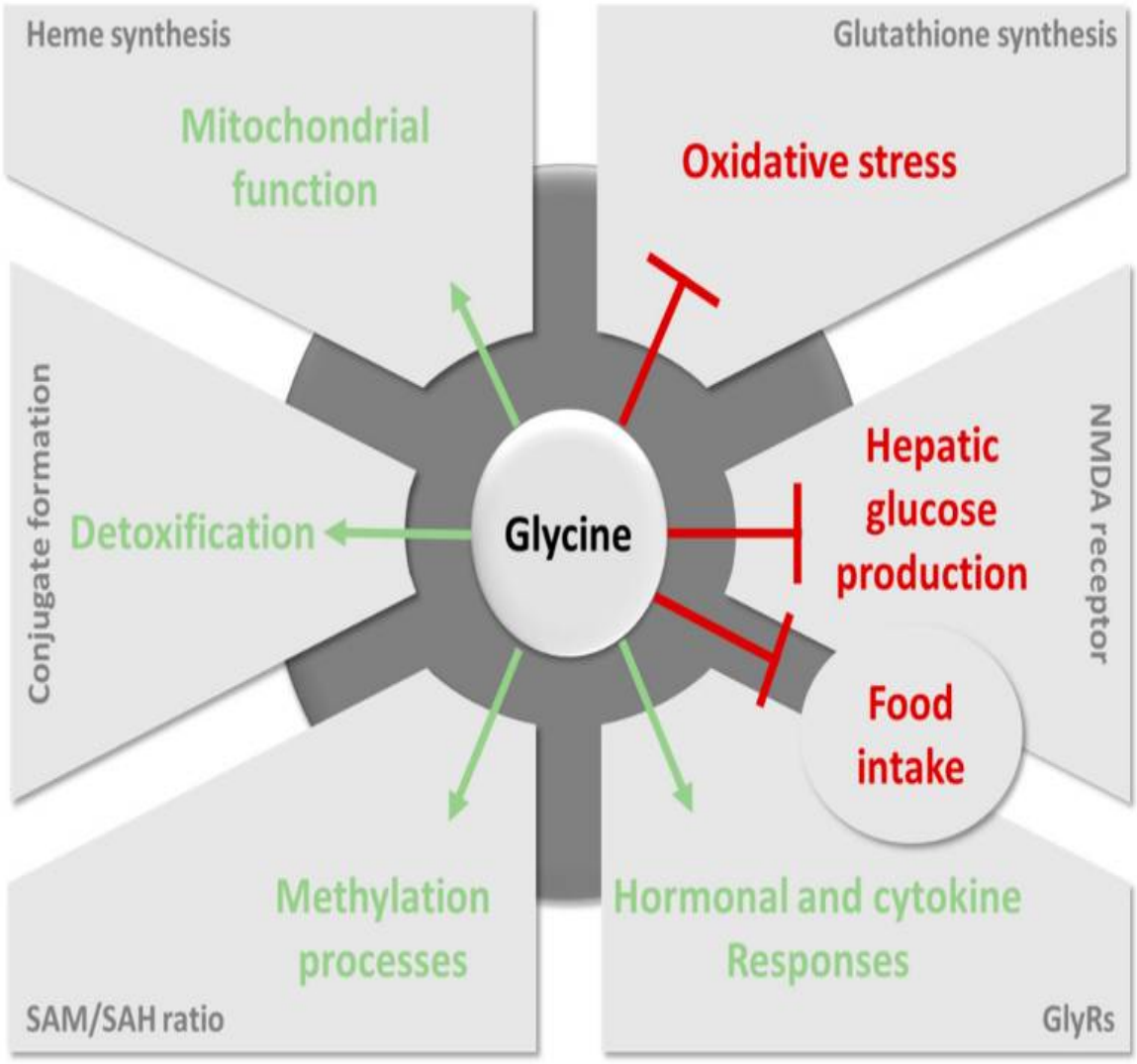

Figure 2.8:Main pathways involving glycine in health benefits**.**

Source: Alves *et al*. (2019)

2.16 Multipurpose channels of glycine metaboilsm

The major Gly participation in metabolic pathways of AA is shown in Figure 2.8. They involve regeneration of methionine and cysteine biosynthesis (transulfuration), production of creatine and threonine degradation. The binding of Ser-Gly and oxidation of Gly produces and transfers methyl groups via folate-mediated remethylation. Betaine as a source of glycine that can be gradually reduced to dimethylglycine (DMG), sarcosine and eventually glycine.

Serine reacts with homocysteine in transulphuration to produce cystathionine. Additionally, generation of cysteine and homoserine requires cystathionine, which has a bioactivity of threonine. S-adenosylmethionine's (SAM) substrate glycine and arginine plus methyl group represent the main precursors of creatine production. Although S-adenosylmethionine is utilised in donating ofmethyl group, Gly plus Ser make excellent methyl group acceptors and donors (Baker, 2006).It is the latter mechanism that explains why excess methionine can be neutralized and regulated by glycine+serine (Alves*et al*., 2019).

Methionine toxicity is important since crystalline methionine is incorporated to chicken diets to fulfill the needs of SAAs (Hafez *et al*., 1978; Namroud *et al*., 2008). This need for free addition of methionine has risenbecause to greater requirements for SAAs caused by genetic advancement in the breeding of broilers. Obviously, lethal methionine quantities are anorexic; decreases weight gain and impair the utilisation of AA (El-Wahab *et al*., 2015; Sigolo *et al*., 2019). Many researchers connected these disorders to buildupof homocysteine fragment in the blood (Namroud *et al*., 2008). Glycine is related to threonine as an end product of threonine oxidation.

The N excretion uses both arginine (through the urea cycle) and Gly (through uric acid). The AAs such as Gly, Arg, Cys, Ser and Thr regulate feather proteins contributing nearly 36% of AA metabolites(Stilborn *et al*., 1997).

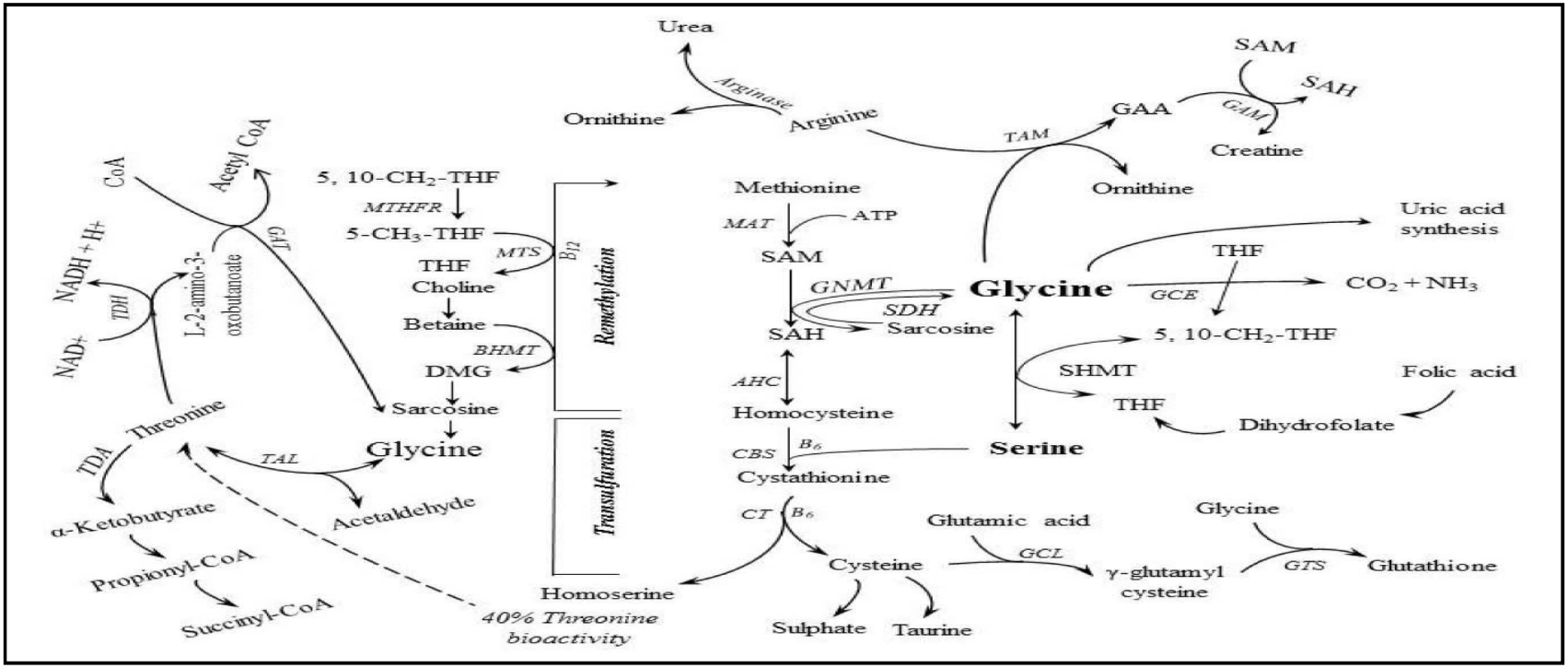

Figure 2.9: Specified amino acid metabolism via glycine pathways (Source: Meléndez-Hevia *et al*., 2009).

AHC, Adenosylhomocysteinase; CBS, Cystathionine βsynthase; CT, Cystathionase; GAM, Guanidinoacetate N-methyltransferase; GAT, Glycine C-acetyl transferase; GCE, Glycine cleavage system; GCL, Glutamate cysteine ligase; GHMT, Glycine hydroxymethyltransferase; GNMT, Glycine N-methyltransferase; GTS, Glutathione synthase; MAT, Methionine adenosyltransferase; MTS, Methionine synthase; MTHFR, Methylenetetrahydrofolate reductase; 5, 10CH2-THF, 5,10-methylenetetrahydrofolate; SDH, Sarcosine dehydrogenase; SHMT, Serine hydroxymethyltransferase; Threonine aldolase (TAL); Threonine deaminase/Threonine dehydratase (TDA); TAM, Transamidinase; TDH, Threonine dehydrogenase; THF, Tetrahydrofolate.

2.17 Relevance of glycine in the nutrition of total suphur amino acid (TSAA)

Supplementation of methionine in diets for poultry is necessary to prevent limitation in cysteine synthesis. Although crystalline cysteine is not a readily available supplement, sources of commercial methionine are added in surplus of the specifications in order to meet overall requirements for TSAAs (Namroud *et al*., 2008). Glycine may account for this cysteine restriction under specified circumstances; alternatively, these assertions can be backed by 5 theories:

1.Impacts on transulfuration-whether dietary glycine supplementation enhances the supply of serine for production of cysteine,consequently boosting the utilisation of sulhur AAs in the feed (Powell *et al*., 2009).

2.Remethylation impacts: In the first place, serine offers the methylsinvolved in methylation of homocystheine. Increase in serine pool in the cell mediated by glycine tends to encourage methioninere-generation (Pillai *et al*., 2006).

Use of supplemental Gly may increase the synthetic availability of choline / betaine, probably through a decrease in betaine oxidation.Enhanced betaine supply has shown to improve folate-basedhomocysteine reethylation efficiency compared to its corresponding betaine pathway (Stipanuk, 2004).

3. Improved ingestion of glycine can enhance the supply of methyl, specifically saving SAM / methionine for compulsory methyl metabolism.These situations are advantageous for elevated physiological needs, such as at thetenderage poultry animals or during stress periods.

4.Glycine can prevent the negative implications of excess or insufficient intake of methionine by improved conversion to cysteine or uric acid as excretory product (Namroud *et al*., 2008; Powell *et al*., 2011).

Glycine has likewise improved the cysteine efficiency for protein accretion. The researchers assumed that increased methyl supply that is obtained from glycine induced the sparing of methionine. Powell *et al*. (2009) questioned existing broiler SAA specifications, claiming that glycine+serine may compromise them. They observed that the amount of 2.32 per cent

dietary glycine+serine alleviated inadequate and surplus dietary SAAs. According to Powell *et al*. (2011), these effects were directly linked to improved production of cysteine, which is as a result ofbiosynthesis of serine, arising from Gly incorporation. The authors furtherreported a significant improvement in feed:gain when additional glycine was coupled with inadequate levels of methionine+cysteine unlike when cysteine was in adequate or surplus concentration.

2.18 Influence of sulfur amino acids (SAA) in protein/AA metaboilsim

Amongst the SAA, methionine is a significant AA that must be included in the ration of birds and is considered as the first critical factor in traditional ration adopted for rising birds (Baker, 2006). Diminished birds's performancegiven rations containing supplemental methionine and cystine was mainly attributed to reduced levels of whole-body protein production linked to decreased RNA production, indicating translational control. The beneficial impact of supplemental Met has also been documented on muscle development, as the incorporation of Met to a Met-deficient feed, otherwise supplemented with respect to other AAs, improves muscle growth and protein synthesis in pectoralis muscles of birds (Barnes *et al*., 1995).

Like methionine, cysteine serves a vital functionfor the production of proteins, but it is formed via the Met metabolic pathway, thus being known as a NEAA. However, the need for SAAmay be fulfilled from M*et al*one, this is accomplished greatly by mixing methionine with cyst(e)ine (cysteine and cystine). In early stage of broiler growth, cyst(e)ine in the diet may replace part of the demand for methionine (Shoveller *et al*., 2003), indicating the relevance of both methionine and cyst(e)ine, in the provision of SAA for production of protein. In a situation such as adverse disorders or toxic states, cysteine is deemed "provisionally essential." In such cases, the need for cysteine is intensified, and this need for cysteine often approaches the body's ability to metabolize it. The biochemical flux and interconversion concentrations have been examined to have a better grasp of the needs of SAA. For reference, acute rat inflammation enhances the formation of a large antioxidant compound called GSH in different tissues such as liver, spleen, lung, skin, etc., with such production responsible for less than 40 per cent of increased use of cysteine during inflammation (Malmezat *et al*., 2000). Therefore, cysteine degradation by sulfate synthesis is substantially reduced, indicating also that cysteine is saved to produce GSH (Malmezat *et al*., 1998). In fact, cysteine is widely used in the liver of septic rats for the creation of taurine and

severe-phase proteins engaged in defense mechainsim of the animal (Malmezat *et al*., 1998; Obled *et al*., 2002). Favorable metabolism of the methionine against cysteine formation was thus detected, indicating an increased demand for SAA in these cases. Interestingly, DL-HMTBA is more easily transformed to Cys and Tau than L-Met, indicating that this Met hydroxy derivative may play a better function in detoxification mechanisms than Met itself (Martin-Venegas *et al*., 2006).

By occupying various roles in protein metabolism, Met + Cysoccupyan important positions between AAs. These are, like other AAs, the constituents of tissue proteins, and thus act as metabolites for protein production. Methionine, for instance, contributes in oxidation and formation of other amino acids in the methyl group, especially cysteine (Figure 2.9). Cysteine is needed for GSH and taurine production, which are vital metabolites for protection of the host against oxidative damage.

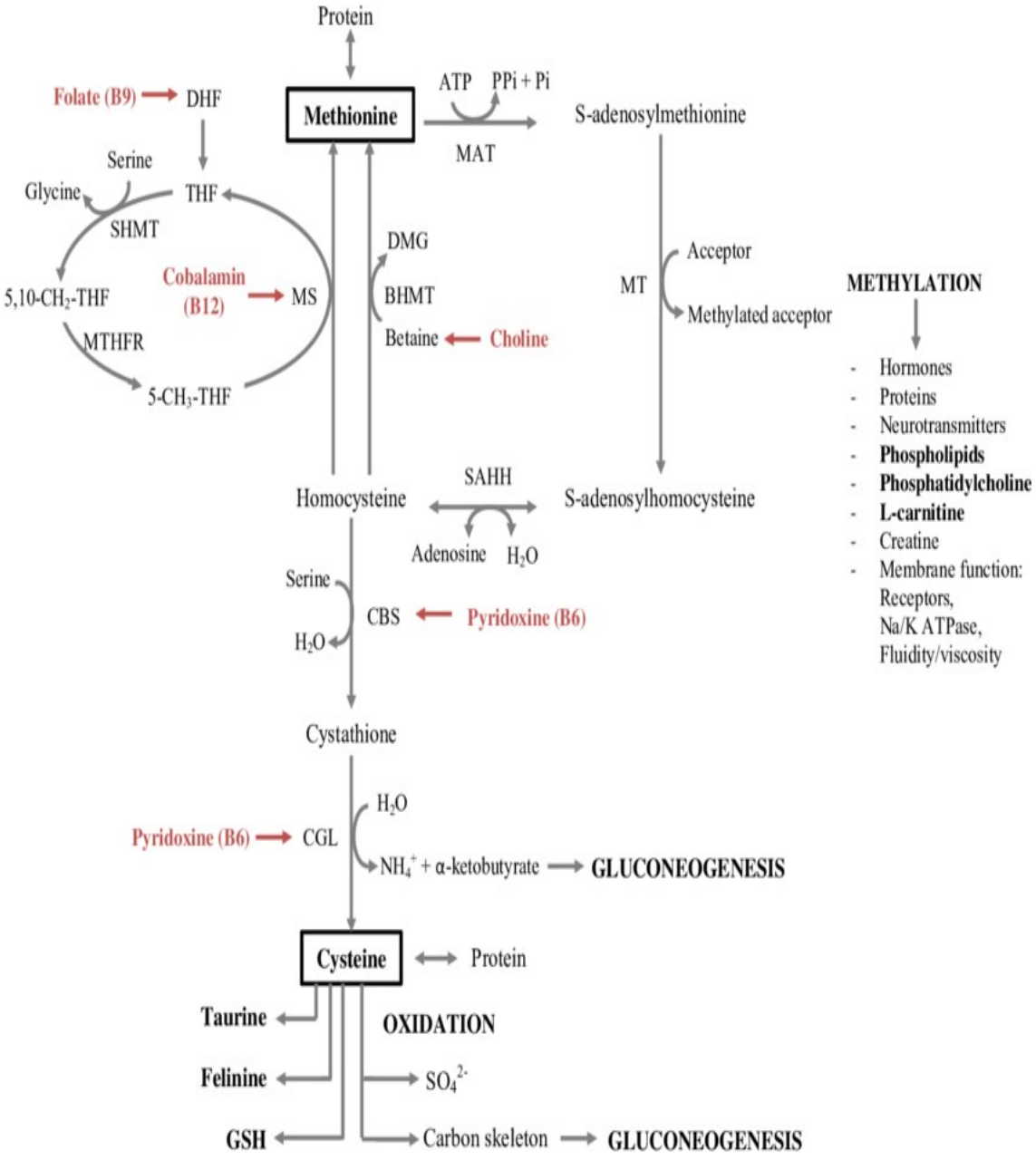

Figure 2.10: Pathways of methionine–cysteine metabolism.

Source: Brosnanand Brosnan (2006)

2.19 The involvement of the sulfur amino acid (SAA) in regulating oxidative condition

Sulfur derivatives such as cysteine, taurine and GSH, play an important function in oxidative damage situations as they are capable of affecting cellular redox status (Shoveller *et al*., 2005) (Fig. 2.10). In turn, the most effective cellular antioxidant in the body is GSH, which is a tripeptide (L-glutamyl–L-cysteinyl–glycine). The GSH and Cys can serve as immediate reactive oxygen species (ROS) scavengers. The ROS and hydrogen peroxide are produced at the mitochondrial stage and might have damaging impact, example, oxidation of lipids and proteins and disruption to the DNA strand-break, influencing biological processes. GSH peroxidase induces the GSH-dependent reduction of hydrogen peroxide, as outlined by Obled *et al*. (2004). Accordingly, GSH is metabolized to a GSSG dimmer, which is either converted down to GSSG reductase or removed from the cell. The GSH and Cys can also shield proteins from permanent oxidative deterioration by reacting with these thiols and proteins and by forming blended disulfides, such as glutathiolated proteins (protein-SSG) (Mallis *et al*., 2002). Moreover, there has been an observation that Met metabolites in proteins can serve as catalytic antioxidants through the mechanism of "Met sulfoxide reductase" (MSR) (Figure 2.10) (Moskovitz, 2005; Weissbach *et al*., 2005). Methionine metabolites are specifically vulnerable to ROS degradation and transformed to methionine sulfoxide (MetO) with the synthesis of two stereoisomers, known as MetO-S and MetOR. Each sequence of hydrolysis and deterioration of methionine will eliminate one analogue of ROS, which could be a huge natural scavenging mechanism for ROS. Pamplona and Barja (2006) and hasconfirmed an impact of methionine concentrations in the feed on oxidative damage and durability; a reduction in the quantities of methionine seems to minimize the susceptibility of proteins to oxidative damage and ROS formation.

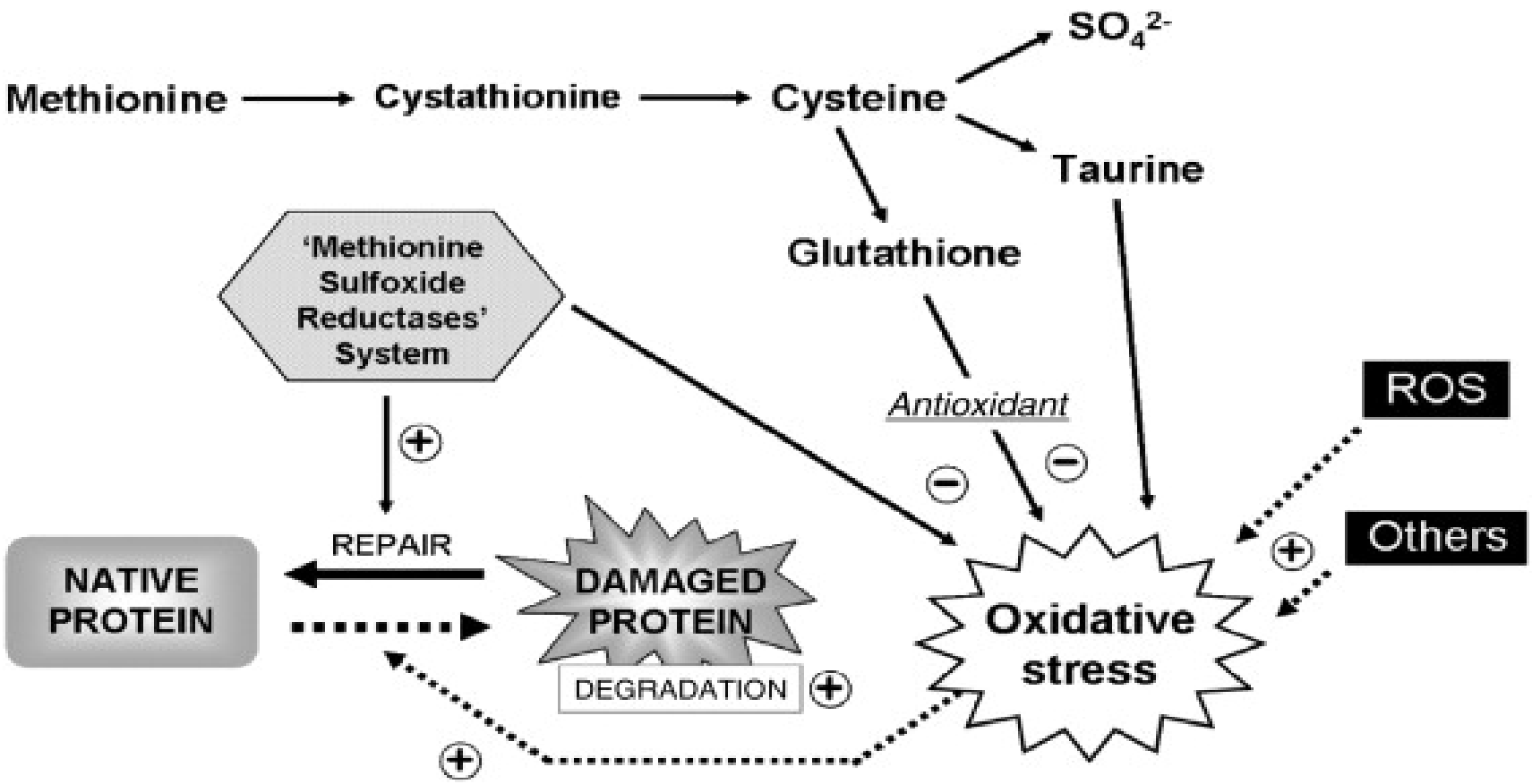

Figure 2.11: Involvement of sulfur amino acids in the regulation of oxidative state

Source: Metayer*et al.* (2008)

2.20 Relationship between threonine and glycine

According to Corzo *et al*. (2009), the metabolic associations between Gly and threonine are possible becuase threonine (Thr) is a source of Gly. However, data trends indicate that sufficient intake of glycine in a situation of Thr deficiency improved growth rate by 3-7%adopting parameters such as body weight gain or carcass traits. The Thr supply is useful in glycine deficiency conditions. Nonetheless, broilersprovided with a Gly-deficient but Thr-adequate basal diet responded positively to Gly supplementation, suggesting that Thr can not substitute all glycine functions (Ospina-Rojas *et al*., 2013b). Glycine and acetaldehyde reverse synthesis of threonine has been considered inconsequential, as previous experiment from Baker *et al*.(2002) shown that Thr aldolase is almost unavailable in poultry.

The biological activity of Thr is potentially viable for Gly under circumstances of sub-optimal threonine utilisation. A typical illustration of the scenario is encountered when diets of high CP levels are usually fed to startingbroilers. The mechanistic justification is that large N consumption causes excessive degradation of Thr to Gly as a metabolic consequence of excretion by nitrogen in form of uric acid. In these circumstances,rapid activities of Thr degrading enzymes were reported (Ciftci and Ceylan, 2004), and indicating strong correlationship between Thr demand and nitrogen consumption, thus,signifyingThr cataboilism.Therefore, supplementary Gly may be suggested as a boost to increasing the efficiency of threonine metabolism for development benefit whenever high protein feed is provided. Several factors that restrict the use of threonine against which glycine can provide relief are identified during the tran-conversion of ingested threonine into mucinproduction, intensified by increased intake of nutritional fibers or under stress situation. Similarly, this is also applicable when threonine is utilised during disease / immune stimulation to produce immunoglobulin and acute phase proteins (Kidd, 2000).

2.21 Nutritional interrelationship of Glycine and Serine (Gly+Ser)

In a reversible one-step reaction, glycine can be synthesized from serine. It is usually believed that Gly and Ser biochemical conversion in chickensis not to be restricted but continuous (Sugahara and Kandatsu, 1976). The amounts of Gly+Ser in feed have same impact on growth efficiency in as much as the same molar amount is considered.Glycine and serine are thus typically measured together. As commonly practice at present, most scientists take into account the analog influence of Glyplus Ser. According to Dean *et al*. (2006), the

physiological significance of glycine and serine joint impact was defined by measuring the Glyc analog (Glyequi) as amount of glycine and molar Glyequi serine, measured as follows:

$$Gly_{equi}\ (gkg^{-1}) = Gly\ (gkg^{-1}) + [0.7143 \times Ser\ (gkg^{-1})]$$

2.22 Growth performance of broilersin responses to dietary glycine/glycine+serine

Dietary Gly supplementation potential has been recognized for enhancing growth rate and has been widely accepted that inadequate Gly+Ser in the rationhinders the capacity to lower CP concentrations inpoultry (Waguespack *et al*., 2009).The Gly+Ser has been identified as the 4th limiting of protein-genic AAs after methionine, lysine and threonine in poultryat early stage of growth fed plant based diet as well as the the first limitingNEAAs in poultry (Waguespack *et al*., 2009; Ospina-Rojas *et al*., 2014).With respect to the acceptance of the significance of Gly+Ser in the feeding of broilers, numerous dose-response investigations have been conducted evaluating the impact of dietary glyequi, although the findings were not compatible.Variations in broiler age may have led to discrepancies, as the demands for amino acid typically decline with age when presented as dietary levels.Nonetheless, it is difficult to compare suggested dietary Gly+Ser concentrations since itsneveraccounted for the levels that induced optimum response or estimated the level required for a particular optimum response proportion (Waguespack *et al*., 2009).The vast array of dietary Gly+Ser concentrations at a 95 percent optimum gain:feed showed that variables affecting the impact of Gly+Ser in rations are important in the feeding of broilers (Dean et al., 2006; Wang et al., 2020).

2.23 Factors affecting the Gly+Ser (Glyequi) response

2.23.1 Impact of metabolic needs of Cysteine

Powell *et al*. (2011) observed that Glyequi's impact on feed efficiency was clarified by Cys production through Met in the presence of Ser and they also recorded improved feed efficiency on the incorporation of supplemental Gly to ration having sufficient Met+Cys, but with inadequate Cys.Additionalsupplementation of Met negatively affected growth rate; however, introducing Cys above the specification of Met+Cys decreased growth enhancing potential of Gly supplementation. Lowered levels of CP are sometimes attributed to the addition of raw feed materials. Since Cys is not generallyincorporated in feeds, the proportion of Met+Cys is doubled, following incorporation with crystalline Met or analogues, irrespective of any particular requirements for Cys. According to Siegert *et al*. (2015b), the

impact of the Met+Cysratios on the dietary Glyequi response was calculated. Consequently, the authors reported that Met+Cys ratios have significantly enhanced the impact to Gly+Ser in feeds, according to this study (Figure 2.11). This effect had implications for the concentration of Glyequi needed to achieve a full benefit of 95 per cent gain:feed. MeetingMet and Cys requirements lowers the need for transformation of Met to Cys.In addition, the synthesis of one cystathionine molecule into Cys molecule in the form of ammonia creates an excess of one molecule of N (Meléndez-Hevia *et al*. 2009).In a Gly-sparring reaction, ammonia is generally detoxified into uric acid. Conversely, ammonia may be used to synthesize de novo NEAAs or other nitrogen related-substances, base on the animal's physiological and nutritional condition. Therefore, Met molecule not transformed to Cys decreases the need of Gly+Ser within the limit of one to two molecules of Glyequi.

2.23.2 Impact of endogenous sources of glycine and serine

Several metabolites have been identified as precursors of Gly or Ser. Threonine and choline possess the greatestefficacy to serve as internalsources of Gly/Ser (Meléndez-Hevia *et al*. 2009). Thr may be transformed to Gly through double metabolic channels. The first is the direct degradationyielding Gly with acetaldehyde. The other method is an intermediate step by transformation to 2-amino-3-ketobutyrate, which reacts further with Gly, acetylCoA, and aminoacetone (Davis and Austic, 1994). Findings have revealed that the second method is the principalroute representing approximately 80 percent of the Thr transformation to Gly in chickens, pigs, and rats (Davis and Austic 1994). When homocysteine is readily accessible (Soloway and Stetten 1953), choline as another precursor, can undergo a 5-step reaction in the liver and transformed to Gly, having dimethylglycine, betaine, betaine aldehyde and sarcosine as intermediates.Glyoxylate, however, is irrelevant in birds, and the prospective amount of Gly produced from trimethyl lysine is very minimal (Meléndez-Hevia *et al*. 2009).

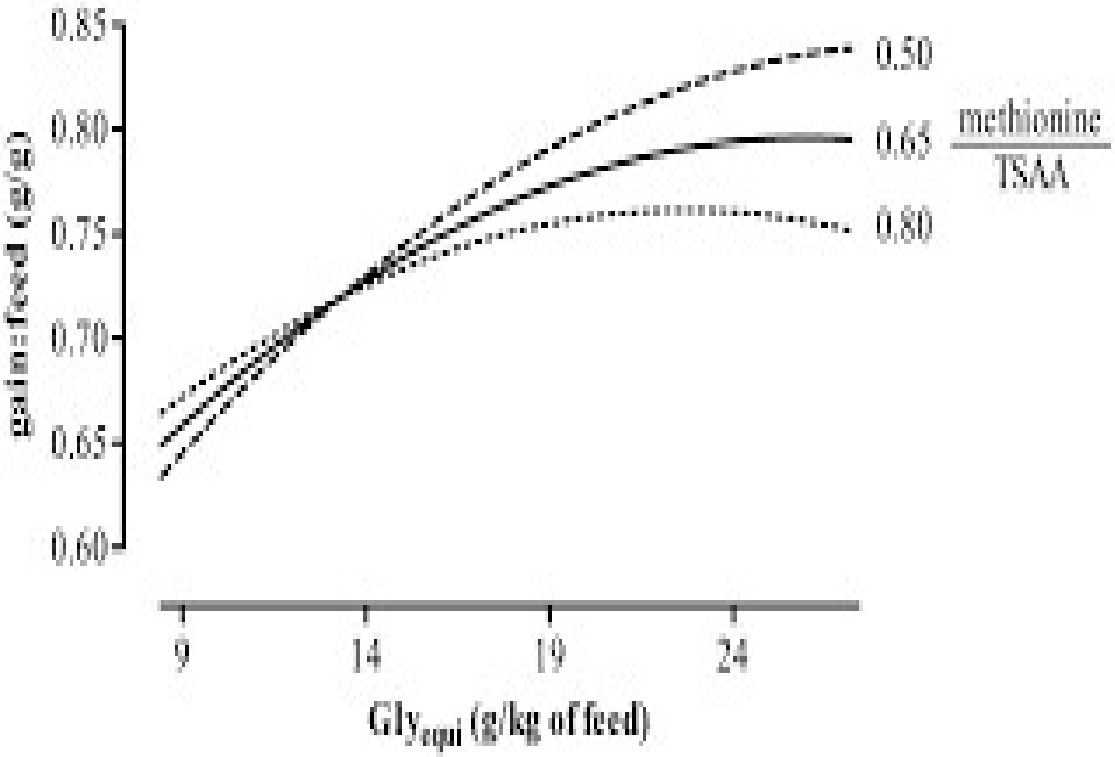

Figure 2.12:A meta-analysis evaluation of response of broiler chickens to methionine/(methionine+cysteine) ratios on gain: feed ratio(Source: Siegert *et al*., 2015b).

2.23.3 Impact of threonine

Relationships between Glyequi and Thr were identified in the feeding experiments and quantified by Lambert *et al.*(2015) and Siegert *et al.*(2015a) (Figure 2.12). Increasing amounts of dietary Thrs have been proven to lower the levels of dietary Glyequi needed to achieve those levels of response The substitution effect was almost consistent for daily weight gain over the calculated range of Glyequi and Thr levels (Siegert *et al*., 2015a), while the replacement benefit showed no linear relationship for gain: feed (Lambert *et al*. 2015). Theinfluence of substitution can not be traced directy to Thr's endogenous conversion to Gly. Single Gly molecule can be synthesized from a molecule of Thr. The Ospina-Rojas *et al*. (2013a) analysis simply suggested that a replacement value had to surpass 0.63. According to Siegert *et al*. (2015a), the substitution values ranged from 0.45 to 1.00 at 95 percent of optimumfeed conversion ratio.This discrepancy in Thr-limited diets is obviously to be due to the relative surplus of several AAs. Rising Thr levels in the feed possibly decreased the degradation of AA (apart from Thr), thus, decreasing the necessity for Gly+Ser in the synthesis of uric acid. When measuring a conversion factor dependent on the response of broiler chicken, the Glyequi-sparing influence can not be excluded via the metabolic transformation of Thr to Gly.

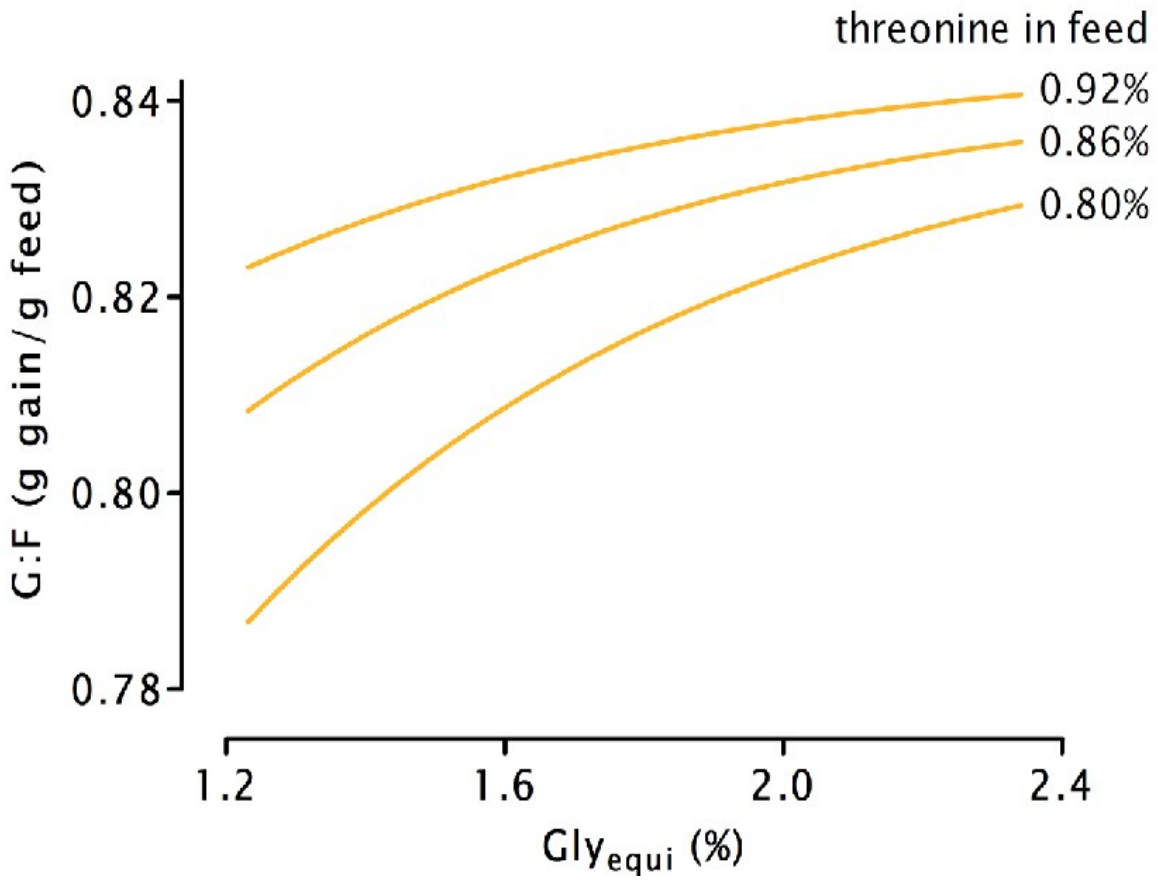

Figure 2.13: Influence of threonine levelsto dietary Gly+Ser on gain:feed ratio of broilers (7–21 d) (Source: Siegert *et al*., 2015a).

2.23.4 The impact of Choline and intermediate products on metabolic route of choline-glycine.

The impact of choline on Gly+Ser response was lowercompare to Thr, however, there exist some substantial effect of choline on the levels of Gly+Ser and Thr required to notice those levels of performance (Hofmann *et al*., 2020). Since the synthesis of Gly from choline generates betaine and dimethylglycine as intermediate compounds, it seems possible for there exist a correlation between betaine or dimethylglycine and Gly+Ser; nevertheless, relationships between these two intermediate metabolites and Gly+Ser have not been studied at all. As noted earlier for Thr, choline's endogenous metabolism to Gly only can not define the proportion of converting choline in the diet to Gly+Ser recorded by Siegert *et al*. (2015a). Metabolically, the transformation of one choline molecule into one Gly molecule is feasible. If choline is entirely transformed to glycine, 1% choline may justify up to 0.54% of glycine being substituted (Siegert *et al*., 2015a). Besides, it was not unidirectional to have the influence of choline fortification on levels of Gly+Ser required to produce exactlevels of response. Gly+Ser levels required to achieve optimum thresholds reduced with higherincorporation of choline at a particular concentration based on Thr level; the needed quantities of Gly+Ser increasedwith increasing supplemental choline (Dilger *et al*. 2007. Certain mechanisms which are not yet understood therefore may be the source of such observations. In this process, choline can not be synthesized from Gly through reversible reactions, since choline betaine formation is irreversible (Siljander-Rasi *et al*. 2003).However, in a nine-step reaction, choline may be produced from Ser through another metabolic pathway. There is no detail on potential drawbacks of the Ser-choline metabolism, according to the available literature (Siegert and Rodehutscord, 2019). Mostly unclear are the nutritional effects on other metabolite roles implicated in the biochemical pathways of choline-Gly and Ser-choline andcertain roles include ability of choline and its intermediate metabolites to act as donors to methyl groups (Meléndez-Hevia *et al*. 2009).

2.23.5 Impacts of glycine, arginine, guanidino acetic acid on synthesis of creatine

Several studies in poultry have evaluated the interrelationship among creatine, Arg, GAA and Glymixtures on growth rate and creatine levels. It involves relationships with arginine and Gly (Savage and Deo, 1989), arginine and GAA (Wu and Morris, 1998; Dilger *et al*., 2013), creatine and arginine,Gly and creatine, and GAA and creatine, respectively (Savage and Deo, 1989; Wyss and Kaddurah-Daouk, 2000; Mitchels*et al*., 2012). Consequently, influence

ofone of those criterias rely on the other's concentration. There is no evidence available that explains combined interacting impact of two or more components from dietary Gly, arginine, GAA, and creatine, based on recent information. Creatine is regarded as a non-suitable feed ingredient, despite its high metabolical activity, because of its volatility and high cost of production. The precursors of creatine namely glycine, arginine, and GAA, possess more desirable features with respect to durability and cost (Baker, 2009). In an experiment conducted by Dilger *et al*. (2013),a reducing trend ofarginine was observed in the feed when there isinclusion of GAA in the diet.The dietary Glyequi intake may be decreased when the Arg demand is lowered with a supplementation of GAA (DeGroot *et al*., 2018). The proportion of replacing arginine with GAA supplementation may be somewhat affected by dietary Glyequi concentrations as Gly restricts the production of GAA (Dilger*et al*., 2013; DeGroot *et al*., 2018). Thereby, quantitative assessments of interactionsamong Gly, arginine, and GAAmay provide more understanding to the influence of dietary Gly.

2.24 Efficiency of nitrogen utilisationand glycine plus serine in low crude protein ration

In formulation of poultry diets, Gly+Ser consideration will greatly increase the efficiency of N uilization in diets that are sufficient for EAAs. Diets containing 17–24 percent CP may result in better N utilization varying from 50 to 65 percent. Published studies have shown that reducing the quantities of CP in the feed, the efficiency of using N tends to increase(Belloir *et al.,* 2017; Kriseldi *et al.,* 2018). Changes that occurred among trials and withintreatments in one study were large, and are possibly based on level of one amino acid such as Gly+Ser, and variations in AA needs of the bird. N usage output of 70–71 percent (Hofmann *et al.,* 2019) and 75 percent (Siegert*et al*. 2016), together with high growth success between the ages of 18 and 21, was recorded in Glyequi with appropriate diets comprising 16.2 and 16.7% CP, respectively. In both experiments, the quantities of total critical AAs were modified by supplementing free AA for compliance with the suggested AA status stated in experiments examining single EAAs, when growth output was best, N utilization efficiency was not at its optimum and concentrations of AA necessary for maximum efficiency usage were smaller than those needed to optimize amino acid synthesis(Lemme *et al*., 2020).Quality of N utilization was higher when there are shortages of NEAAs in the feed (other than Gly+Ser), though there was decreased in growth rate (Hofmann *et al*., 2019). Such authors claimed this was attributed to the possible production of NEAAs utilizing reabsorbed urine N, and reduction in loss of tissue proteinwith respect to adjustment ofinadequate AA.

2.25 Recommendation for dietary glycine and serine (Glyequi), and feed assessment

Determining optimal nutritional resources and assessing feedstuffs are main factors in the feeding of livestock. The reported optimal amounts of glycine+serine reveal very large differences andthis is to be expected in light of the different metabolic functions of Gly (Dean *et al*., 2006). In this regard, since the produced pectoral muscle creatine (an impotant metabolic final product of glycine) is linked linearly to age, the requirement for glycine becomes higher throughout bird initial period of production but reduces as the animal grows older (Waguespack *et al*., 2009). Certain factors that determine response of Gly+Ser include concentrations of metabolically related AAs in the diet such as sulphur amino acids, serineand arginine (Powell *et al.,* 2011; Siegert *et al.,* 2015b). On an equimolar level, Serine can completely negate the glycine function (Waguespack *et al*., 2009). There is a suggestion that Gly+Ser need in broilers is stronger when fedexcess CP diets (Heger and Pack 1996), but this theory was refuted by Dean *et al*. (2006), who confirmed that efficiacy of Gly+Ser in reduced protein diet. The amount of Glyequi varies greatly across feed ingredients and within them (Table 2.4). Even though the variability of Glyequi in CP is small, the use of only vegetable raw materials in feed formulation renders the concentration of Glyequi in feed largely dependent on the concentration of CP (Chrystal *et al*., 2020). The quantity of Gly_{equi} in CP of vegetable feed ingredients can hardly be increased or reduced. The use of animal by-products raises the content of Glyequi in feed but feeding to poultry with animal-derived proteins is officially banned in some parts of the world.

Table 2.3: Levels of glycine+serine and ratios of glycine, serine and glycine+serine in selected protein-feedstuffs (Siegert and Rodehutscord, 2019)

Feed	Gly_{equi} (g/kg DM)	Gly (g/16 g N)	Ser (g/16 g N)	Gly_{equi} (g/16 g N)
Cereal grains				
Barley	8.5	4.0	4.2	7.0
Corn	6.6	3.9	4.8	7.3
Durum	11.1	3.7	4.6	7.0
Oats	8.8	4.0	4.7	7.3
Rye	7.9	4.4	4.3	7.4
Triticale	9.5	4.1	4.5	7.3
Winter wheat	8.3	4.1	4.5	7.4
Cereal byproducts				
Corn bran	8.6	4.5	4.1	7.9
Corn gluten feed	17.1	4.6	4.6	7.5
Wheat bran	11.4	3.2	4.7	8.2
Wheat gluten feed	56.4	5.1	4.2	6.6
Brewery byproducts				
DDGS (wheat)	22.5	4.0	4.4	7.2
Brewer's dried yeast	35.3	4.4	4.9	7.8
Pulses				
Field beans	21.6	4.1	4.6	7.4
Field peas	18.5	4.3	4.6	7.6
Lupins	30.4	3.9	4.8	7.4
Oilseeds and oilseed meals				
Rapeseed (full fat)	17.6	5.2	4.3	8.2
Rapeseed meal	31.7	5.0	4.1	7.9
Soybean meal	41.6	4.2	5.0	7.8
Sunflower expeller	28.5	5.9	4.2	8.7
Milk byproducts				
Casein	55.2	1.8	5.6	5.8
Milk powder	16.2	1.9	5.3	5.7
Whey powder	6.4	1.9	4.5	5.1
Animal byproducts				
Blood meal	76.6	4.6	5.0	8.2
Blood plasma protein	67.6	3.6	6.1	8.0
Feather meal	130.1	7.4	10.2	14.7
Fish meal	64.3	4.7	2.4	6.4
Meat and bone meal	86.9	14.9	3.8	17.7
Meat meal	84.5	11.4	4.8	14.8

Feed additives sufficient to increase the concentration of Glyequi in feed are crystallineGly and Ser, but have no approval in most countries. Adequate concentrations of Glyequi in low CP feed can hardly be achieved in countries where animal by-products are banned in broiler ration and the use of free glycine and L-serine is not accepted (Siegert and Rodehutscord, 2019). A Glyequi deficit may be decreased by an excess of endogenous precursors such as threonine and choline. Betaine and dimethylglycine may also be appropriate endogenous precursors as intermediate steps of the synthesis of glycine from choline, but no evidence is available on this (Dilger *et al*., 2007; Siegert *et al*., 2015a; Van Krimpen, 2016).

2.26 Impact of glycine onimmunity and gut health

It has been observed that Glyplays a crucial function in health and immunity, for instance, Gly generated in response to stress signals, is widely integrated in immunogenic proteins (Takahashi*et al*., 2008). Therefore, during an immune test, the blood and liver levels of AAs that are immune-active basedmight be changed, causing quantitative adjustments in their feed intake (Le Floc'h *et al*., 2004).Through laboratory salmonellosis, degradation of liver and blood free Gly serine and SAAs were documented;nevertheless, treatment with arginine without Gly increased the recovery period of birds infected by Salmonella pullorum (Dahiya *et al*., 2005). It was documented modulated cytokine secretions of glycine+cysteine treatment in the endo-toxaemia rat model of the tumour necrosis factor (Takahashi*et al*., 2008).According to Takahashi *et al*. (2008), glycine supplementation had halted broilersinflammation through the inhibition of cytokine development. It has been shown that utilization of AAs could be inhibited by (Le Floc'h *et al*., 2004), thus, making Gly tobecome necessary as a replacement to enhance AA use into synthetic pathway.Such mechanisms are arranged to enable digested nutrients to migrate selectively while at the same time conserving the status of the villi surface.

Glycine is used in the body and intestinal protection against the attack of microorganism and damage to body organs as an AA variable in the skin, feathers, blood, bones, cartilage, collagen, and intestinal tissues (Van Harn *et al.,* 2018).Low strength of the skin induced by deterioration of infected carcasses in broiler production is an economic issue.Glycine probably elicited this response to execute the gut repair via facilitating the de novo production of intestinal collagen (Meléndez-Hevia *et al.*2009).

Glycine works in antioxidant defence as a substrate in glutathione production and glutathione is known as a peptide which play a significant role in free radical neutralization. This functions through its group of sulphydryls that has similarity to charged toxicants (Friedman, 1994). The enhanced need for methionine usually found during aflatoxicosis is correlated with a metabolic requirement of glutathione in detoxification process identified a long time hindranceto glutathione production in older humans, and a combination of cysteine + glycine can resolve this problem(Pacheco *et al*., 2018). Research is required to decide whether and under what conditions glycine restricting factor in the glutathione synthesis in birds. This has consequences for low antibiotic, eco-sustainable development of poultry systems.Ironically high consumption of glycine causes excessive weight and necrotic enteritis through Clostridia perfringens (Dahiya *et al*., 2005), which has deterimentalimpact on safety of feed.

2.27 Recent developments in nutrition of amino acids

The aforementioned premises illustrate the insufficient supply of some amino acids by using diets with minimal protein levels. Another problem in amino acid feeding is formulating healthy diets with growing development requirements and enhancing animal health. Thr, for instance, is often deficient in reduced crude protein diets, given its vital significance in preserving gastrointestinal purity and immune function (Wu, 2013).Recent developments in dietary amino acids have resulted from a variety of methods including meta-analysis, nutritional and biochemical research. The key problems for the future are the synthesis of the evidence collected from cell to animal at various rates and the firm grasp of the utilization of amino acids for appropriate dietary use.Although,investigations have been committed to studingessential functions of SAAs, additional studies are mostly required to receive concrete proof of some of their impact on dietary synthesis, cellular mechanisms, and metabolic approaches in animals to nutritional or physiopathological situations such as disease and environmental pressure (Belloir *et al*., 2017).In fact, in conditions involving oxidative status and gene expression, the consequences of sulfur amino acids must be analysed. Suggestions for consumption of Met and Cys must be reconsidered in the context of their functions and the complexities of utilization of sulfur amino acids. There is also a need for further research to enhance the comprehension of various functions of sulfur AAs at the whole body phase. Because of the high Met and Cys needs for feather production these results are particularly noteworthy in poultry.Cys content has to be addressed when modifying Met's supply while also considering their possible interrelationship with Gly-Ser, as outlined in Figure 2.14. Due

to the equimolar and reversible conversion between Gly and Ser, Gly must also be incorporated in adequate amounts in the diet, since Gly is often not metabolised sufficiently satisfy to satisfy its metabolic demands especially as a precursor for uric acid production. There has been confirmation that Gly enrichment has beneficial impact on poultry performance provided with inadequate CP intakes (Dean *et al*., 2006).Nonetheless, there have been contradicting accounts in the publications and a current meta-analysis shows that accurate Gly+Ser specifications for broilers are now hard to determine as a result of smaller number of results available and are likely to restrict variables or prejudice in these results (Lambert*et al*., 2015). Therefore, relationship of Gly and Thr (Figure 2.14) must be studied, since the empirical significance of Gly formation from Thr is possibly based on the abundance of Thr in the ration (Wang *et al*., 2013; Lambert*et al*., 2015).

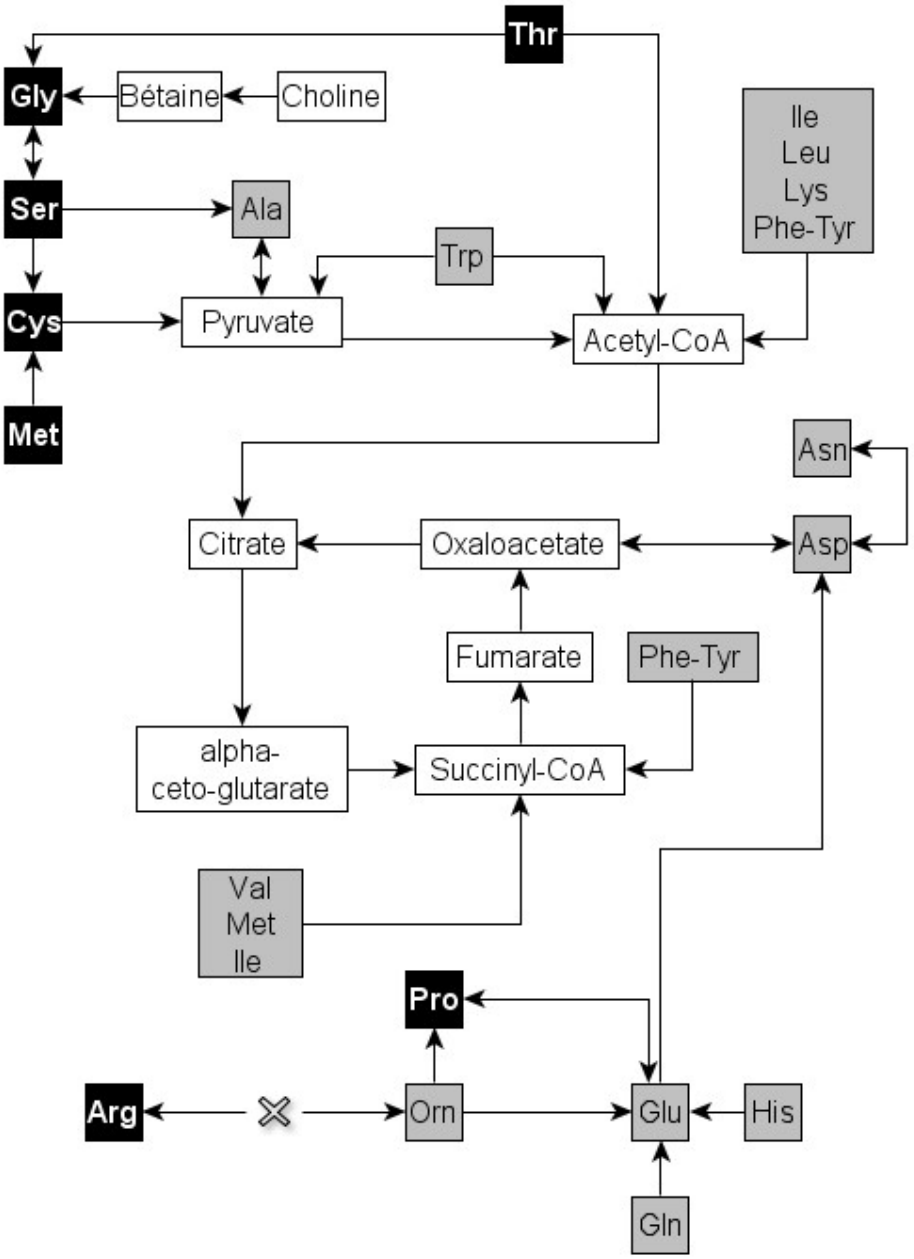

Figure 2.14: Metabolic pathways, relationships and specific characteristics of amino acids in poultry

Source: Belloir *et al*. (2015)

2.28 Current position and Perceptions of glycine+serine recommendations

As with other non-essential AAs, in many nutritional guideline tables, Gly and Ser are not listed. During the first 3 weeks of post-hatch, the National Research Council (1994) suggests 1.25% of Gly+Ser level in the ration of poultry birds. Compared to modern breeds commonly used widely, and purified feeds, the five experiments were conducted with slower growing breeds. This contributed to reports of specifications of 0.3–1.8% (Dean *et al*. 2006).Recent Brazilian guidelines propose the use of 1.97% Gly+Ser for 1–7d post-hatch and 1.92% Gly+Ser for 8–21d post-hatch diets of male broiler chickens with' standard / high performance' (Rostagno *et al*., 2017). Such guidelines do not specify how they defined certain principles. The National Research Council (1994) and Rostagno *et al*. (2017) recognize the Gly+Ser in the feed as the total amount of the two AAs, as has been presently widespread practice. The National Research Council (1994) guidelines measured Glyequi to cover a range of 1.01 to 1.20% Glyequi based on Gly's contribution to Gly+Ser (taking into account the levels of Gly and Ser in feedstuffs.Similarly, a range of 1.59 and 1.90% of Glyequi concentration for 1–7 d post-hatch has been suggested according to Rostagno *et al*. (2017) standards.

Since Gly+Ser possess the ability to restrict broiler chicken's growth rate, both AAs should preferably be present in nutritional guidelines appropriate for low CP diets, particularly on a digestible basis, as these diets are likely to become more useful in the future (Siegert *et al*., 2015a). Considering of the several variables affecting optimum Gly+Serlevels in the feed is unrealistic when simplifying guidelines in tables. If the tabular guidance program is extended with versatile components henceforth, suggested concentrations of Glyequi may be provided as parameters because of the fundamental dietary features (Siegert and Rodehutscord, 2019).Changeable suggestions may include modifications to either the Met+Cys proportion or levels of precursor in the ration (Siegert *et al*., 2015b). According to Wang *et al*. (2013), it is necessary to model effects on the Glyequi criterion, such as purine synthesis, hippuric acid and haem. If such a comprehensive itemization effectively improves diet formulation, consistency relies on the quantitative data being available. Conversely, these effects could be interpreted as criteria for the level performance and maintenance.Experiments that are quantitatively examining the impact of dietary attributes have demonstrated that incremental changes in factors that influence Glyequi's dietary response have not altered the response in a linear trend (Siegert and Rodehutscord,

2019). Alternatively, a non-linear trend in response to the Glyequi levels in the diet was reported (Lambert *et al*. 2015; Siegert *et al*. 2015a, 2015b). Easy correction variables could therefore restrict the probability of suggesting concentrations of nutrients closer to the animal's need. Another way to eliminate this limitation would be to incorporate parameter adjustments and obtain guidelines through dietary models that meet the requirements.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Facility

Three experiments were conducted at two different facility locations. In experiment 1, the study was conducted at the Poultry Unit, Teaching and Research Farm of the Department of Animal Science, University of Ibadan, Ibadan, Oyo state, Nigeria. Experiment 2 and 3 were conducted at the Poultry unit, Iguatemi experimental farm of the Universidade Estadual de Maringá, Parana state, Brasil. In experiment 1, all broiler chicks were raised in open ventilated shelter under deep litter with appropriate management practices throughout the 21 days duration. Every pen had a chick tube feeder and a bell drinker. In experiment 2 and 3, birds were maintained in thermostatically-controlled deep litter floors pens in an environmentally-controlled room comprising cooling condensers, heater, fans, and foggers with rice husks as deep litter material and each pen had 1 pipe feeder and 3 nipple drinkers.

3.2 Bird management

The birds were raised using standard procedures and conditions. Prior to the arrival of the experimental birds, the poultry house, pens and equipment used were cleaned and disinfected properly. Consistent bright lighting was supplied throughout the research period. Provision of experimental feed and clean water were supplied on regular basis in appropriate troughs while vaccination and medication schedules were properly administered as appropriate. All the requisite daily poultry management proceduresand control of temperature were observed throughout the experimental period.

3.3 Experimental birds and design:

Experimental chicks (1-d old)used for the study were supplied from a reputable licensed hatchery enterprise. A computer sorting allotment programme for experimental animal of Kim and Lindemann (2007) software package was used for grouping birds by weight and assigned them to pens to obtain similar starting pen weights in a completely randomized

experimental design (CRD). In study 1, a total of 288Arbor acre®birds were allotted in a 3 by 3 factorial pattern of 9 treatments having 4 replications of8 chicks each giving a total of 36 pens while in study 2 and 3, a total of 1275 Cobb-Vantress® chicks were randomly allotted into a 3 × 5 factorial design, having fifteen treatments withfivereplicates perseventeenchicks.

3.4 Experimental diets

3.4.1 Experiment 1

Nine diets were formulated to providethree concentrations of CP (22%, 20% and 18% CP) in combination with three levels of supplemental glycine (0.0%, 0.2% and 0.4%)by adjusting the levels of maize and soybean meal, as shown in experimental dietary layout (Table 3.1). The experimental diets were formulated to be isocaloric containing 3120kcal ME/kg withMet, Lys, calcium and available phosphorus levels maintained at or exceeding NRC (1994) recommended specificationsfor broiler chicks at 1 – 21 d of age (Table 3.2).

Table 3.1: Study 1 experimental dietary treatments layout design

Layout	Adequate CP Diet			Sub-adequate CP Diet			Low CP Diet		
CP levels	22%			20%			18%		
Gly levels	0.0%	0.2%	0.4%	0.0%	0.2%	0.4%	0.0%	0.2%	0.4%
Gly+Ser	1.87%	2.06%	2.25%	1.67%	1.86%	2.05%	1.47%	1.66%	1.85%
No of birds	8	8	8	8	8	8	8	8	8
No of Replicates	4	4	4	4	4	4	4	4	4
No of treatments	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9

Table 3.2: Gross composition (g/100g DM) of experimental basal diets with varying levels of crude protein (Values in parenthesis are analyzed values)

Ingredients	**22% CP**	**20% CP**	**18% CP**
Maize	52.85	59.42	65.60
Soybean meal	39.00	33.20	27.50
Soya-oil	4.00	3.00	2.20
Di calcium phosphate	2.00	2.00	2.10
Limestone	1.00	1.00	1.00
Salt	0.40	0.40	0.40
Vitamin-Premix	0.40	0.40	0.40
DL-Methionine	0.25	0.28	0.30
L-Lysine	0.10	0.30	0.50
L-Glycine	0.00	0.00	0.00
Total	100	100	100
Nutrients composition			
ME Kcal/Kg	3121.50	3123.95	3125.18
Crude protein %	22.50 (22.13)	20.45 (20.80)	18.41 (18.73)
Calcium %	0.92	0.91	0.91
Available Phosphorus	0.49	0.48	0.48
Methionine %	0.60 (0.59)	0.60 (0.57)	0.60 (0.56)
Lysine %	1.37 (1.34)	1.37 (1.38)	1.37 (1.36)
Glycine %	1.05 (1.09)	0.95 (1.01)	0.85 (0.92)
Threonine %	0.84 (0.78)	0.78 (0.75)	0.72 (0.70)
Met+Cys %	0.9 (0.82)	0.86 (0.78)	0.82 (0.74)
Gly+Ser %	1.87 (1.90)	1.67 (1.66)	1.47 (1.45)
Leucine %	1.92 (1.85)	1.81 (1.80)	1.70 (1.74)
Arginine %	1.48 (1.44)	1.36 (1.39)	1.24 (1.21)
Histidine %	0.59 (0.56)	0.55 (0.52)	0.51 (0.49)
Isoleucine %	0.93 (0.85)	0.90 (0.81)	0.87 (0.76)
Phenylalanine %	1.06 (0.97)	0.99 (0.91)	0.91 (0.86)
Tryptophan %	0.33(0.29)	0.28 (0.22)	0.25 (0.20)
Valine %	1.03 (0.98)	0.96 (0.92)	0.89 (0.87)

3.4.2 Experiment 2

Fifteen diets were formulated to consist of 0.2, 0.4, 0.6, 0.8 and 1.0%levels of supplemental Gly (SGly) to provide dietary total Gly+Ser of 1.72, 1.87, 2.0, 2.17 and 2.32%, respectively, in combination with three levels of dietary standardized ileal digestible (SID) Met (0.30, 0.50 and 0.70%, corresponding to 85, 100 and 115% of the required dig Met+Cys respectively, for broilers as recommended (Rostagno *et al*., 2017). The composition of experimental feed (Table 3.4) were prepared based on analytical values of corn and soybean meal to contain 17% CP and 2975kcalME/kg, andalso to me*et al*l other nutrient needs of broilers (Rostagno *et al.*, 2017)with the exception of the concentrations of Gly (or total Gly+Ser) and SID Met (Table 3.3). All remaining experimental rations were produced by adding crystalline Gly and DL-Met to the basal formulated feed as substitutes for the inert filler(kaolin).

Table 3.3: Study 3 experimental dietary treatment layout design

Layout	Deficient Met diet					Adequate Met diet					Excess Met diet				
Met %	0.30%					0.50%					0.70%				
Met+Cys	0.77%					0.90%					1.03%				
Treatments	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
SGly level %	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0
Gly+Ser %	1.72	1.87	2.02	2.17	2.32	1.72	1.87	2.02	2.17	2.32	1.72	1.87	2.02	2.17	2.32
Gly_{equi}%	1.49	1.64	1.79	1.94	2.06	1.49	1.64	1.79	1.94	2.06	1.49	1.64	1.79	1.94	2.06
Replicates	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
No of birds/replicate	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Table 3.4: Gross composition (g/100g DM) of basal diets containing varying concentrations of methionine+cysteine (Met+Cys)

Ingredients	Deficient Met diet	Adequate Met diet	Excess Met diet
Corn	69.20	69.20	69.20
Soybean 45%	22.20	22.20	22.20
Inert	1.50	1.40	1.30
Dicalcium phosphate	1.80	1.80	1.80
Limestone	0.90	0.90	0.90
Soybean oil	0.80	0.80	0.80
L-lysine	0.73	0.73	0.73
Salt	0.50	0.50	0.50
L-arginine	0.44	0.44	0.44
Premix	0.40	0.40	0.40
L-valine	0.32	0.32	0.32
L-threonine	0.30	0.30	0.30
L-isoleucine	0.27	0.27	0.27
L-trytophan	0.06	0.06	0.06
DL-methionine	0.34	0.48	0.61
L-glycine	0.27	0.27	0.27
Nutrient			
Available Phosphorus	0.431	0.431	0.431
Glycine+Serine	1.72	1.72	1.72
Glycine equivalent	1.49	1.49	1.49
SID Isoleucine	0.834	0.834	0.834
SID Leucine	1.299	1.299	1.299
SID Lysine	1.243	1.243	1.243
Met + Cys	0.770	0.900	1.030
Potassium	0.589	0.589	0.589
Crude protein	17.79	17.87	17.87
Sodium	0.215	0.215	0.215
SID Threonine	0.809	0.809	0.809
SID Trytophan	0.212	0.212	0.212
SID Valine	0.958	0.958	0.958

3.4.3 Experiment 3

Fifteen experimental diets were prepared comprising 0.2, 0.4, 0.6, 0.8 and 1.0%levels of supplemental Gly (SGly) to supply total Gly+Ser dietary levels of 1.72, 1.87, 2.0, 2.17 and 2.32%, respectively and three Thr concentrations (0.69, 0.81 and 0.93 g / kg, equivalent to 85, 100 and 115 per cent of the Thr requirement respectively, for chicksas recommended (Rostagno *et al*., 2017). The basal feed was prepared to consist of 17% CP and 2975 kcalME/kg based on corn and soyabean meal (Table 3.6). The experimental diets were developed in compliance with the chemical analysed values of feeds and the acceptable nutrient specifications for broilers (Rostagno *et al*., 2017), without considering the concentrations of Gly (or total Gly+Ser) and Thr.

Table 3.5: Study 3 experimental treatment layout design

Layout	Deficient Thr diet					Adequate Thr diet					Excess Thr diet				
SThr levels	0.2%					0.3%					0.4%				
SID Thr	0.69%					0.81%					0.93%				
Treatments	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
SGly level %	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0
Gly+Ser %	1.72	1.87	2.02	2.17	2.32	1.72	1.87	2.02	2.17	2.32	1.72	1.87	2.02	2.17	2.32
Gly_{equi} %	1.49	1.64	1.79	1.94	2.06	1.49	1.64	1.79	1.94	2.06	1.49	1.64	1.79	1.94	2.06
Replicates	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
No of birds	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Table 3.6: Gross composition (g/100g DM) of experimental basal diets containing varying dietary Threonine (Thr) concentrations and glycine+serine (Gly+Ser) levels

Ingrediente	**0.69% Dig Thr**	**0.81% Dig Thr**	**0.93% Dig Thr**
Corn	69.30	69.30	69.30
Soybean 45%	22.10	22.10	22.10
Inert	1.50	1.40	1.30
Dicalcium phosphate	1.80	1.80	1.80
Limestone	0.90	0.90	0.90
Soybean oil	0.70	0.70	0.70
Salt	0.50	0.50	0.50
Premix	0.40	0.40	0.40
DL-methionine	0.46	0.46	0.46
L-arginine	0.44	0.44	0.44
L-lysine	0.73	0.73	0.73
L-valine	0.32	0.32	0.32
L-glycine	0.29	0.29	0.29
L-isoleucine	0.27	0.27	0.27
L-threonine	0.20	0.30	0.40
L-trytophan	0.06	0.06	0.06
Nutrients			
ME (Kcal/kg)	2975.00	2975.00	2975.00
Crude protein	17.76	17.76	17.76
Available phosphorus	0.43	0.43	0.43
Glycine+Serine	1.72	1.72	1.72
Glycine equivalent	1.49	1.49	1.49
SID Isoleucine	0.83	0.83	0.83
SID Leucine	1.30	1.30	1.30
SID Lysine	1.24	1.24	1.24
Met+Cys	0.90	0.90	0.90
Potassium	0.59	0.59	0.59
Sodium	0.22	0.22	0.22
SID Threonine	0.69	0.81	0.93
SID Trytophan	0.21	0.21	0.21
SID Valine	0.958	0.958	0.958

3.5 Data collection

3.5.1 Measurement of growth Performance:

Performance traits were determined by measuring average feed intake (AFI), average weight gain (AWG) and feed conversion ratio (FCR) on weekly basis and at the termination of the experiment.Initial body weight of birds was measured at the start of each trial on day 1 and final pen weight rmeasured on 21 day of age. The AWG was obtained by subtracting average initial body weight from the average final body weight. The AFI per replicate was measured by subtracting the quantity of feed leftover from the quantity of feed supplied at the termination of the experiment. Records of mortality werekept regularly, and dead birds were weighed and their weights were used to modify the FCR by summing up the weight dead birds and pen weight, then dividing feed consumed by gain.

3.5.2 Carcass characteristics evaluation

After termination of the feeding trial on day 21, twobroilersper pen replicate were chosen with respect to their mean pen weight, killed and subsequent bled for the assessment of carcass and organ qualities. The dead birds were dipped in hot boiling water at 70^{o}C, de-feathered and thereafter, was manuallyeviscerated. After the evisceration, carcass traits such as the weight of the whole breast, thigh, liver, pancreas and abdominal fats were recorded. All carcass data were expressed as percentage of live weight of each broiler chicken.

3.5.3 Serum biochemical evaluation

On day 21 of the experiment, individual weight of birds per replicate pen were taken and two birds that weighed closest to the pen mean weight were chosen for blood collection, which was taken through the jugular vein. The serum samples were collected by blood centrifugation for 10min at 3000xg at 4°C accompanied by preservation at -70°C until laboratory analysis were conducted. Serum total protein, albumin, uric acid, glucose, triglyceride, creatinineand ammonia were assessed colorimetrically with the aid of reagent kits (Gold Analisa Ltd, Belo Horizonte, Minas Gerais, Brazil).

3.5.4 Collection of ileal digesta - Ileal amino acid digestibility

On day 21, known quantity of appropriate treatment diets were incorporated with chromic oxide as an indigestible marker and fed to the experimental chicks for 7 daysto monitor nutrient digestibility. Experimental diets and water were offered *ad libitum*. On day 28, feed and water were withdrawn and after 3 hours of feed withdrawal, four birds were taken from each penand killed by CO_2 strangulation and dissected rapidly to extract ileal digesta, a small intestine product from Meckel's diverticulum about 4 cm before ileocecal junction. After rapid removal of the section, digesta were gently squeezed into a collection vessel by rinsing the ileal digested with deionized water. Subsequently, the ileal contentcollected from birds were pooled by replicate pen, freeze-dried, milled and preserved in air-tight plastic sample containers at -4^{o}C until required for analysis (Adedokun*et al*., 2007; Bandegan *et al*., 2009)

3.5.5 Pectoral muscles creatine evaluation

Two birds of similar weights were selected at 21 d of the experiment and killed by electrical stunning and bled subsequently before the removal ofpectoral breast meat. Examination of creatine concentration in pectoral breast muscles was carried out using the procedure of Chamruspollert *et al*. (2002). Using a meat grinder, the pectoral breast muscles were ground and 1 g of ground meat was taken and put inside a test tube (25/150 mm) with 20ml of 1M sulfuric acid. Thereafter, samples were autoclaved for 45 minutes at pressure rate of 1.1kg/cm^2 and kept at 4°C pending further analysis. Samples were further takenintovolumetric flasks (100 ml). Tubes and samples were rinsed with deionized water twice for removal of all contents.18ml of 2M NaOH were added to each volumetric flask accompanied by 5ml of sodium tungstate (100gkg^{-1}) addition. At the same period, the overall volume of deionized water in the flasks was reduced to 100 ml. Thereafter, samples were rattled and then permitted to stand for 5mins prior filtering. Subsequently, 10ml of the clear filtrate was poured into another 100ml of graduated flask and thereafter, 5ml of alkaline picrate and 10ml of NaOH (100gkg^{-1}) were introduced to transform creatine to creatinine. Samples were combined and made to remain for 10mins and added with deionized water to make up to a 100ml volume. Creatinine was adpoted as a standard solution (500mg/ml), and the sample absorbance and standard were determined at a 450nm wavelength using spectrophotometer (Semi-auto Biochemistry Analyser, System BIO-2000, São Paulo, Brazil).

3.5.6 Evaluation ofthiobarbituric acid reactive substance (TBARS) for oxidative stability

The oxidative stability of breast meat was evaluated by measuring mg of malondialdehyde per kg of meat. Five gram samples in 15mL distilled water were homogenized for 1 minute at 1130x g in the test tube. The homogenated sample (1mL) was taken and transferred to a test tube. After that, the test tube was filled with 50uL butylated hydroxyanisol (7.2 %) and 2mL TBARS-TCA solution (20mM TBARS in 15% TCA). The tubes were transfered into a boiling water bath and heated at 90^{o}C for 30min, allowed to cooled and then centrifuged for 10 min at 3000 rpm. The Supernatant absorbance was determined with a spectrophotometer at 532 nm. The lipid oxidation was calculated as the TBARS value represented as mg of malondialdehyde per kg of meat (mg MDA / kg) as described by the recommended methodsof Ahn *et al*. (1998).

3.6 Analytical procedures

In experiment 1,diets and ileal digesta were analysed for N by the Kjedhal procedure (AOAC, 2006) and the CP contents wereobtained by multiplying the N contents by 6.25. Chromic oxide (Cr_2O_3) determination of feed and ileal digesta werecarried out spectrophotometrically through post wet ash digestion with nitric and perchloric acids in accordance to the procedures of Fenton and Fenton (1979). The concentrations ofAAs in the diets and ileal digesta were assessed spectorophotometerically using ninhydrin chemical reaction techniquein a24 hourhydrolysis with 6M of HCL acid at 110°C asdescribed by Schroeder *et al*. (1990)procedures. However, in experiment 2 and 3, representative samples from the basal experimental diet were collected and prepared in duplicate for CP (method 968.06) and AAs (994.12) analysis according to the guidelines of AOAC (2006). Samples for AAs determination were hydrolyzed with 6M HCL at 110°C for 24 hours and key AAs composition of the hydrolysates were analyzed through the aid of HPLC. The compositions of Met and Cys were gotten through performic acid oxidation before hydrolysis.

3.7 Digestibility Calculations

The CP and AA apparent ileal digestibility was estimated using the index method based on ratios of indigestible marker according to the equation as defined by Iyayi *et al.* (2013).

$$(1 - [(Cr_2O_{3\ feed}/\ Cr_2O_{3\ digesta})x\ (AA_{digesta}/AA_{feed})])\ x\ 100$$

where, $Cr_2O_{3\ feed}$is chromium in diet; $Cr_2O_{3\ digesta}$isileal digesta chromium

$AA_{digesta}$is AA in ileal digesta; $AA_{feed}$is dietary AA.

All analyzed values were expressed as g/kg DM.

3.8 Data analysis:

In all analysis, replicate cages were considered as the experimental unit. The data were analyzed by two-way ANOVA via 3×3 (study 1) and 3×5 (study 2 and 3) factorial arrangement of treatments in a completely randomized design to determine the main effects with a template comprising the defined effects of Gly and CP (Study 1), Gly and Met (Study 2), and Gly and Thr (Study 3), and their interactions using the GLM procedure of SAS (2012). The models were:

$$Y_{ijk}= \mu+CP_i + Gly_j +CP_i\times Gly_j +e_{ijk}\text{……...(Study 1)}$$

$$Y_{ijk}= \mu + Met_i + Gly_j + Met_i\times Gly_j +e_{ijk}\text{……(Study 2)}$$

$$Y_{ijk}= \mu + Thr_i + Gly_j + Thr_i\times Gly_j+e_{ijk}\text{…….(Study 3)}$$

The Yijk = response variable, μ = overall mean, CP_i = fixed effect of CP level, Met_i = fixed effect of Met level, Thr_i = fixed effect of Thr level, Gly_j = fixed effect of Gly level, $(CP\times Gly, Met\times Gly, Thr\times Gly)_{ij}$ = first order interaction, and e_{ijk} = random residual error.

Significant differences between means were separated by the Least Significant Difference test and a probability level of $p < 0.05$ was considered to be statistically significant. In addition, regression analysis was performed by using linear and quadratic effects to determine the optimum level of Gly supplementation/total Gly+Ser requirement estimates where considered relevant.

CHAPTER FOUR

4.0 RESULTS

4.1 EXPERIMENT 1

4.1.1Effect of glycine supplementation and varying concentrations of dietary crude protein on growth performance traits

Effect of varying concentrations of CP and SGly on AWG and FCR of the chicks were affected ($p < 0.05$) while no difference ($p >$ diet.05) was recorded on AFI (Table 4.1). Experimental chicks fed 22% CP diet recorded a higher ($p < 0.05$) AWG of 778.13g, followed by those on 20% CP diet having 729.57g while the lowest ($p < 0.05$) AWG of 610.51g was recorded in chicks offered low CP (18%) diet. An increase in FCR was recorded as the level of CP decreases in the diet, thus, birds fed 20% CP diet showed lower ($p < 0.05$) FCR of 1.57 compared to a higher ($p < 0.05$) FCR of 1.95 recorded in group that consumed 18% CP ration. The AFI values ranged from 1193.06g in 18% CP diet to 1221.51g in 20% CP diet and showed no variation ($p > 0.05$) among the means. Supplemental Glycine influenced ($p < 0.05$) the AWG and FCR of experimental birds but did not affect ($p > 0.05$) AFI. The AWG increased and that of FCR decreased with increasing Gly supplementation.Thus, birds fed 0.4% SGly diet gave higher ($p < 0.05$) AWG (776.68g) and lower ($p < 0.05$) FCR (1.60) compared to those on 0.2 and 0.0% SGly diets.

Table 4.1: Effect of varied CP and supplemental glycine concentrations on performanceindices

Nutrient levels %		AWG (g/bird)	AFI (g/bird)	FCR
CP	22	778.13[a]	1216.39	1.57[c]
	20	729.57[b]	1221.51	1.69[b]
	18	610.51[c]	1193.06	1.95[a]
	SEM	9.16	13.09	0.03
GLY	0	623.55[c]	1188.37	1.94[a]
	0.2	717.97[b]	1222.34	1.72[b]
0.4		776.68[a]	1220.26	1.60[c]
	SEM	9.16	13.09	0.03
Anova		----------**P-values**----------		
	CP	<.0001	0.2781	<.0001
	Gly	<.0001	0.14	<.0001
	CP*Gly	0.0039	0.1734	0.0052

[a-c] Mean values without common superscriptsdiffers significantly ($P \leq 0.05$).

4.1.2 Interaction of supplemental glycine and protein levels on performance traits in broilers

Interaction between varied dietary CP and supplemental Gly levels on performance characteristics of broiler chicks is displayed in Table 4.2. An interaction ($p < 0.05$) was observed between different dietary CP and SGly concentrations on AWG and FCR. An improved ($p < 0.05$) AWG and FCR were obtained with increasing levels of SGly at all levels of CP. Birds fed diet with 22% CP and 0.4% SGly combination gave the highest ($p < 0.05$) AWG (830.85g) and best FCR (1.44) while the least ($p < 0.05$) AWG (517.91g) and FCR (2.23) was observed in birds fed 18% CP and 0% SGly diet. Thus, the interaction of CP and supplemental Gly levels indicated that increasing supplemental glycine up to 0.4% in the different levels of dietary CP resulted to better FCR of the experimental birds (Figure 4.1).

Table 4.2: Interaction between dietary levels of crude protein and glycine supplementation on performance parameters of broiler chickens (1-21 d).

Nutrient levels (%)		**Treatments**	**AWG (g/bird)**	**AFI (g/bird)**	**FCR**
CP	**Gly**				
22	0	T_1	733.97^{b}	1227.35	1.68^{c}
	0.2	T_2	769.57^{b}	1225.44	1.59^{d}
	0.4	T_3	830.85^{a}	1196.38	1.44^{e}
20	0	T_4	618.79^{c}	1180.97	1.92^{b}
	0.2	T_5	755.07^{b}	1224.35	1.62^{cd}
	0.4	T_6	814.85^{a}	1259.22	1.55^{d}
18	0	T_7	517.91^{d}	1156.78	2.23^{a}
	0.2	T_8	629.28^{c}	1217.22	1.94^{b}
	0.4	T_9	684.35^{c}	1244.19	1.82^{b}
SEM			15.86	22.67	0.05
Anova			**----------P-values----------**		
CP*Gly			0.0039	0.1734	0.0052

[a-c] Mean values withdifferent letters are not the same ($P \leq 0.05$).

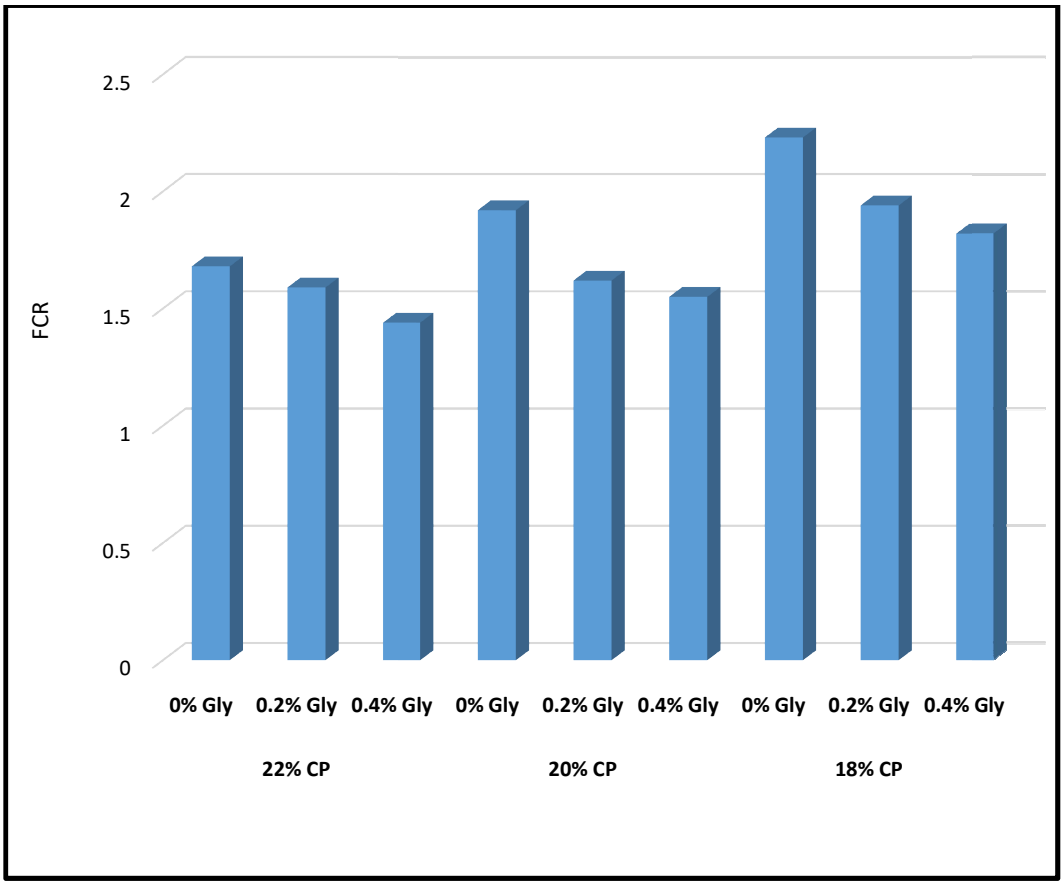

Figure 4.1:Impact of dietary crude protein andsupplemental Gly interaction on feed conversion ratio of experimental chicks.

4.1.3 Impact of glycine supplementation and varying concentrations of crude protein on serum biochemistry of broiler chickens (1-21d)

The result of serum biochemistry of broilers fedvaried concentrations of CP and supplemental Gly is displayed in Table 4.3. Serum protein, albumin, glucose, and creatinine were not affected ($p > 0.05$) by different concentrations of CP and SGly. The SUA of the birds was observed to increase ($p < 0.05$) whendietary CP concentrations reduced from 22 to 18% while a reduced ($p < 0.05$) SUA was obtained when supplemental Gly increases from 0 to 0.4%. Birds fed 22 and 20% CP diet have similar ($p > 0.05$) uric acid concentration of 5.30 and 5.44, respectively, and significantly lower ($p < 0.05$) from those fed 18% CP diet with higher serum uric acid of 6.29mg/dL. Moreover, Figure 4.2 shows that as the level of supplemental glycine increased from 0 to 0.4%, a decreasing trend in serum uric acid concentration was observed. However, serum triglyceride concentrations increased ($p < 0.05$)when dietary CP levels reduces to 18%,but no significant ($p < 0.05$) impact was recorded byGly supplementation. Broilers that consumed 18% CP feed recorded higher ($p < 0.05$) triglyceride concentration (120.87mg/dL) compared to those fed 20% and 22% CP diets with mean values of 115.29 and 115.12mg/dL, respectively.

Table 4.3: Serum biochemical indices of broilers fed corn-soybean based diets containing different concentrations of crude protein with glycine supplementation

Nutrient levels	Glucose	Total Protein	Triglyceride	Uric acid	Creatinine	Albumin
Crude protein %						
22	208.57	2.75	115.12[ab]	5.30	1.34	1.73
20	220.75	2.69	85.29[b]	5.44	1.16	1.94
18	220.9	2.79	128.87[a]	6.29	1.05	1.72
SEM	11.34	0.11	12.96	0.64	0.20	0.10
Glycine %						
0	211.35	2.8	106.39	6.69[a]	1.19	1.76
0.2	220.06	2.77	98.57	5.88[ab]	1.08	1.83
0.4	218.82	2.65	124.31	4.46[b]	1.28	1.8
SEM	11.34	0.11	12.96	0.64	0.2	0.1
ANOVA	**----------p-values ----------**					
Crude protein	0.6829	0.7963	0.0777	0.0503	0.6017	0.2159
Glycine	0.8427	0.6030	0.3749	0.0675	0.7786	0.8842
CP*GLY	0.7578	0.6161	0.0116	0.2194	0.8233	0.2963

[a-c] Mean values without the same superscrips are different ($P \leq 0.05$).

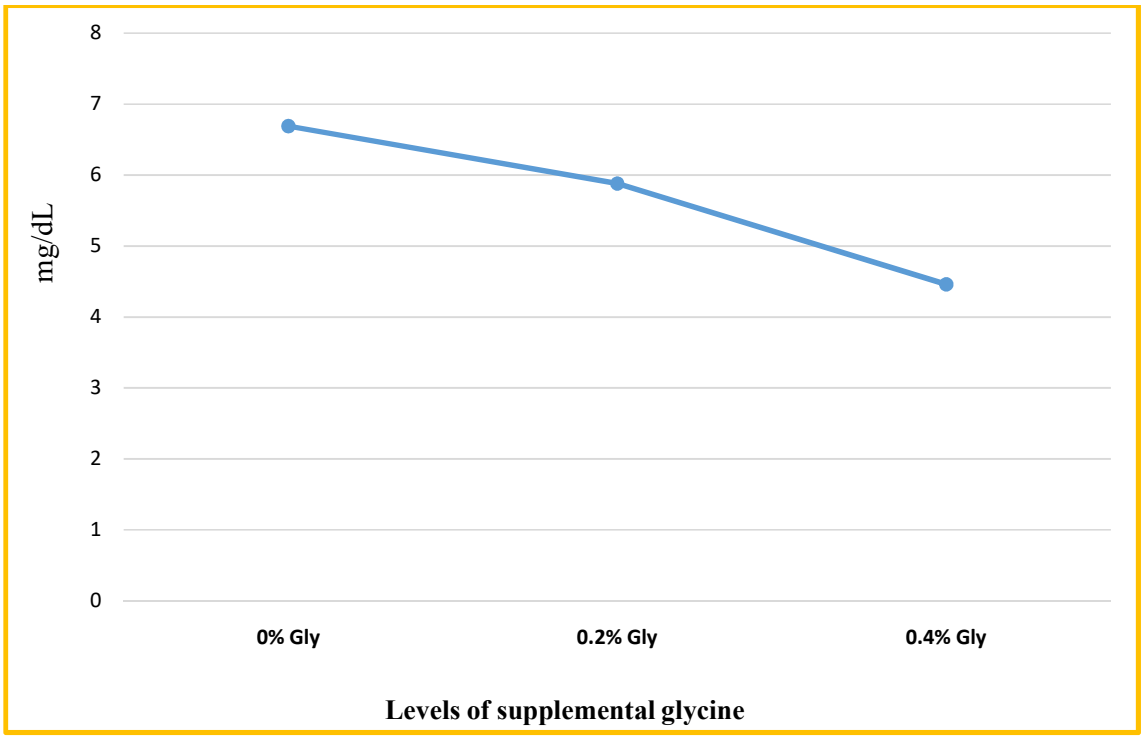

Figure 4.2:Serum uric acid of birds given feeds with varied glycine supplementation

4.1.4 Interactive impact of varying dietary crude protein and supplemental glycine levels on serum biochemistry of broiler chickens (1-21 d)

The result of the interactionbetween different levels of crude protein and supplemental glycine on serum biochemical indices of broiler chicks is shown in Table 4.4. The interaction between levels of CP and supplemental Gly significantly ($p < 0.05$) affected SUA and triglyceride of the chicks but did not show any significant ($p > 0.05$) difference on all other serum biochemical indices measured. TheSUA was observed to decrease with increasing Gly concentration at all CP levels. Thus, SUA of chicks was same ($p > 0.05$) among groups given the dietary combinations of 18% CP and 0% Gly (8.48mg/dL), and 20% CP and 0% Gly (7.05mg/dL) and greater ($p < 0.05$) in the groups that consumed other dietary combinations. On the other hand, supplementation of Gly decreased SUA up to 0.4%across the various CP levels and the minimum ($p > 0.05$) SUA concentration of 4.16mg/dL was noticed in broilers given ration combination of 18% CP and 0.4% Gly.

Table 4.4: Interaction of dietary crude protein andsupplemental glycine levels on serum biochemistry of experimental chicks

CP %	Gly %	Treatments	Glucose	Total Protein	Triglyceride	Uric acid	Creatinine	Albumin
22	0	T_1	204.79	2.86	114.78^{b}	4.54^{b}	1.50	1.67
	0.2	T_2	215.37	2.51	117.29^{b}	6.36^{ab}	1.04	1.65
	0.4	T_3	205.57	2.87	113.28^{b}	4.99^{b}	1.47	1.89
20	0	T_4	218.56	2.60	72.93^{c}	7.06^{a}	1.01	1.78
	0.2	T_5	232.73	2.75	78.69^{c}	5.03^{b}	1.07	2.19
	0.4	T_6	210.97	2.71	104.26^{bc}	4.24^{b}	1.41	1.84
18	0	T_7	210.71	2.95	131.47^{ab}	8.48^{a}	1.07	1.84
	0.2	T_8	212.09	2.70	99.74^{c}	6.24^{ab}	1.12	1.65
	0.4	T_9	239.91	2.72	155.41^{a}	4.16^{c}	0.95	1.66
SEM			19.64	0.19	22.45	1.10	0.35	0.17
P-values								
CP*Gly			0.7578	0.6161	0.0116	0.0219	0.8233	0.2963

[a-c] Mean values without common superscript letters are statistically different ($P \leq 0.05$).

4.1.5 Impact of varied crude protein and supplemental glycine levels on carcass prime cut-parts of broilers chickens (1-21 d)

The result of carcass prime-cuts of birds fed rations containing varied concentrations of CP and glycine supplementation are presented in Table 4.5. The relative breast, drumstick, thigh, abdominal fat and liver weights for different levels of CP diets ranged between 258.33g to 281.11g, 147.22g to 155.00g, 170.33g to 176.78g, 12.11g to 20.11g and 46.00g to 53.44g, respectively. No variation ($p > 0.05$) was noticed in experimental chicks given various dietary CP levels on the carcass cut-parts indices measured except for abdominal fat. The abdominal fat deposition increased with a decrease in CP levels. An increase ($p < 0.05$) in relative weight of abdominal fat (20.11g) was obtained in the group that consumed 18% CP diet, which differed from 14.67g and 12.11g obtained in those fed 20 and 22% CP diets, respectively. The influence of SGly affected ($p < 0.05$) relative breast meat and abdominal fat weight but showed no difference ($p > 0.05$) on other indices measured. Increasing levels of supplemental Gly increased ($p < 0.05$) breast weight and decreased abdominal fat weight of experimental broiler chicks**.** Birds fed diet with 0.4% Gly showed higher ($p < 0.05\%$) breast weight of 292.44g and was similar to those on 0.2% SGD (277.56g) but differed from those on 0% SGD (233.00g). Abdominal fat (10.57g) was significantly ($p < 0.05$) lower in birds fed 0.4% SGly compared to those on diet with 0.0% (16.77g) and 0.2% (16.11g) SGly levels.

Table 4.5:Carcass indices of broilers fed rations containing different concentrations of crude protein with glycine supplementation

Nutrient level	**%**	Breast (g/bird)	Drum stick (g/bird)	Thigh (g/bird)	Abdominal fat (g/bird)	Liver (g/bird)
CP	22	281.11	151.33	176.22	12.11[b]	46.00
	20	263.56	147.22	170.33	14.67[b]	48.44
	18	258.33	155.00	176.78	20.11[a]	53.44
	SEM	12.29	6.68	10.07	1.66	4.03
Gly	0.0	233.00[b]	159.78	181.11	16.77[a]	54.22
	0.2	277.56[a]	148.89	173.33	16.11[a]	48.22
	0.4	292.44[a]	144.89	168.89	10.57[b]	45.44
	SEM	12.29	6.68	10.07	1.66	4.03
		----------Anova----------				
CP		0.4083	0.7168	0.8825	0.015	0.4291
GLY		0.0390	0.2893	0.6907	0.025	0.3129
CP*GLY		0.0459	0.0838	0.3970	0.009	0.9628

[a-b] Mean without similar lettersvaried significantly ($P < 0.05$).

4.1.6 Interaction of crude protein and supplemental Gly levels on carcass prime-cut characteristics

The result of interaction between different levels of CP and supplemental Gly on the carcass prime-cuts is displayed in Table 4.6. There was a significant ($p < 0.05$) interaction between CP and supplemental Gly levels on relative breast and abdominal fat weight of the broiler chicks. Breast weight of chicks fed dietary combination of 22% CP and 0.4% Gly gave the highest ($p < 0.05$) mean value of 307.00g and did not differ ($p > 0.05$) from those fed diet containing 22% CP and 0.2% Gly (283.67g), 20 (288.67g) and 18% (281.67g) CP at 0.4% Gly combinations. However, chicks fed 18% CP and 0% Gly combination gave the lowest ($p < 0.05$) breast yield of 218.67g and was similar ($p > 0.05$) to those on 20% CP and 0% Gly (227.67g). In Figure 4.4, it shows that at different dietary CP level, increasing level of Gly supplementation up to 0.4% in the diets increased ($p < 0.05$) breast meat yield. Increasing SGly level decreased abdominal fat weight at all CP concentrations.Thus, abdominal fat weight (9.00g) was lower ($p < 0.05$) in experimental birds offered diet combination of 20% CP and 0.4% SGly and did not differ ($p > 0.05$) from those on 20% CP and 0.4% SGly diet while a higher ($p < 0.05$) mean value of 23.67g was obtained in those group that consumed feed with 18% CP and 0.0% SGly combination and similar ($p > 0.05$) to 22.33g and 22.00g recorded in those fed 18% CP and 0.0% SGly, and 20% CP and 0.0% SGly diet combinations.

Table 4.6: Interaction between graded concentrations of crude protein and glycine supplementation on carcass indices of broilers

Nutrient level %		Breast (g/bird)	Drum stick (g/bird)	Thigh (g/bird)	Abdominal fat (g/bird)	Liver (g/bird)
CP	**Gly**					
22	0	252.67^{bc}	140.00	160.00	15.00^{b}	46.33
	0.2	283.67^{ab}	153.00	177.67	11.67^{bc}	53.67
	0.4	307.00^{a}	172.00	192.67	9.67^{c}	60.33
20	0	227.67^{cd}	126.67	153.67	22.00^{a}	46.33
	0.2	274.33^{ab}	157.67	182.67	13.00^{b}	47.67
	0.4	288.67^{a}	157.33	174.67	9.00^{c}	51.33
18	0	218.67^{d}	168.00	193.00	22.33^{a}	43.67
	0.2	274.67^{ab}	136.00	159.67	23.67^{a}	43.33
	0.4	281.67^{ab}	150.00	176.00	13.00^{b}	51.00
	SEM	21.29	11.57	17.43	2.88	6.97
Anova		----------p-values----------				
CP*GLY		0.0459	0.0838	0.397	0.009	0.9628

[a-d] Mean values without similar letters were different ($p < 0.05$).

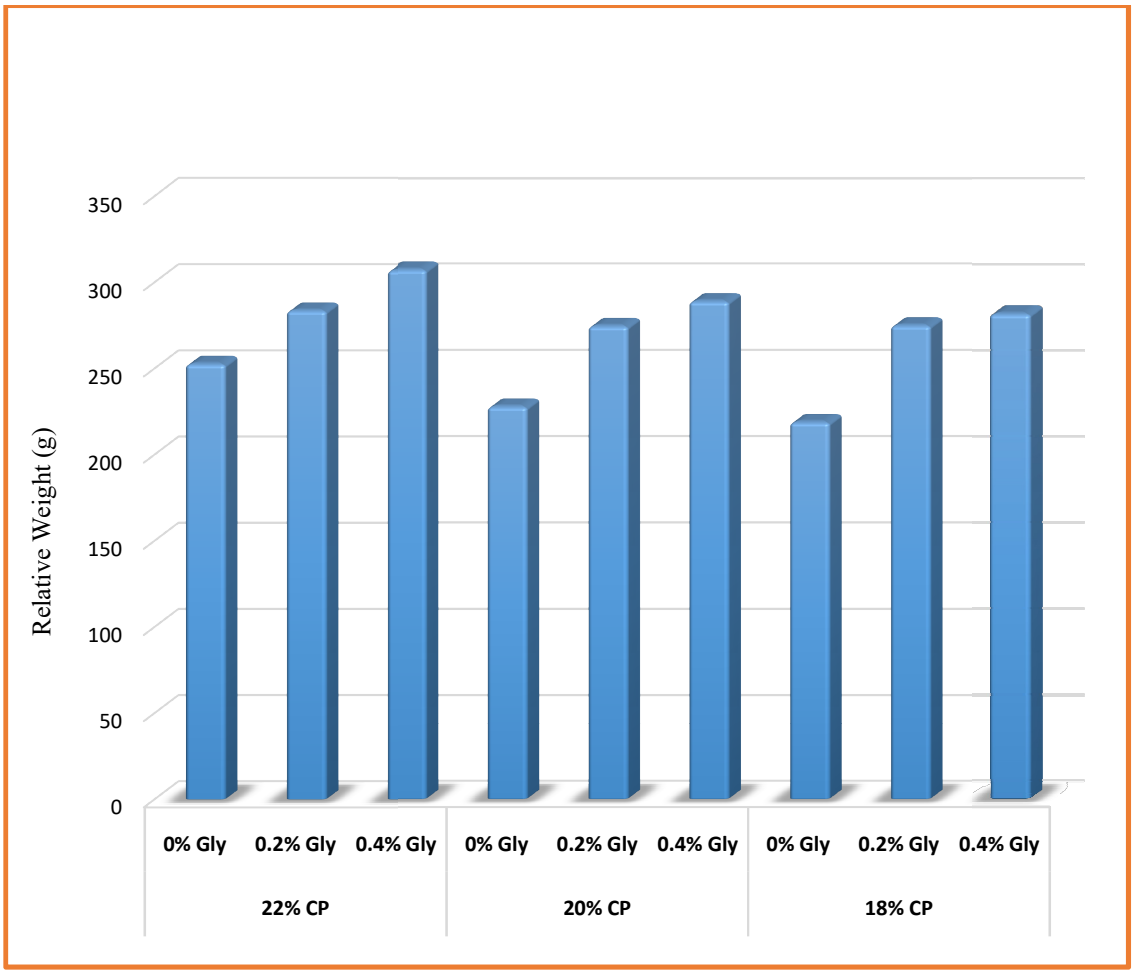

Figure 4.3: Interaction of dietary CP and supplemental glycine levels on relative breast meat yield of experimental chicks.

4.1.7 Ileal digestibility of crude protein and amino acid in chicks fed different dietary levels of crude protein and supplemental glycine

Table 4.7 shows the result of varied concentrations of crude protein and glycine supplementation on apparent CP and AA ileal digestibility of broiler chicks. Significant ($p < 0.05$) variation was noticed on apparent ileal digestibility of chicks supplied with different dietary CP and SGly levels. Apparent crude protein digestibility values for birds fed diet containing different CP levels ranged from 64.55 to 80.24%, while those on supplemental Gly diets ranged from 62.37 to 81.67%. Birds fed 22% CP diet had the highest ($p < 0.05$) CP digestibility of 80.24% than those on 20% CP (74.50%) and 18% CP (64.55%). Higher (CP digestibility recorded in birds fed 0.4% SGly (81.67%) was similar ($p > 0.05$) to those fed 0.2% SGly (75.25%) but significantly ($p < 0.05$) different from those on 0% SGly with the least digestibility value of 62.37%. Ileal AAs digestibility of the broiler chicks was significantly ($p < 0.05$) affected by different levels of dietary CP except that of glutamic acid digestibility. However, glycine supplementation did not affect statistically ($p > 0.05$) the ileal AAs digestibility of the broiler chicks. A decreasing trend was observed in apparent ileal AA digestibility when levels of CP decreased from 22 to 18%. Birds fed 22%CP diets had the highest ($p < 0.05$) ileal digestibility values for all the essential and non-essential AA but differs significantly from those birds fed 20 and 18% CP diets. However, chicks fed diets containing 18% CP recorded significantly ($p < 0.05$) lower digestibility values for all AAs measured except for cystine and showed no significant ($p > 0.05$) difference with birds fed 20% CP diet.

Table 4.7:Ileal digestibility Crude protein and amino acidS of broiler chickens (1-21 d) fed diets containing varied concentrations of crude protein and supplemental glycine.

	Crude Protein Levels %			**Supplemental Glycine %**				----------P-values----------		
Nutrients %	**22.00**	**20.00**	**18.00**	**0.00**	**0.20**	**0.40**	Pooled SEM	CP	GLY	CP × GLY
Dry matter	75.58	73.44	72.15	64.02	76.58	80.57	1.05	0.0054	0.7712	<.0001
Crude protein	80.24	74.50	64.55	62.37	75.25	81.67	1.33	0.0087	0.1047	<.0001
Essential AA										
Lysine	75.50	63.08	58.88	51.99	68.02	77.44	2.85	0.0018	0.6795	0.0001
Methionine	74.93	61.42	60.07	50.04	66.73	78.29	3.03	0.007	0.2388	<.0001
Phenylalanine	74.64	63.56	58.46	51.13	67.24	78.29	3.00	0.0147	0.1347	<.0001
Trytophan	76.58	62.42	56.89	49.86	65.50	80.53	3.57	0.0091	0.2001	<.0001
Isoleucine	76.84	63.03	55.75	49.16	64.81	81.66	3.76	0.0093	0.1694	<.0001
Leucine	74.31	59.93	48.95	46.07	54.68	82.44	4.27	0.0061	0.2251	<.0001
Threonine	76.36	64.13	60.17	53.73	65.59	81.34	3.97	0.0585	0.4174	0.0007
Valine	75.57	62.29	60.39	52.05	67.79	78.40	2.82	0.0068	0.1927	<.0001
Histidine	75.72	62.79	60.41	52.38	68.19	78.36	2.80	0.0069	0.1975	<.0001
Arginine	75.76	62.56	59.80	51.81	67.57	78.74	2.92	0.0074	0.1948	<.0001
Non-essential AA										
Glycine	74.13	62.52	59.99	50.23	67.45	78.96	2.97	0.0068	0.1927	<.0001
Serine	75.82	62.67	60.48	52.39	68.16	78.41	2.8	0.0064	0.1875	<.0001
Aspartic	75.37	63.76	63.75	52.11	69.40	81.37	2.74	0.0101	0.0906	<.0001
Proline	75.64	64.08	62.82	52.50	68.31	81.73	2.77	0.0075	0.1943	<.0001
Asparagine	76.16	62.52	62.43	52.07	66.75	82.29	3.04	0.0065	0.1852	<.0001
Tyrosine	75.76	63.15	62.57	51.82	67.58	82.09	2.92	0.0075	0.1938	<.0001
Cystine	78.61	54.82	66.57	54.22	60.66	85.12	4.03	0.0075	0.2858	0.0002
Alanine	75.68	63.62	63.09	52.37	68.27	81.75	2.77	0.0071	0.1806	<.0001
Glutamine	76.56	62.40	56.91	49.84	65.49	80.53	3.57	0.0092	0.1996	<.0001
Cysteine	77.83	64.81	53.86	50.62	62.05	83.84	4.31	0.0109	0.2797	0.0002
Glutamic acid	58.30	68.14	58.24	52.55	61.39	78.05	4.08	0.2137	0.4595	<.0001

4.1.8 Effect of interaction between CP and supplemental Gly levels on apparent ileal AA digestibility of broiler chickens (1-21d)

The result of interaction between CP and supplemental Gly levels on apparent ileal CP and AA digestibility of birds are displayed in Table 4.9. There was significant ($p < 0.05$) interactions observed in all the ileal digestibility values for both EAA and NEAAs among the various dietary treatment combinations. Increasing supplemental glycine at 0.4% in combination with all levels of dietary CP significantly increased ($p < 0.05$) ileal amino acids digestibility (Figure 4.4). Birds fed diet with 22 and 20% CP diets at 0.4% SGly combination gave the same (*$p > 0.05$*) ileal digestibility values but higher ($p < 0.05$) than other dietary combinations. However, chicks fed diet containing 18% CP and 0% Gly recorded the lowest ($p < 0.05$) cumulative ileal digestibility values for essential and non-essential AA of 41.45% and 43.50%, respectively.

Table 4.8: Interaction of varied levels of dietary crude protein and supplemental glycine on ileal nutrient digestibility of experimental chicks

CP Levels	22% High Protein			20% Medium Protein			18% Low Protein			
SGly Levels	0%	0.2%	0.4%	0%	0.2%	0.4%	0%	0.2%	0.4%	
Dry matter	65.75	78.09	82.89	65.33	76.88	78.11	60.98	74.76	80.72	1.65
Crude protein	68.94	83.86	87.91	63.08	78.76	81.66	55.09	63.12	75.43	2.98
Essential AA										
Lysine	66.89	75.90	83.70	45.81	65.43	77.99	43.28	62.73	70.64	4.93
Methionine	64.42	75.48	84.89	44.87	62.65	76.73	40.82	62.07	73.26	5.25
Phenylalanine	65.95	74.42	83.54	46.49	66.21	77.99	40.96	61.09	73.34	5.19
Trytophan	67.65	74.45	87.64	42.64	64.16	80.47	39.30	57.89	73.47	6.18
Isoleucine	67.72	73.57	89.24	41.97	65.26	81.86	37.79	55.59	73.88	6.51
Leucine	64.59	65.83	92.51	39.37	57.69	82.72	34.24	40.52	72.08	7.39
Threonine	69.36	71.89	87.84	47.10	63.43	81.86	44.73	61.45	74.32	6.88
Valine	66.21	75.75	84.75	44.55	64.90	77.41	45.40	62.72	73.05	4.89
Histidine	66.68	76.06	84.42	45.67	65.11	77.60	44.79	63.39	73.06	4.84
Arginine	66.59	75.66	85.03	44.78	64.85	78.04	44.05	62.20	73.16	5.06
Mean EAA	66.61	73.90	86.36	44.33	63.97	79.27	41.54	58.97	73.03	5.71
Non-essential AA										
Glycine	61.49	75.65	85.25	44.41	64.77	78.39	44.78	61.94	73.25	5.15
Serine	66.85	76.12	84.48	45.15	65.10	77.75	45.17	63.26	73.00	4.85
Aspartic	66.48	76.04	83.58	44.78	63.46	83.03	45.06	68.69	77.49	4.74
Proline	66.47	76.11	84.34	45.53	63.52	83.18	45.48	65.30	77.67	4.8
Asparagine	67.10	75.78	85.59	44.36	60.03	83.17	44.75	64.43	78.11	5.26
Tyrosine	66.60	75.66	85.04	44.08	62.19	83.18	44.78	64.88	78.05	5.06
Cystine	70.78	70.91	94.14	50.01	63.33	86.38	41.86	47.74	74.85	6.98
Alanine	66.68	75.99	84.37	44.81	63.19	82.88	45.63	65.62	78.01	4.8
Glutamine	67.62	74.43	87.62	42.56	64.14	80.49	39.34	57.90	73.49	6.19
Cysteine	69.35	71.90	92.25	46.04	63.45	84.95	36.47	50.80	74.32	7.46
Glutamic acid	65.45	72.44	80.09	46.97	57.22	79.05	45.22	54.50	75.00	7.07
Mean NEAA	66.81	74.64	86.07	45.34	62.76	82.04	43.50	60.46	75.75	5.67

4.2 EXPERIMENT 2

4.2.1 Impact of methionine and supplemental glycine levels on performance of broiler chickens (1-21 d)

The results of performance characteristics of experimental birds provided with varying dietary methionine and SGly levels are shown in Table 4.9. The AWG, AFI and FCR were affected ($p < 0.05$) by the different levels of dietary methionine and SGly. Birds fed 0.50% Met diet had the superior ($p < 0.05$) AWG of 859.00g, which was similar ($P > 0.05$) to 850.40g obtained in chicks offered 0.30% Met diet, while birds on 0.70% Met diet shown a lower ($p < 0.05$) AWG of 801.4g. A similar ($p > 0.05$) AFI of 1158.60g and 1157.20g was obtained in birds fed 0.50 and 0.70% Met diets, respectively, but were lower ($p < 0.05$) than 1184.80g recorded in birds fed 0.30% Met diet. The FCR of birds fed varying levels of Met diets showed that birds on 0.50% Met diet recorded significantly ($p < 0.05$) lower mean value of 1.35 than those fed 0.30% (1.40) and 0.70% Met diets.

The AWG, AFI and FCR were influenced ($p < 0.05$) by supplemental Gly addition and ranged from 807.30 to 863.20g, 114.90 to 1201.70g and 1.33 to 1.48, respectively. Increasing level of SGly produced a quadratic ($p < 0.05$) response on AWG and FCR with an estimated optimal point of 2.28 and 2.29% Gly+Ser,respectively. Birds fed 0.80% SGly diet had superior ($p < 0.05$) AWG (863.20g) and recorded the minimum ($p < 0.05$) FCR (1.33) while the AFI of the experimental birds decreased linearly ($p<0.001$) with increasing level of SGly. Similar ($p < 0.05$) feed intake was consumed by birds fed 0.6, 0.8 and 1.0% SGly diets compared to those on 0.2 and 0.4% SGly diets. The linear relationship of SGly and feed intake is shown in Figure 4.5, while the quadratic relationship of supplemental Gly on AWG and FCR is represented in Figure 4.6 and 4.7. From the graphs, it could be depicted that the 60.40, 90.70, and 88.25% of the total improvement in feed intake, weight gain and FCR, respectively, were accounted for by glycine supplementation.

Table 4.9: Impact of varied concentrations of methionine and supplemental glycineon growth paramenters of experimental birds.

Nutrient levels	AWG (g/bird)	AFI (g/bird)	FCR (g/g)[1]
Glycine (%)			
0.20	807.3	1190.5	1.476
0.40	826.0	1201.7	1.456
0.60	837.7	1144.9	1.370
0.80	863.2	1145.3	1.328
1.00	850.4	1152.1	1.357
SEM	7.02	10.51	0.015
Methionine (%)			
0.30	850.4[a]	1184.8[a]	1.396[b]
0.50	859.0[a]	1158.6[b]	1.350[c]
0.70	801.4[b]	1157.2[b]	1.447[a]
SEM	5.44	8.14	0.011
ANOVA			
SGly	Q*	L***	Q*
Met	0.001	0.03	0.01
SGly*Met	0.27	0.12	0.05

[abc]Means with the different letters in a column differ significantly (P <0.05).

[1]Values corrected for mortality.

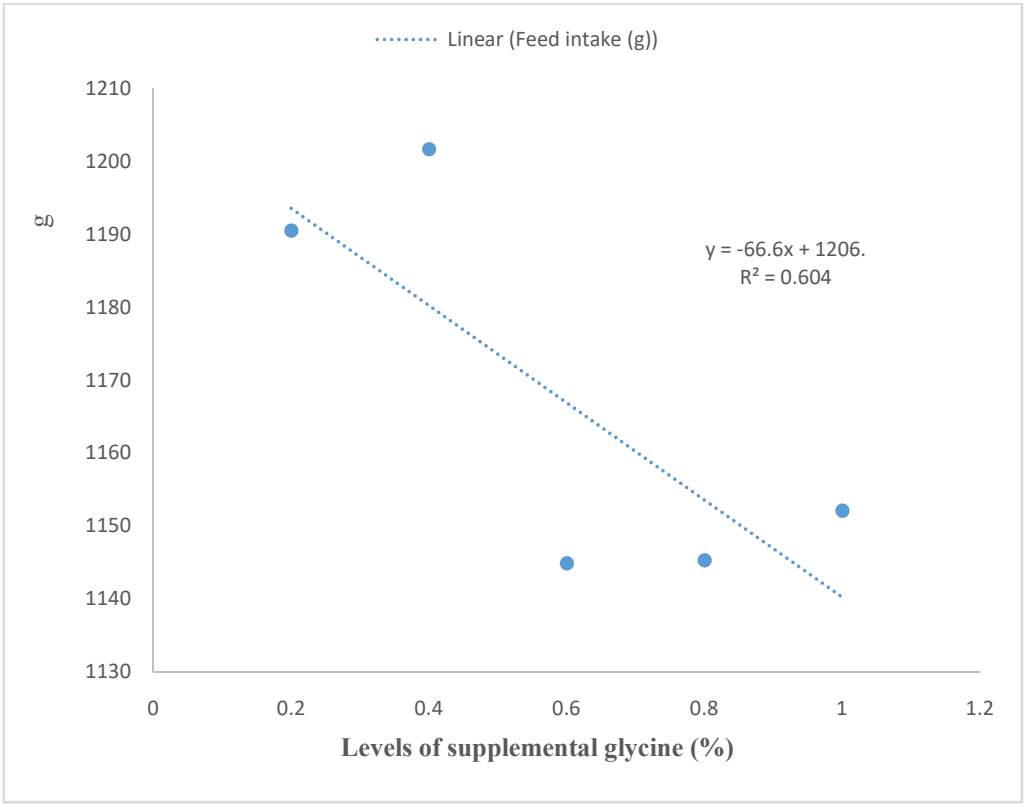

Figure 4.5: Relationship between supplemental glycine and feed cosumption of broilers fed low crude protein diet

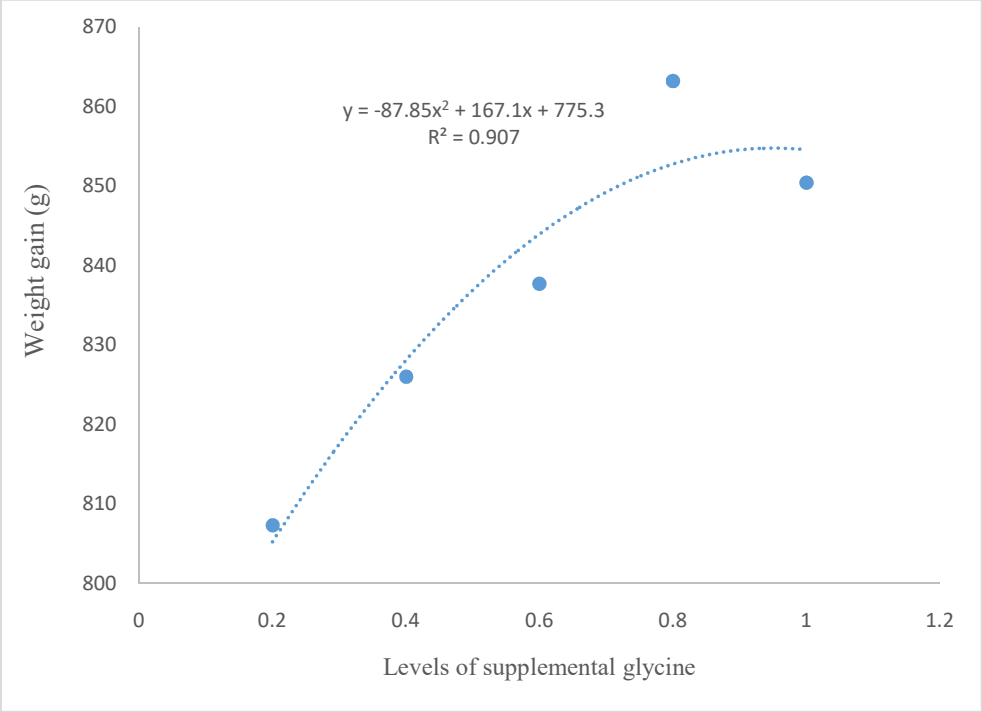

Figure 4.6: Relationship between supplemental glycine and average weight gain of birds given low crude protein feed

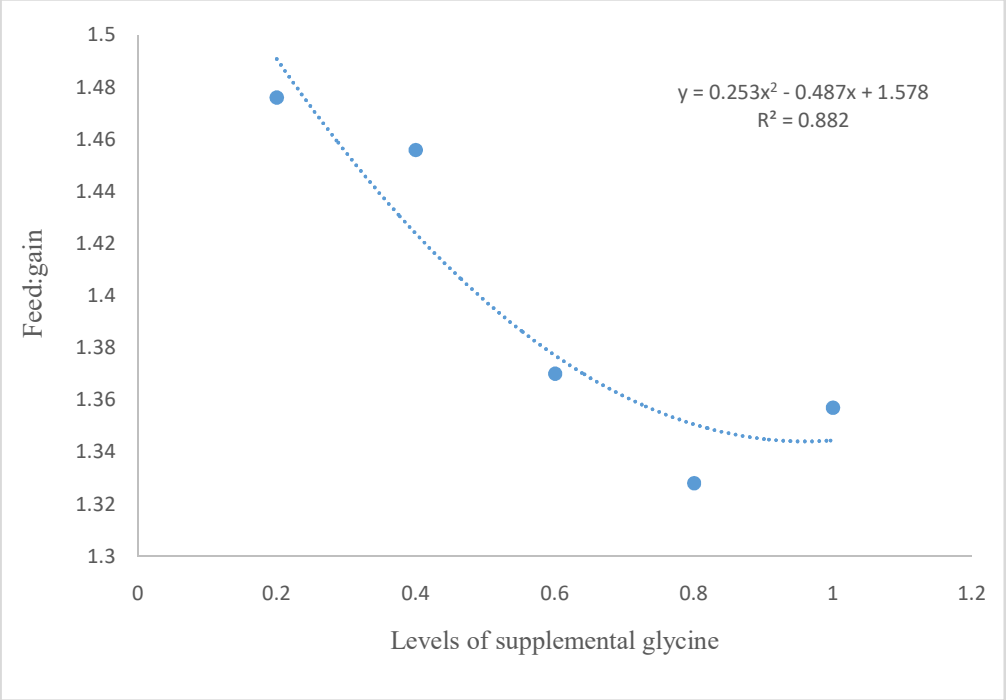

Figure 4.7: Relationship between supplemental glycine and FCR of broilers fed low crude protein diet

4.2.2 Effect of interaction between dietary concentration of Met and Gly supplementation on performance of birds (1-21 d)

The interaction between SGly and Met concentrations for FCRwas significant ($p < 0.05$) while the AWG and feed intake was not affected ($p > 0.05$) (Table 4.10). The treatment interaction showed that lower ($p < 0.05$) FCR of 1.30, 1.31and 1.32 were obtained in birds placed on T9, T5 and T8 diets compared to the group that consumed T1, T2, T11, T12and T13 diets. Unfolding the treatment interactions disclosed that a decreasing linear ($p < 0.05$) effect for FCR was recorded by increasing SGly levels in birds fed diets with 0.3 and 0.7% Met levels, while a quadratic ($p < 0.05$) effect was recorded for those fed 0.5% Met diet with an estimated optimum level of 2.16% Gly+Ser as provided by 0.8% SGly diet. The linear relationship observed when the levels of SGly increased in 0.3 and 0.7% Met diet are presented in Figure 4.8 and 4.9, respectively, while the quadratic relationship observed by increasing SGly levels and 0.5% Met diet is shown in Figure 4.10. The regression equations from the graphs show that 88.02, 57.97 and 91.82% of the total improvement on FCR in diets containing 0.3, 0.7 and 0.5% Met, respectively, were as a result of influence of Gly supplementation in the diets.

Table 4.10: Impact of interaction between methionine and glycine levels on performance indices in broiler chickens (1-21d)

Treatments	Met (%)	SGly (%)	AWG (g)	AFI (g)	Feed:gain
T_1	0.30	0.20	817.8	1232.5	1.51^{a}
T_2	0.30	0.40	832.1	1237.3	1.49^{a}
T_3	0.30	0.60	849.1	1145.1	1.35^{bcd}
T_4	0.30	0.80	883.9	1171.3	1.33^{bcd}
T_5	0.30	1.00	869.1	1137.8	1.31^{cd}
T_6	0.50	0.20	819.3	1159.8	1.42^{abcd}
T_7	0.50	0.40	848.2	1172.8	1.38^{abcd}
T_8	0.50	0.60	883.5	1162.0	1.32^{cd}
T_9	0.50	0.80	867.2	1130.2	1.30^{d}
T_{10}	0.50	1.00	876.7	1168.3	1.33^{bcd}
T_{11}	0.70	0.20	784.7	1179.1	1.50^{a}
T_{12}	0.70	0.40	797.7	1194.8	1.49^{a}
T_{13}	0.70	0.60	780.5	1127.5	1.45^{ab}
T_{14}	0.70	0.80	838.7	1134.4	1.35^{bcd}
T_{15}	0.70	1.00	805.3	1150.3	1.43^{abc}
	SEM		12.16	18.20	0.025
	ANOVA				
	SGly*Met		0.27	0.12	0.05
	SGly*0.30% Met		NS	NS	L^{***}
	SGly*0.50% Met		NS	NS	Q^{*}
	SGly*0.70% Met		NS	NS	L^{***}

[abc]Means withvaried superscript letters are statistically different ($P < 0.05$).
[1]Values corrected for mortality.

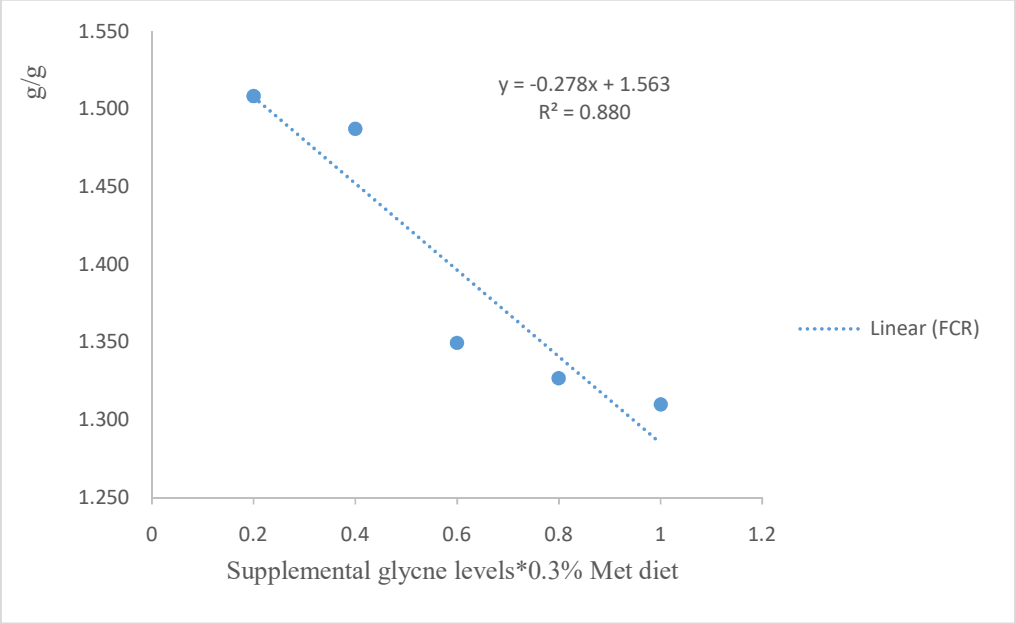

Figure 4.8: Linear relationship of supplemental glycine levels and 0.3% Methionine on FCR

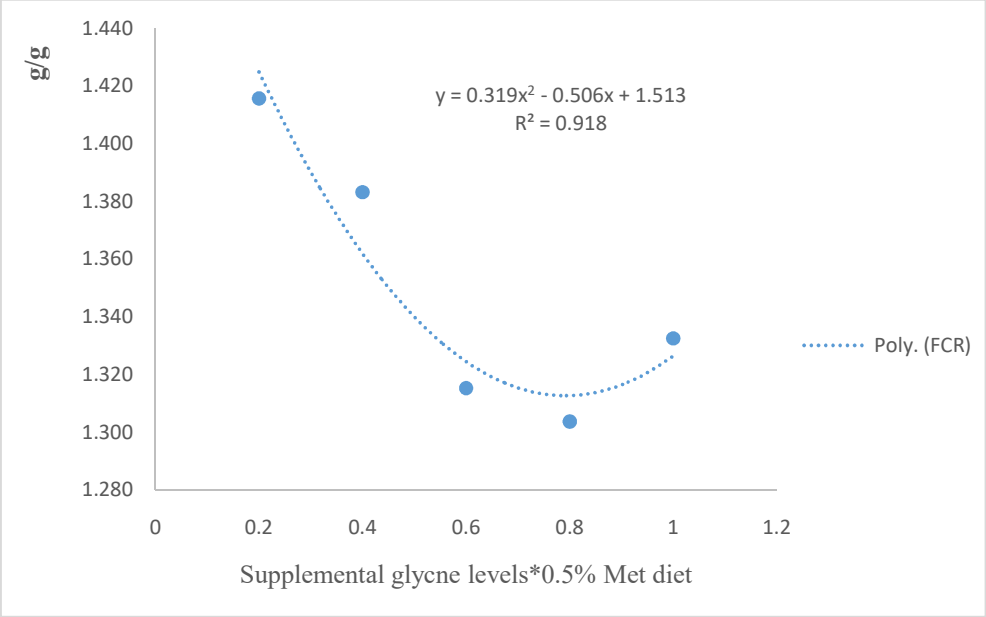

Figure 4.9: Quadratic relationship of supplemental glycine levels and 0.5% Methionine on FCR

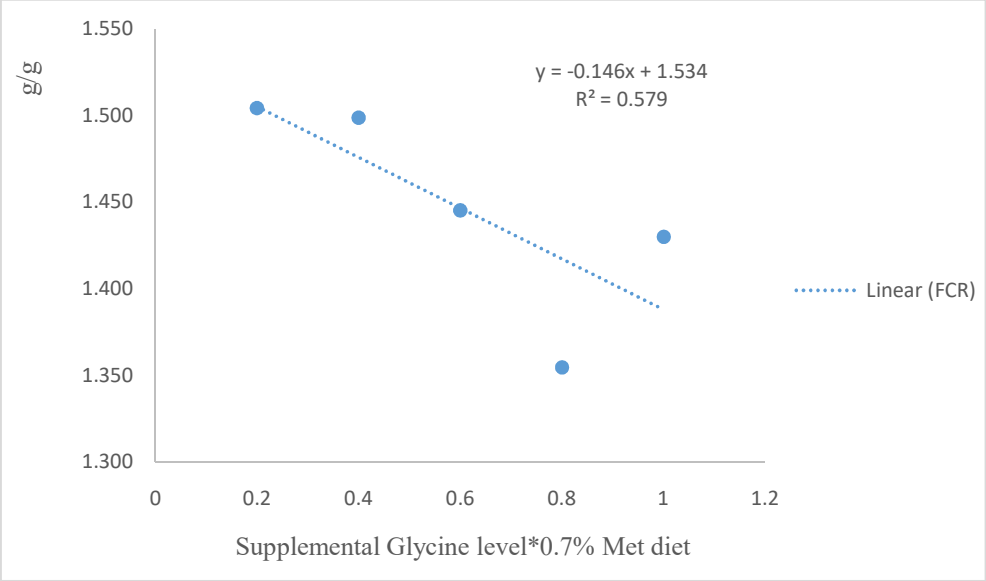

Figure 4.10: Linear relationship of supplemental glycine levels and 0.7% methionine on FCR

4.2.3 Pectoral muscle creatine, breast and liver attributes of broilers chickens fed diferent levels of dietary methionine and supplemental glycine

The result of effect of supplemental Gly to minimal CP feed with varying concentrations of methionine on pectoral muscle creatine, breast and liver weight is presented in Table 4.11. Dietary Met and Glylevels affected ($p < 0.05$) breast weight and pectoral muscle creatine of experimental birds. Bothrelative breast weight and pectoral muscle creatine in broilers rised ($p < 0.05$) quadratically with SGly levels, having an estimated optimal points at 2.11 and 2.22% dietary Gly+Ser levels, respectively. Thus, maximum ($p < 0.05$) breast weight (135.30g) and pectoral creatine concentration (3.43mg/g) was recorded in birds fed 0.8% SGly diet while those group on lower SGly diet (0.2%) had the lowest ($p < 0.05$) mean values of 123.02g and 3.11mg/g, respectively. The quadratic relationship observed with increasing levels of SGly on breast weight and pectorial creatine is shown in Figure 4.11 and Figure 4.12, respectively. The regression equations from the graph showed that 99.85% and 99.81% of the total variation observed in relative breast weight and pectoral creatine respectively were accounted by Gly supplementation. However, chicks provided feed with 0.50% Met level had higher ($P < 0.05$) pectoral muscle creatine (3.45mg/g) and was comparable to those group on 0.70% Met diet (3.35mg/g), while those on 0.30% Metdiet showed the lowest ($P < 0.05$) mean value of 3.16mg/g.Similarly, birds fed 0.50% Met diet gave the highest ($P < 0.05$) relative breast weight (133.82g/kg) compared to 130.61 and 128.76g/kg recorded on those fed 0.30% and 0.70% Met diet, respectively.

Table 4.11: Pectoral muscle creatine and relative breast, liver, and pancreas weights of broilers fed low protein diets containing supplemental Gly with varying levels of Met

Nutrient levels	Creatine (mg/g muscle)	Breast (g/kg of BW)	Liver (% BW)
Glycine (%)			
0.20	3.11	123.02	3.35
0.40	3.32	128.92	3.26
0.60	3.41	133.43	3.04
0.80	3.43	135.30	2.93
1.00	3.34	134.65	2.96
SEM	0.10	1.94	0.09
Methionine (%)			
0.30	3.16^{b}	130.61^{b}	3.09^{ab}
0.50	3.45^{a}	133.82^{a}	3.01^{b}
0.70	3.35^{a}	128.76^{b}	3.23^{a}
SEM	0.08	1.51	0.07
ANOVA		**P-values**	
SGly	Q=0.06	Q=0.02	0.02
Met	0.03	0.03	0.05
SGly*Met	0.75	0.02	0.16

$^{a-b}$Means withvaried lettersare statistically different ($P < 0.05$).

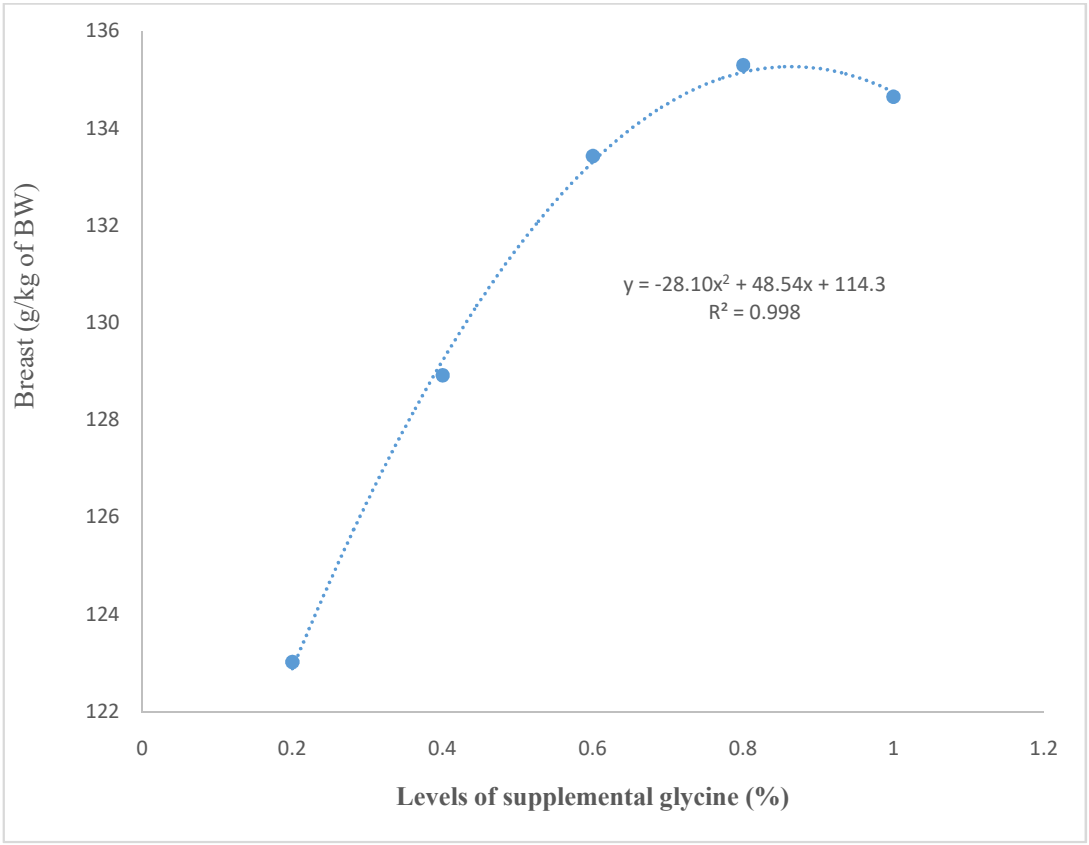

Figure 4.11: Quadratic relationship between glycine and relative breast weight in broilers (1-21 d) fed low crude protein diets

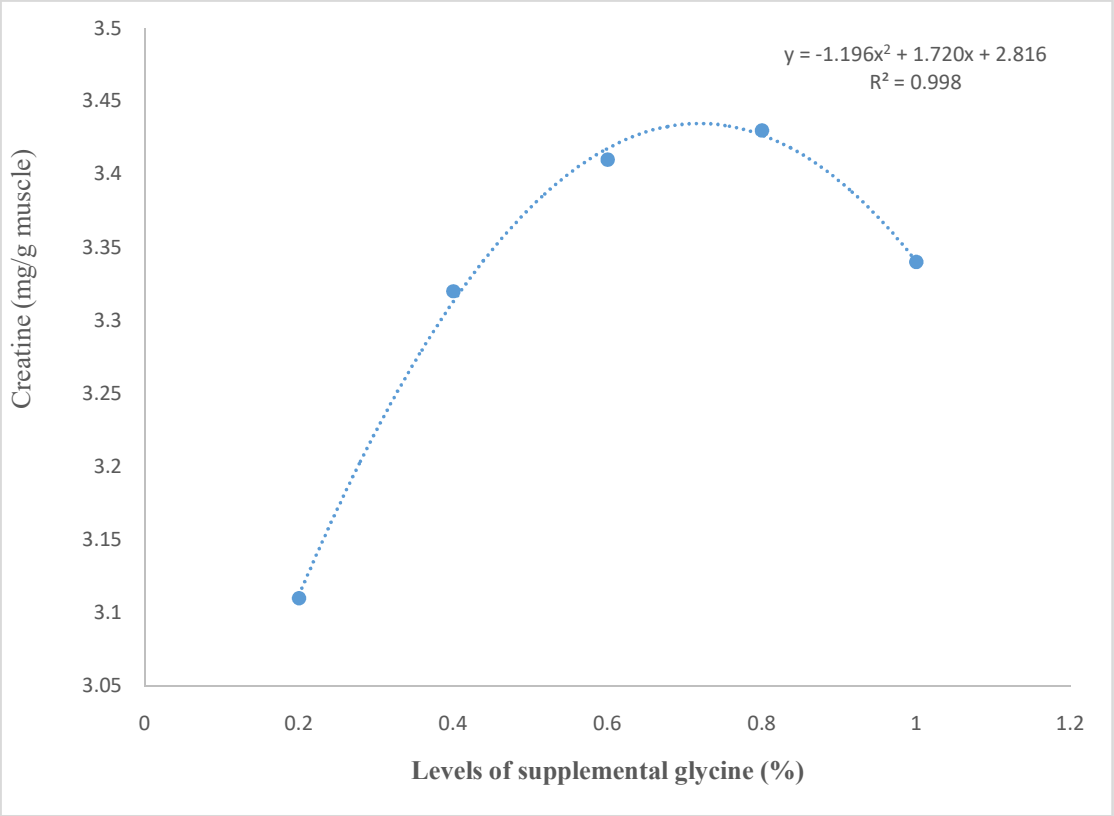

Figure 4.12: Quadratic relationship between glycine and pectoral muscle creatine in broilers (1-21 d) fed low crude protein diets

4.2.4: Effect of interaction between varied levels of methionine and glycine supplementation on pectoral muscle creatine, breast and liver weights of broiler chickens

The interaction between SGly and Met levels in the diets wassignificant ($P < 0.05$) for relative breast weight but no effect ($P > 0.05$) was obtained for pectoral muscle creatine and liver weight (Table 4.12). The treatment interaction showed that birds provided with T1 and T6 rations recorded lower ($P < 0.05$) breast weight compared to the group fed T5, T9 and T10 rations. Unfolding the interaction showed that diets with 0.3% and 0.5% Met concentrations, increasing SGly levelsdisplayed a positive linear ($P < 0.05$) influence on breast meat yield, while a quadratic ($p < 0.05$) effect was obtained for diet containing 0.7% Met with an estimated optimal response point of 2.06% Gly+Ser level. In diets containing lower (0.3%) and adequate (0.5%) Met levels, greater relative breast weight was obtained at higher SGly levels up to 1.0% (2.32% Gly+Ser) as 139.21 and 140.78g, respectively; while maximum breast weight of 131.56g was recorded up to 0.8% SGly level (2.17% Gly+Ser) in those group fed higher Met diet (0.7%). The relationship of SGly with 0.3, 0.5 and 0.7% Met levels for breast weight is shown in Figure 4.13, 4.14 and 4.15, respectively. The regression equations from the curve showed that 92.48, 96.88 and 90.14% of the total variation observed in relative breast weight of dietary treatments containing 0.3, 0.5 and 0.7%, respectively, is attributed to the influence of Gly supplementation.

Table 4.12: Pectoral muscle creatine, relative breast and liver weights of birdssupplied withdietarymethionine and supplemental glycine concentrations

Met (%)	SGly (%)	Treatments	Creatine (mg/g muscle)	Breast (g/kg of BW)	Liver (% BW)
0.30	0.20	T_1	2.99	119.32[b]	3.30
	0.40	T_2	3.15	127.92[ab]	3.09
	0.60	T_3	3.19	133.01[ab]	2.98
	0.80	T_4	3.27	133.57[ab]	3.01
	1.00	T_5	3.22	139.21[a]	3.06
0.50	0.20	T_6	3.25	124.07[b]	3.19
	0.40	T_7	3.27	130.51[ab]	3.14
	0.60	T_8	3.60	136.02[ab]	3.02
	0.80	T_9	3.64	140.78[a]	2.88
	1.00	T_{10}	3.48	137.75[a]	2.83
0.70	0.20	T_{11}	3.10	125.67[ab]	3.57
	0.40	T_{12}	3.53	128.33[ab]	3.54
	0.60	T_{13}	3.45	131.27[ab]	3.12
	0.80	T_{14}	3.37	131.56[ab]	2.91
	1.00	T_{15}	3.31	126.97[ab]	3.00
		SEM	0.18	3.37	0.15
ANOVA			**----------P-values----------**		
SGly*Met			0.75	0.02	0.16
SGly*0.30% Met			---	L=0.001	---
SGly*0.50% Met			---	L=0.01	---
SGly*0.70% Met			---	Q=0.06	---

[a-c] Valueswith similar letters are not statistically different (P > 0.05).

SGLY: Supplemental Glycine; Met: Methionine; L: Linear; Q: Quadratic; BW: Body weight

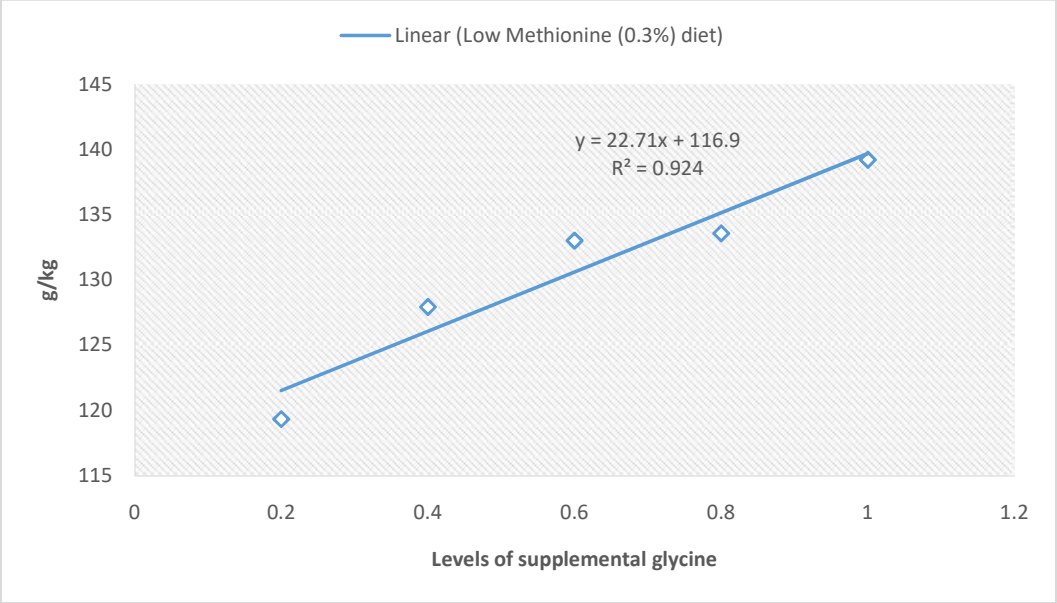

Figure 4.13: Linear relationship of levels of supplemental glycine and diet containing 0.3% methionine

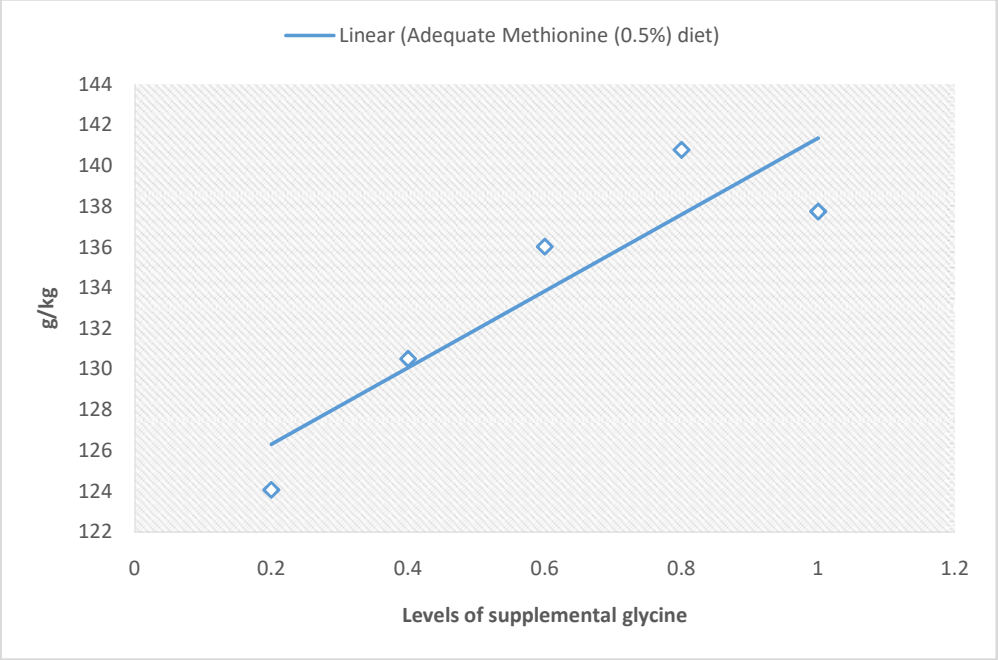

Figure 4.14: Quadratic relationship of levels of supplemental glycine and diet containing 0.5% methionine

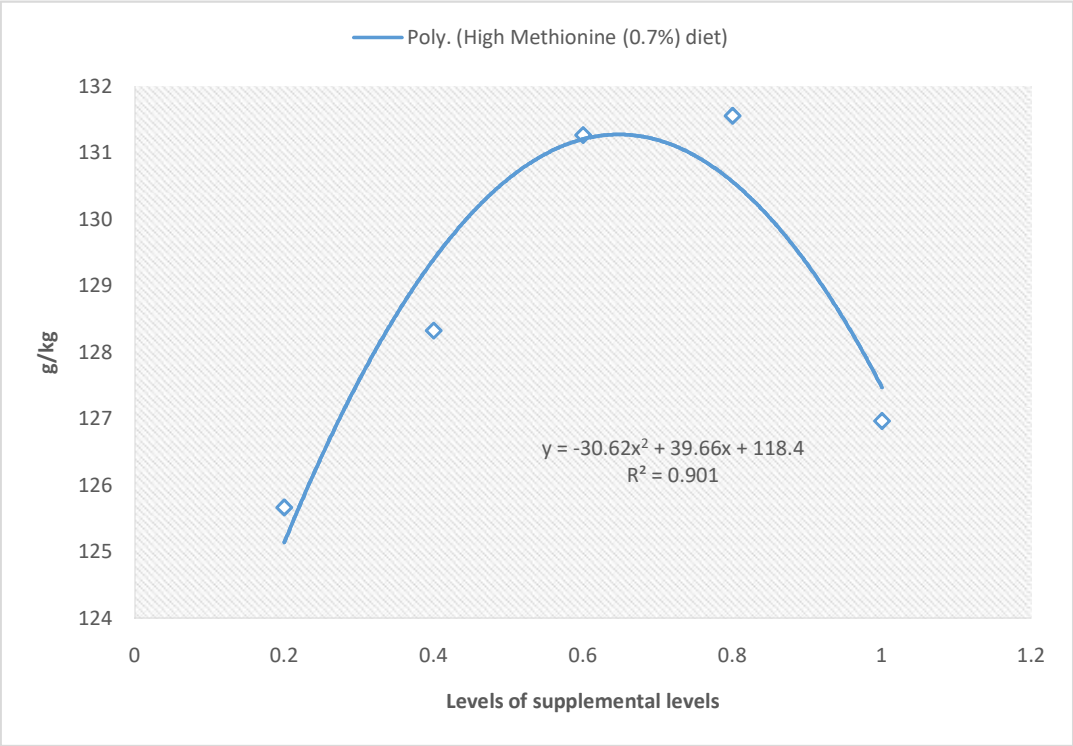

Figure 4.15: Quadratic relationship of levels of supplemental glycine and diet containing 0.70% methionine

4.2.5 Serum biochemistry of broiler chickens fed different dietary concentrations of methionine and supplemental glycine

The outcome of the serumparameters of birds offered diets containing varied levels of Met and SGly is shown in Table 4.13. Serum total protein, albumin, creatinine, glucoseand triglycerides were not affected ($P < 0.05$) by increasing levels of SGly and Met concentrations in the diets except for the serum uric acid (SUA) and serum ammonia (SA) concentrations. IncreasingMetlevels from 0.3% to 0.7% resulted to an increase($P < 0.05$) in SUA concentration of the birds. Thus, birds fed diet containing 0.30 and 0.50% Met levels recorded a lower ($P < 0.05$) concentration of SUAvalues of 2.95 and 3.06mg/dL, respectively, than those on diet with 0.7% Met (3.43mg/dL). A decrease in SA concentration was obtained ($P < 0.05$) in treatment containing 0.50% Met (3.96mg/dL) than those with 0.30% (4.27mg/dL) and 0.70% (4.34mg/dL) Met concentrations. Increasing levels of SGly in the feed showed a decreasing quadratic ($P < 0.05$) effect on SUA and SA concentration of the birds, with estimated optimalresponse level of 2.10% Gly+Ser.Thus, birds fed 0.60% SGly diet had the least ($p < 0.05$) concentration of SUA (2.90mg/dL) and was similar to those group fed 0.8 and 1.0% SGly diet when compared to 0.2 and 0.4% SGly diets. Also, a lower ($p < 0.05$) SA concentration of 4.03mg/dL was recorded in birds fed 0.6 and 0.8% SGly diets and were similar ($p > 0.05$) to 4.09mg/dL obtained by those fed 1.0% SGly diet but differed from those group on 0.2 and 0.4% SGly diets with mean values of 4.54 and 4.30mg/dL, respectively. The significant relationship of increasing levels of SGly on SUA and SA is represented in Figure 4.16 and 4.17, respectively. The regression equation from the graphs depicted that 88.9% and 97.68% of the total improvement observed in SUA and SA, respectively, were attributed to glycine supplementation.

Table 4.13: Blood biochemical indices of broilers (1-21 d) fed low protein diets containing different concentration of methionine with varying levels of supplemental glycine.

Nutrient levels (Main effects)	Creatinine (mg/dL)	Uric acid (mg/dL)	Total protein (g/dL)	Glucose (mg/dL)	Ammonia (mg/dL)	Albumin mg/dL	Triglycerides (mg/dL)
SGly (%)							
0.20	3.19	3.45[a]	2.53	246.46	4.54[a]	1.28	85.53
0.40	3.27	3.24[ab]	2.65	253.92	4.30[a]	1.21	88.35
0.60	3.30	2.90[c]	2.46	243.47	4.03[b]	1.19	83.77
0.80	3.39	3.04[c]	2.60	249.06	4.03[b]	1.19	85.04
1.00	3.09	3.13[bc]	2.56	246.74	4.09[b]	1.22	83.97
SEM	0.12	0.14	0.16	6.77	0.14	0.04	4.23
Met (%)							
0.30	3.11	2.95[b]	2.54	254.38	4.27[a]	1.23	86.14
0.50	3.26	3.06[b]	2.57	247.00	3.96[b]	1.24	87.95
0.70	3.37	3.43[a]	2.57	242.41	4.34[a]	1.18	81.91
SEM	0.09	0.11	0.12	5.24	0.11	0.03	3.10
Anova	**----------P-values----------**						
Met	0.35	0.01	0.38	0.78	0.02	0.29	0.41
SGly	0.12	Q=0.03	0.96	0.89	Q=0.06	0.51	0.47
SGly*Met	0.28	0.37	0.37	0.83	0.96	0.13	0.88

[a-b]Means of thesame letters are similar ($P \leq 0.05$).

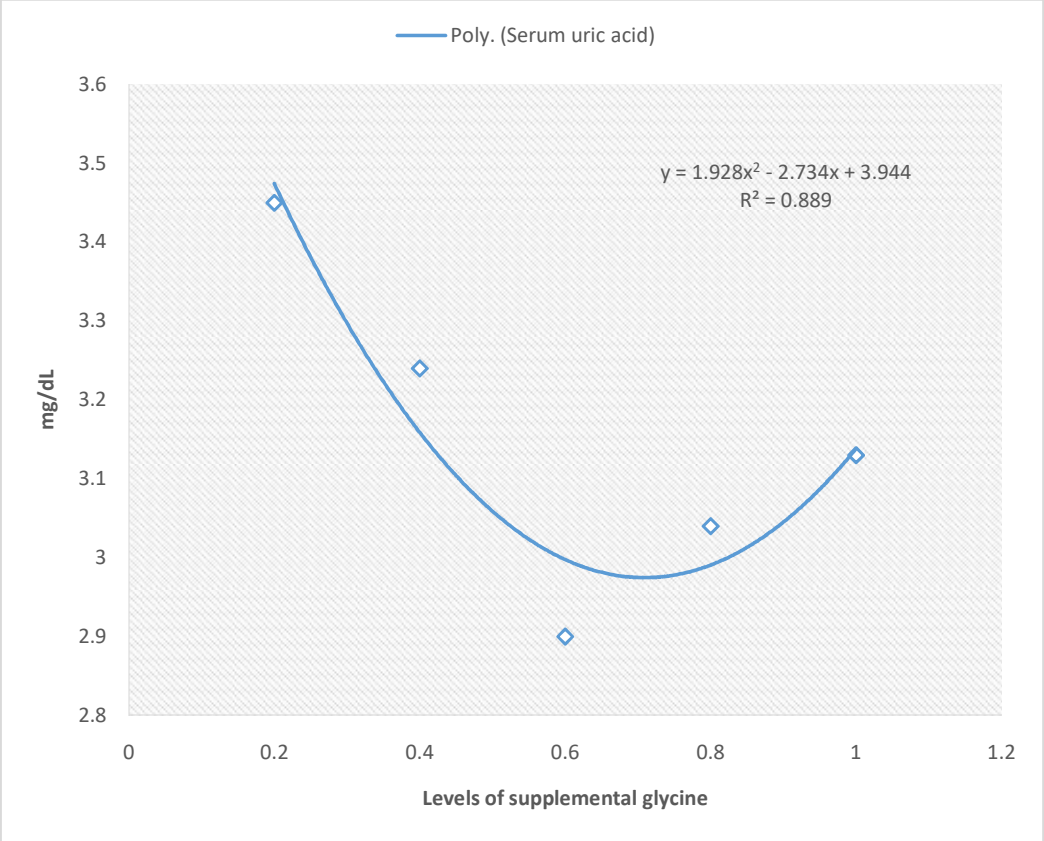

Figure 4.16: Quadratic relationship between SGly and SUA in broilers supplied with low crude protein diet

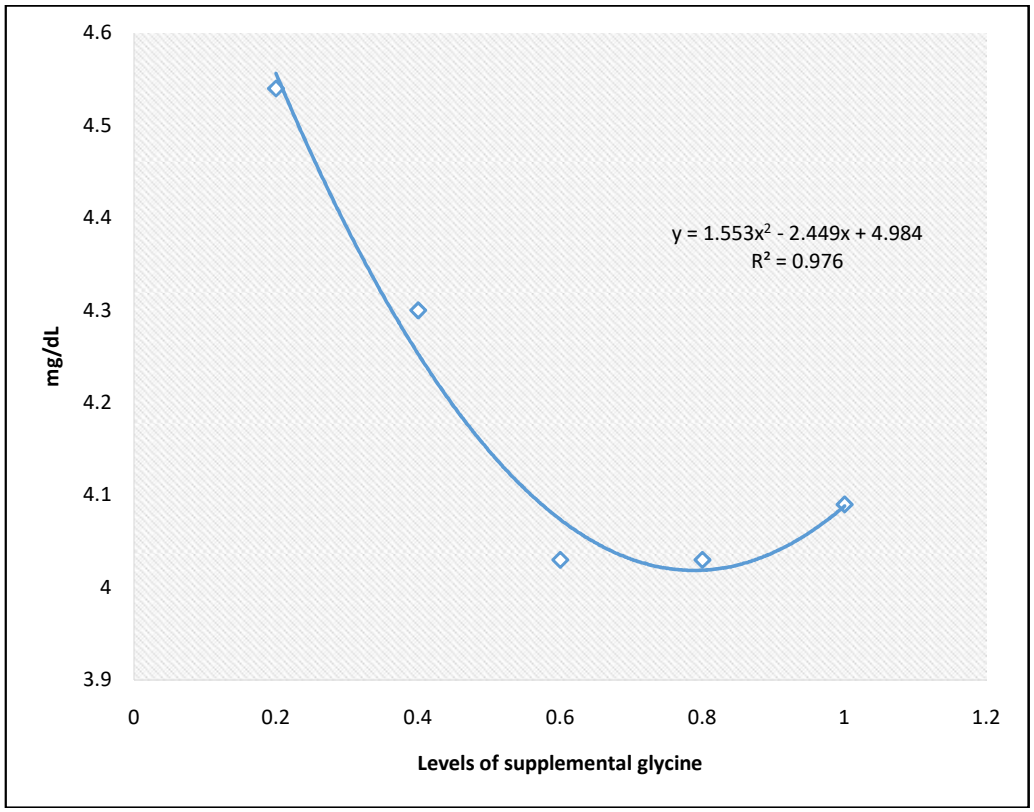

Figure 4.17: Quadratic relationship between SGly and SA in broilers supplied with low CP diets

4.2.6 Interaction effect between levels of methionine and glycine supplementation in the diet of birds on serum biochemistry

The result of interaction effects of dietary Met and glycine supplementation is displayed in Table 4.14. The interaction between dietary Met and SGly levels was not significant ($p > 0.05$) as observed for all evaluated serum indices. Thus, means of serum total protein, albumin, creatinine, uric acid, glucose, ammonia, and triglyceride were statistically similar among the various dietary combinations of Met and SGly.

Table 4.14: Interaction between varied concentrations of methionine and glycine supplementation on serum biochemical parameters of broiler chickens (1-21 d)

Met (%)	SGly (%)	Creatinine (mg/dL)	Uric acid (mg/dL)	Total protein (g/dL)	Glucose (mg/dL)	Ammonia (mg/dL)	Albumin mg/dL	Triglycerides (mg/dL)
0.30	0.20	3.20	3.28	2.70	250.18	4.60	1.26	87.44
	0.40	3.16	3.04	2.46	269.35	4.48	1.26	87.54
	0.60	3.09	2.91	2.65	255.09	3.95	1.29	91.61
	0.80	3.05	2.87	2.21	243.44	4.28	1.20	84.73
	1.00	3.04	2.67	2.67	253.82	4.04	1.16	79.38
0.50	0.20	3.26	3.19	2.31	248.89	4.36	1.20	93.24
	0.40	3.29	3.11	2.66	244.10	3.98	1.22	98.33
	0.60	3.42	2.81	2.27	233.24	3.89	1.19	81.15
	0.80	3.44	2.89	2.94	263.04	3.74	1.26	84.49
	1.00	2.90	3.29	2.66	245.75	3.83	1.34	82.55
0.70	0.20	3.11	3.87	2.59	240.30	4.55	1.37	75.91
	0.40	3.35	3.56	2.82	248.31	4.43	1.16	79.17
	0.60	3.38	2.98	2.45	242.09	4.23	1.09	78.56
	0.80	3.67	3.35	2.64	240.71	4.08	1.12	85.90
	1.00	3.33	3.41	2.36	240.64	4.40	1.14	89.99
SEM		0.21	0.24	0.28	11.72	0.25	0.07	9.59
Anova		**----------P-values----------**						
SGly*Met		0.28	0.37	0.37	0.83	0.96	0.13	0.88
SGly*0.30% Met		---	---	---	---	---	---	---
SGly*0.50% Met		---	---	---	---	---	---	---
SGly*0.70% Met		---	---	---	---	---	---	---

[a-b] Mean valueshaving same letterss are statistically similar ($P < 0.05$).

4.2.7 Lipid oxidative stability (TBARS Evaluation) of broiler chickens fed dietary levels of methioinine and glycine supplementation.

TBARS values (lipid oxidation) of birds fed supplemental Gly and Met diets are presented at Table 4.15. The interaction effects ofdietary Met concentrations and SGly levels was found to be significant ($p < 0.05$) for lipid oxidative stability. Birds fed 0.50% Met diet with 1.00% Gly level had the lowest ($p < 0.05$) TBARS value of 0.67mg/kg while birds fed 0.50% Met and 0.20% Gly diet combination had a significantly higher ($p < 0.05$) TBARS value (1.03mg/kg) compared to other dietary treatment combinations. At lower (0.30%) and adequate (0.50%) Met diets, increase in TBARS values were recorded with increasing levels of SGly while at higher Met diet (0.70%), TBARS values decreased with increasing SGly levels.

Met levels had no significant influence ($p > 0.05$) on the TBARS values of the breast meat of the broiler chickens. The TBARS values ranged from 0.81mg/kg (0.30% Met) to 0.89 mg/kg (0.50% Met). Supplemental Gly levels did not affect($p > 0.05$) TBARS values observedon the breast meat of the birds. The TBARS values obtained for birds fed SGly diets were 0.87, 0.90 and 0.788 mg/kg for diets with 0.20, 0.60, and 1.0%, respectively.

Table 4.15: Lipid oxidative stability of broiler chickens fed low protein diets containing different dietary concentrations of methionine and supplemental glycine.

Met (%)	SGly (%)	TBARS (mg malondialdehyde /kg)
0.30	0.20	0.802^{b}
	0.60	0.782^{bc}
	1.00	0.841^{abc}
0.50	0.20	1.034^{a}
	0.60	0.950^{ab}
	1.00	0.673^{c}
0.70	0.20	0.765^{bc}
	0.60	0.971^{ab}
	1.00	0.852^{abc}
	SEM	0.09
Main effects		
SGly levels (%)		
	0.20	0.867
	0.60	0.901
	1.00	0.788
	SEM	0.06
Metlevels (%)		
	0.30	0.808
	0.50	0.885
	0.70	0.862
	SEM	0.06
Anova		**P-values**
	SGly	0.16
	Met	0.43
	SGly x Met	0.01

[a-c]Means bearingvaried superscripts show statistical difference ($P < 0.05$).

4.3 EXPERIMENT 3

4.3.1 Performance traits of chicks on low protein diets with varying SID Thr and supplemental glycine levels

The impact of varied dietary concentrations of SID Thr and SGly levels on performance response of birds on low protein diet is shown in Table 4.16. Increasing dietary SID Thr level had no significant effect on AWG (P=0.49), however, significant differences ($p < 0.05$) were recorded on AFI (P=0.04) and feed:gain ratio (P=0.001) of the birds. Birds fed 0.93% SID Thr diets consumed more ($p < 0.05$) feed (1211.9g) and was similar to those group on 0.69% Thr diet (1208.50g) compared to those fed 0.81% SID Thr diet that had the lowest ($p < 0.05$) feed consumption (1166.30g). A similar trend was observed for FCR where birds fed 0.81% SID Thr diet recorded the lowest ($p < 0.05$) FCR of 1.39 compared to higher ($p < 0.05$) of 1.46 and 1.48 obtained in birds fed 0.69 and 0.93% SID Thr diets, respectively. The AWG and FCR of the birds were significantly ($p < 0.05$) influenced by the increasing levels of SGly while AFI was not affected ($P > 0.05$). As the level of Gly increased in the diets, there was an increasing quadratic effect (P=0.02) for AWG with an estimated optimum point of 2.16% dietary Gly+Ser concentration, while a decreasing quadratic effect (P=0.06) of SGly was observed for FCR with an estimated optimum point of 2.14% Gly+Ser concentration. The quadratic relationship of SGly on AWG and FCR as represented in Figure 4.18 and 4.19, respectively. The regression equation obtained from the graph depicted that 96.03% and 99.02% of the total variations encountered in AWG and FCR, respectively can be attributed to the impact of supplemental glycine inclusion.

Table 4.16: Response of broilers to low protein diet containing varying concentrations of SID threonine and supplemental glycine levels on growth parameters.

SGly (%) SID Thr (%)	AWG (g/bird)	AFI (g/bird)	FCR
SGly (%)			
0.20	784.7	1161.8	1.481
0.40	828.7	1203.2	1.452
0.60	840.3	1200.7	1.429
0.80	842.1	1203.6	1.430
1.00	842.0	1208.6	1.437
SEM	11.77	18.99	0.014
SID Thr (%)			
0.69	829.0	1208.5[a]	1.459[a]
0.81	834.0	1166.3[b]	1.399[b]
0.93	819.7	1211.9[a]	1.480[a]
SEM	9.12	14.71	0.012
Anova	----------P-values----------		
SGly	Q=0.02	0.11	Q=0.06
SID Thr	0.49	0.04	0.001
SGly x SID Thr	0.09	0.78	0.04
SGly*0.69% SID Thr	----	----	L=0.04
SGly*0.81% SID Thr	----	----	L=0.03
SGly*0.93% SID Thr	----	----	Q=0.04

[a-c]Means having different alphabets varied statistically ($P \leq 0.05$).
[1]Values corrected for mortality

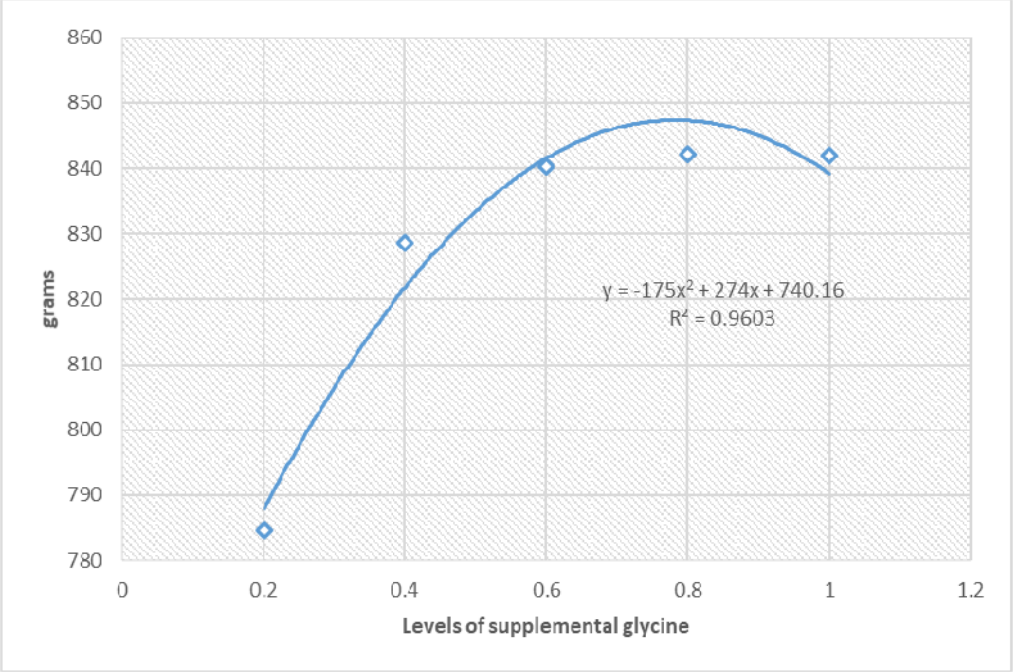

Figure 4.18: Quadratic relationship between glycine levels and body weight gain

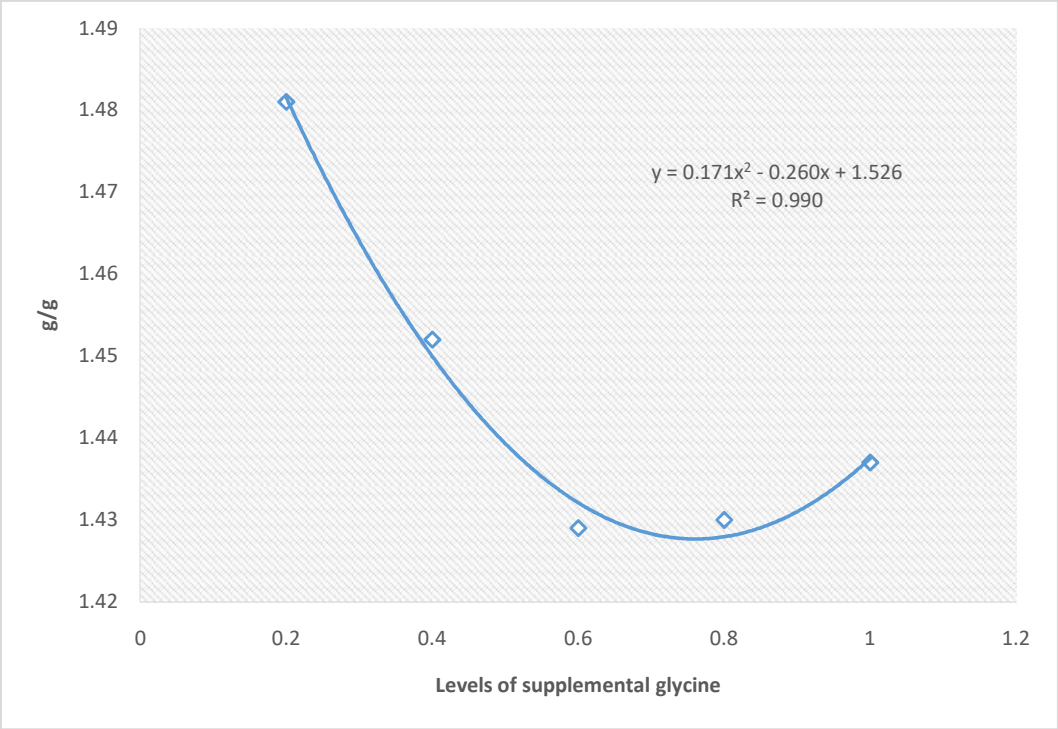

Figure 4.19:Quadratic relationship between glycine levels and feed conversion ratio

4.3.2 Impact of interaction between levels of SID threonine and supplemental glycine on performance of broiler chickens

An interaction between the dietary concentration of SID Thr and SGly levels was only observed to be significant (P=0.04) for FCR, while other variables were not significantly ($p > 0.05$) affected (Table 4.17). The treatment interaction effect disclosed that birds provided with T10 diet had the lowest ($P < 0.05$) FCR of 1.37 compared to birds fed T1, T11 and T15 diets having mean values of 1.50, 1.50 and 1.52, respectively. Moreover, the FCR reducedin a linear ($P < 0.05$) manneras theconcentrations of SGly increases in the diets containing low (0.69%) and adequate (0.81%) SID Thr levels; thus, the higher the level of SGly in these diets, the better the FCR recorded in the birds. However, the FCR of birds fed 0.93% SID Thr diet showed a quadratic ($p < 0.05$) response as levels of SGly increased with an optimal point of 2.0% Gly+Ser being estimated. The relationship of SGly on 0.69, 0.81 and 0.93% SID Thr concentrations was shown in Figure 4.20, 4.21 and 4.22, respectively. The regression equation from these graph revealed that SGly levels accounted for 95.62%, 93.58% and 96.31% of the total improvement recorded in the FCR of birds fed rations having 0.69, 0.81 and 0.93% SID Thr, respectively.

Table 4.17: Interaction between dietary SID threonine and glycine supplementation on growth traits of broiler chickens (1-21 d)

Treatments	SID Thr (%)	SGly (%)	AWG (g)	AFI (g)	Feed:gain
T_1	0.69	0.20	773.7	1160.5	1.500^{ab}
T_2		0.40	826.1	1215.7	1.471^{abc}
T_3		0.60	837.1	1214.3	1.452^{abc}
T_4		0.80	849.1	1226.3	1.445^{abc}
T_5		1.00	858.8	1225.5	1.427^{abc}
T_6	0.81	0.20	777.2	1120.1	1.444^{abc}
T_7		0.40	836.6	1184.4	1.416^{abc}
T_8		0.60	847.1	1176.0	1.388^{abc}
T_9		0.80	853.8	1179.0	1.381^{bc}
T_{10}		1.00	855.4	1172.1	1.369^{c}
T_{11}	1.03	0.20	803.2	1204.8	1.499^{ab}
T_{12}		0.40	823.2	1209.6	1.471^{abc}
T_{13}		0.60	836.9	1211.7	1.448^{abc}
T_{14}		0.80	823.5	1205.4	1.465^{abc}
T_{15}		1.00	811.8	1228.2	1.517^{a}
	SEM		20.38	32.89	0.03
Anova		----------P-values----------			
SGly x SID Thr			0.09	0.78	0.04
SGly*0.69% SID Thr			----	----	L=0.04
SGly*0.81% SID Thr			----	----	L=0.03
SGly*0.93% SID Thr			----	----	Q=0.04

[a-c]Means with thesame letters are statistically similar (P > 0.05).
[1]Values corrected for mortality

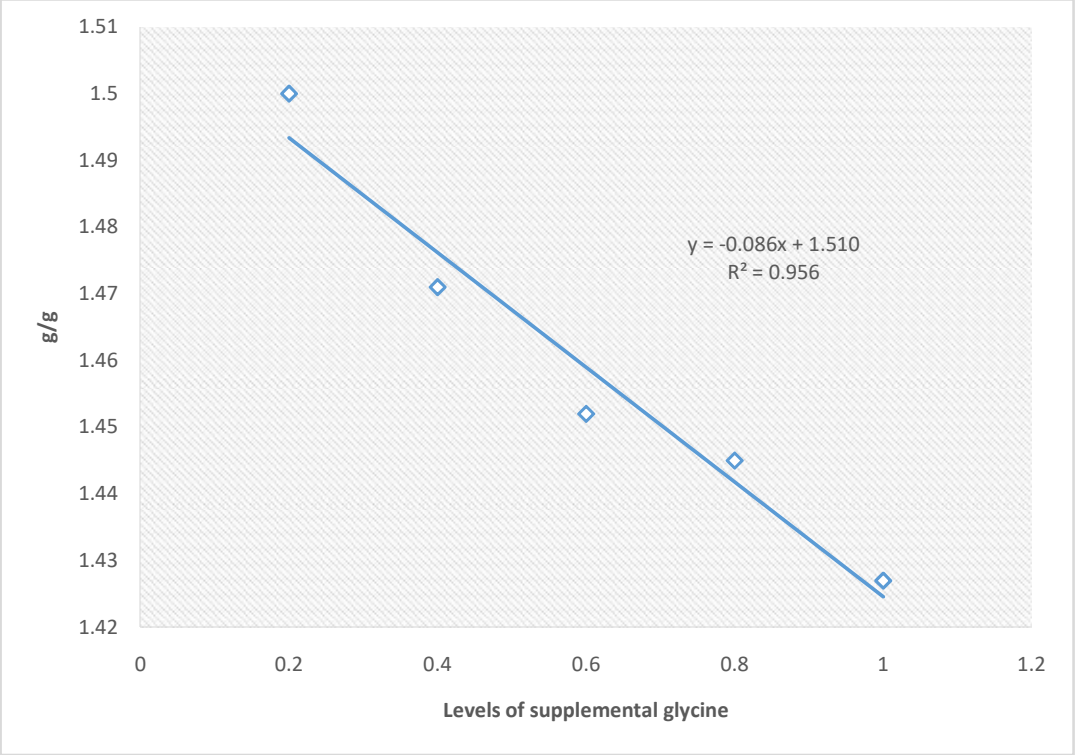

Figure 4.20: Linear relationship of supplemental glycine and low threonine diet (0.69%) on FCR

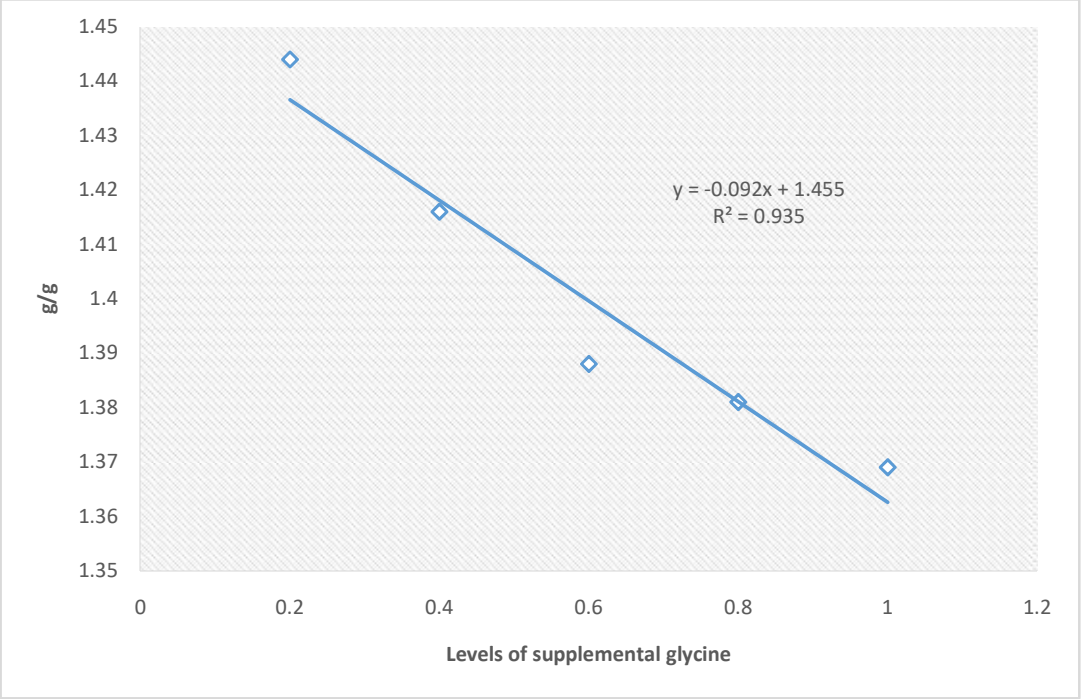

Figure 4.21: Linear relationship of supplemental glycine and adequate threonine diet (0.81%) on FCR.

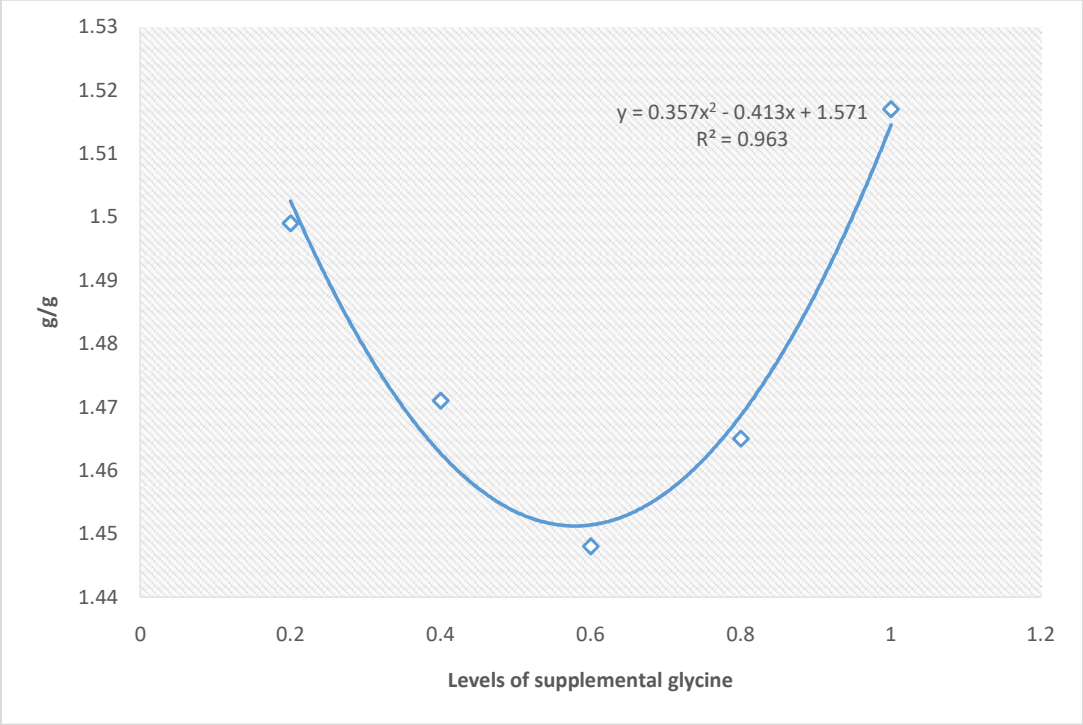

Figure 4.22: Quadratic relationship of supplemental glycine and excess threonine diet (0.93%) on FCR

4.3.3 Serum biochemical indices of experimental birds fed low protein ration with different SID threonine and supplemental glycine levels.

Serum biochemical metabolites of broilers on low protein diet containing varying concentrations of SID Thr and SGly levels are presented in Table 4.18. All serum parameters evaluated did not show any statistical difference ($p > 0.05$) among the graded concentrations of SGly. However, dietary SID Thr levels resulted to a significant difference ($P < 0.05$) onSUA and SA concentrations while other serum variables were not significantly affected ($P > 0.05$). Broilers provided withfeedhaving 0.81% SID Thr level had the lowest ($p < 0.05$) concentrations of SUA (3.71mg/dL) and SA (3.96mg/dL) compared to thoseconsuming 0.69 and 0.93% SID Thr diets.

Table 4.18: Serum biochemical metabolites of broilers fed low protein diets containing varying concentrations of SID threonineand supplemental glycine levels

Nutrient levels (Main effects)	GLU (mg/dL)	TP (mg/dL)	ALB (mg/dL)	SUA (mg/dL)	CRE (mg/dL)	TG (mg/dL)	SA (mg/dL)
SGly (%)							
0.20	248.64	2.72	1.56	4.00	0.25	82.61	4.55
0.40	259.39	2.77	1.58	4.27	0.23	87.61	4.32
0.60	271.28	2.81	1.59	3.64	0.25	79.90	4.23
0.80	266.29	2.75	1.66	4.23	0.23	84.82	4.30
1.00	261.72	2.81	1.59	3.66	0.24	80.25	3.96
SEM	8.44	0.15	0.05	0.13	0.01	3.12	0.22
SID Thr (%)							
0.69	250.58	2.74	1.57	4.16[a]	0.23	84.32	4.55[a]
0.81	267.07	2.84	1.61	3.71[b]	0.24	84.99	3.96[b]
0.93	266.75	2.74	1.62	4.01[a]	0.25	79.80	4.30[a]
SEM	6.54	0.12	0.04	0.09	0.01	2.7	0.17
Anova		----------p-values----------					
SGly	0.21	0.72	0.38	0.14	0.61	0.43	0.09
SID Thr	0.12	0.76	0.64	0.03	0.10	0.22	0.04
SGly x SID Thr	0.62	0.84	0.68	0.01	0.78	0.34	0.06

[a-c]Means with different superscript letters are statistically different ($P < 0.05$).
GLU-Glucose, TP- Total protein, ALB- Albumin, SUA- Serum Uric acid, CRE- Creatine, TG- Triglyceride, SA- Serum Ammonia

4.3.4 Interaction of varied dietary concentrations of SID threonine and supplemental glycineon serum biochemistry of experimental birds

The impact of interaction between SGly and SID Thr levels was not significant ($p > 0.05$) for all the serum indices evaluated except SUA and serum ammonia (SA) concentration (Table 4.19). Treatment interactions showed that birds fed T1 diet recorded a lower ($P < 0.05$) SUA (3.00mg/dL) compared to those offered T1, T2, T3, T9 and T12 diets, whereas a lower ($P < 0.05$) SA (3.46 mg/dL)was recorded in birds fed T6 diet compared to those given T1 and T11 diets. Birds fed 0.69% SID Thr had a decreased linear ($p<0.05$) response on SUA and SA as the levels of SGly increases in the diet. The linear relationship of SGly and SUA in 0.69% SID Thr diet is represented in Figure 4.23. and the regression equation from the curve depicted that 96.95% of the observed variations on SUA of birds fed 0.69% of SID Thr diet is attributed to effect of glycine supplementation. In contrast, no significant ($p > 0.05$) linear or quadratic effect was recorded with increasing levels of SGly in birds fed feedswith 0.81 and 0.93% SID Thr concentrations.

Table 4.19: Interaction effects of varying concentrations of SID threonineand supplemental glycine levelson serum biochemical metabolites of broilers fed low protein diets

Treatments	SGly (%)	SID Thr %	GLU	TP	ALB	SUA	CRE	TG	SA
T_1	0.69	0.20	243.09	2.73	1.60	4.90^{a}	0.22	82.31	5.37^{a}
T_2		0.40	244.32	2.66	1.50	4.39^{ab}	0.22	88.21	4.45^{abc}
T_3		0.60	241.77	2.58	1.47	4.13^{abc}	0.25	79.45	4.37^{abc}
T_4		0.80	255.88	2.90	1.61	3.95^{abcd}	0.21	87.87	4.27^{abc}
T_5		1.00	267.82	2.79	1.67	3.43^{bcd}	0.23	83.77	4.30^{abc}
T_6	0.81	0.20	243.65	2.74	1.55	3.19^{cd}	0.24	83.97	3.46^{c}
T_7		0.40	267.25	2.90	1.70	3.83^{abcd}	0.23	89.01	4.06^{abc}
T_8		0.60	287.23	2.74	1.63	3.00^{d}	0.26	79.94	4.10^{abc}
T_9		0.80	280.53	2.90	1.62	4.70^{a}	0.23	88.52	4.19^{abc}
T_{10}		1.00	256.68	2.93	1.55	3.82^{abcd}	0.23	83.50	4.00^{bc}
T_{11}	0.93	0.20	259.17	2.67	1.54	3.91^{abcd}	0.27	81.56	4.83^{ab}
T_{12}		0.40	266.60	2.75	1.56	4.58^{a}	0.23	85.61	4.44^{abc}
T_{13}		0.60	284.83	3.11	1.66	3.79^{abcd}	0.25	80.30	4.22^{abc}
T_{14}		0.80	262.47	2.44	1.76	4.03^{abcd}	0.26	78.07	4.44^{abc}
T_{15}		1.00	260.66	2.72	1.57	3.73^{bcd}	0.25	73.47	3.59^{bc}
		SEM	14.60	0.27	0.09	0.22	0.02	4.70	0.39
Anova				----------P-values----------					
SGly x SID Thr			0.62	0.84	0.68	0.01	0.78	0.34	0.04
SGly*0.69%Thr			----	----	----	L=0.001	----	----	L=0.04
SGly*0.81% Thr			----	----	----	----	----	----	----
SGly*0.93% Thr			----	----	----	----	----	----	----

[a-c]Means with different superscript letters are statistically different ($P < 0.05$).
GLU-Glucose, TP- Total protein, ALB- Albumin, SUA- Serum Uric acid, CRE- Creatine, TG- Triglyceride, SA- Serum Ammonia

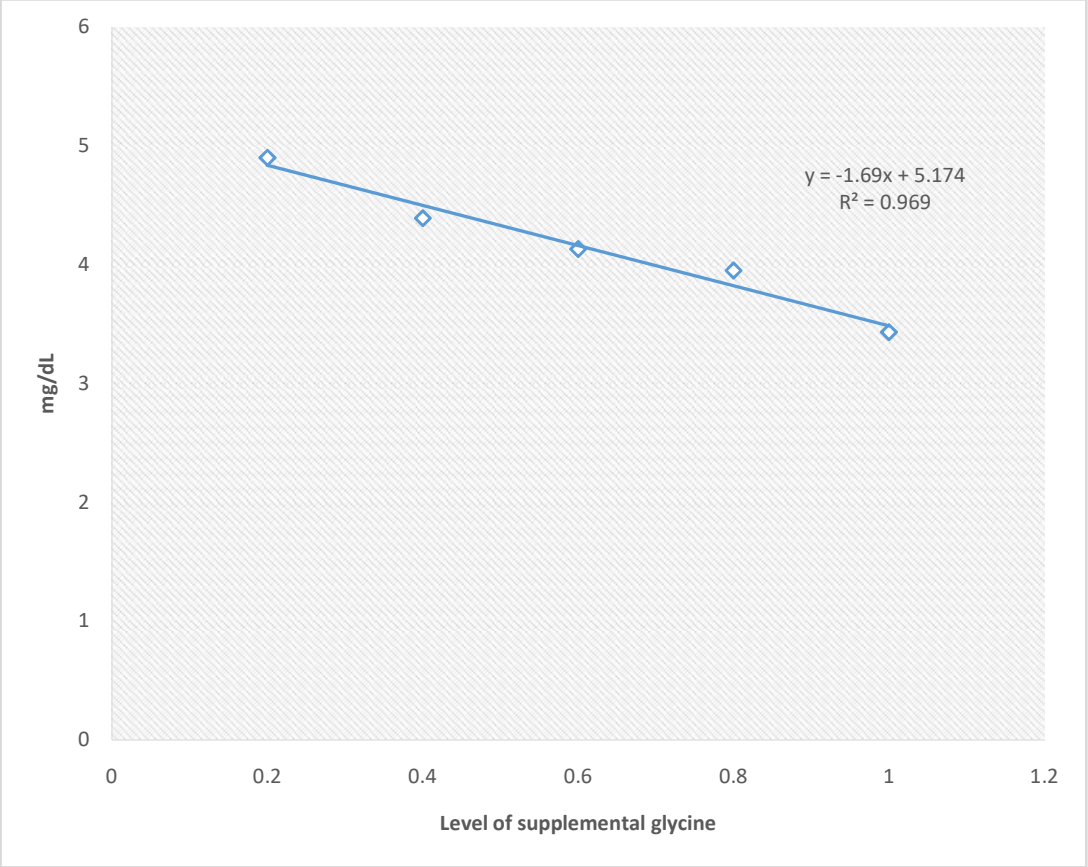

Figure 4.23: Linear relationship of supplemental glycine and serum uric acid concentration in 0.69% SID threonine diet.

4.3.5 Pectoral creatine, breast and liver weight of experimental birds provided reduced CP ration containing different concentrattions of SID threonine and supplemental glycine

Table 4.20presents the results of broilers response to dietary levels of SID Thr and SGly on pectoral muscle creatine concentration, relative breast and liver weight. The interaction between increasing level of SGly and SID Thr showedno impact ($P > 0.05$) on pectoral muscle creatine concentrations, breast and liver weights of the broilers. Considering the main effects, varied SID Thr concentrations did not have any significant ($P>0.05$) effect on the pectoral muscle creatine and other carcass traits measured. However, increasing SGly levels significantly ($p < 0.05$) influenced pectoral muscle creatine and relative breast weight of the birds. Thepectoral muscle creatine had a quadratic ($P<0.03$) response with increasing levels of SGly and a maximum point was estimated at 2.16% dietary Gly+Ser. Thus, birds fed diets with 0.60% SGly level had the highest ($p < 0.05$) concentration of pectoral muscle creatine (3.79mg/g) and were similar to those fed 0.4% (3.49mg/g), 0.8% (3.74mg/g) and 1.0% (3.73mg/g) SGly diets compared to those fed 0.2% SGly diet (3.03mg/g). The breast yield of experimental birds increased ($p < 0.05$) linearlywith response to increasing SGly levels and ranged from 127.09g/kg in 0.20% SGly diet to 133.99g/kg in 1.00% SGly diet. Moreover, the relationship between increasing levels of SGly on pectoral muscle creatine and relative breast weight are displayed in Figure 4.24 and 4.25, respectively. The regression equation from the curve establishes that 97.80% and 98.45% of the observed improvement in muscle creatine and breast meat weight, respectively, were as a result of Gly supplementation to low CP diets.

Table 4.20: Pectoral muscle creatine and carcass traits of broilers on low protein diet containing varying concentrations of SID threonineand supplemental glycine levels.

Treatments	SID Thr %	SGly %	Creatine (mg/g)	Breast (g/kg)	Liver (g/kg)
T_1	0.69	0.20	2.93	128.22	3.39
T_2		0.40	3.49	136.33	3.49
T_3		0.60	3.83	121.03	3.38
T_4		0.80	3.40	128.37	3.81
T_5		1.00	3.48	133.20	3.67
T_6	0.81	0.20	2.99	125.82	3.61
T_7		0.40	3.53	124.46	3.30
T_8		0.60	3.60	131.18	3.22
T_9		0.80	3.88	142.93	2.57
T_{10}		1.00	3.98	133.69	3.66
T_{11}	0.93	0.20	3.17	127.22	3.51
T_{12}		0.40	3.43	127.54	3.10
T_{13}		0.60	3.94	139.06	3.14
T_{14}		0.80	3.95	124.56	3.42
T_{15}		1.00	3.74	135.07	3.26
		SEM	0.27	4.94	0.26
Main effects					
SGly %	0.20		3.03	127.09	3.50
	0.40		3.49	129.45	3.30
	0.60		3.79	130.42	3.25
	0.80		3.74	131.95	3.27
	1.00		3.73	133.99	3.53
	SEM		0.16	2.85	0.15
SID Thr %	0.69		3.43	129.43	3.55
	0.81		3.60	131.62	3.27
	0.93		3.65	130.69	3.29
	SEM		0.12	2.21	0.12
ANOVA	Gly x Thr		0.522	0.330	0.430
	Gly*0.69% Thr		---	---	---
	Gly*0.81% Thr		---	---	---
	Gly*0.93% Thr		---	---	---
	Gly		Q=0.025	L=0.006	0.080
	Thr		0.374	0.770	0.550

[a-c]Mean valueshaving different letters differedsignificantly ($P < 0.05$).

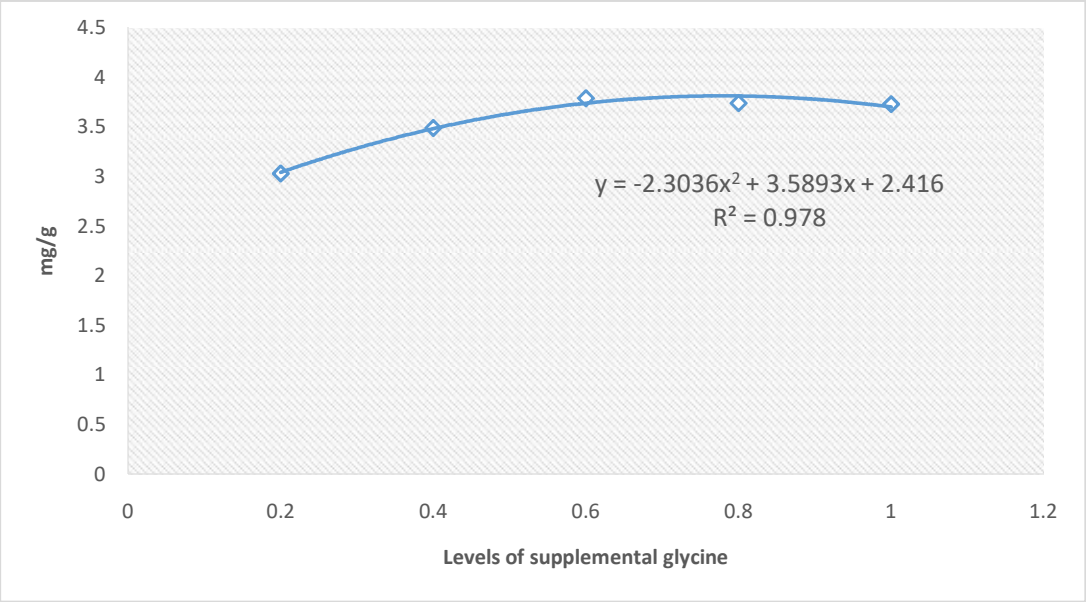

Figure 4.24: Quadratic regression between supplemental glycine levels and pectoral muscle creatine of experimental chicks

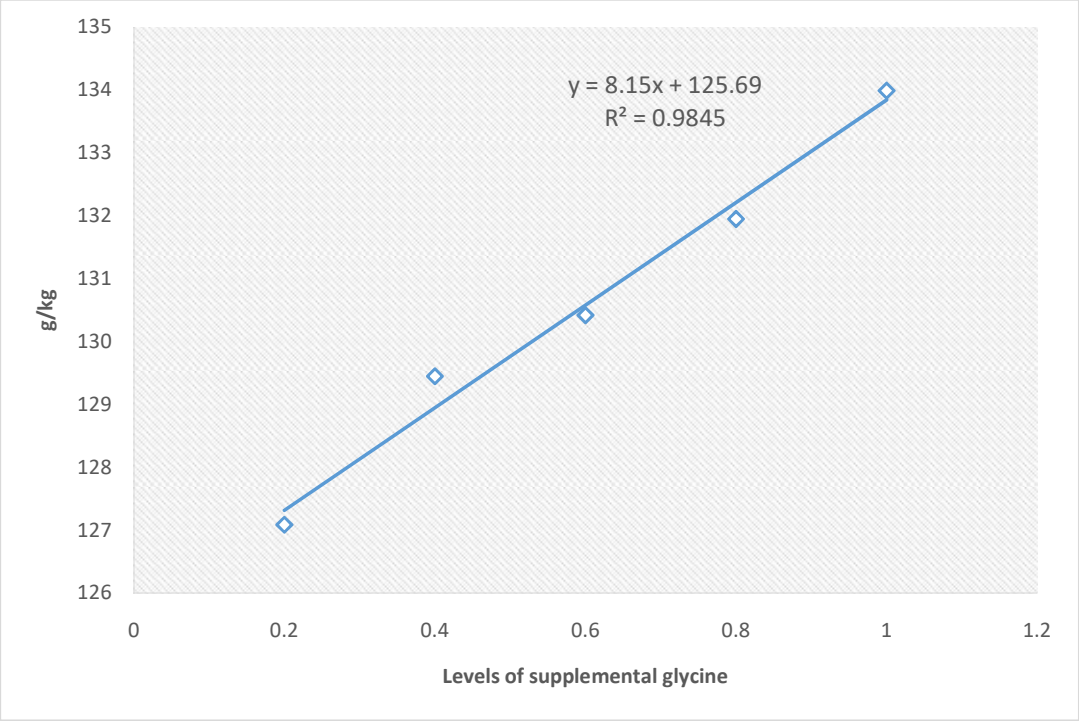

Figure 4.25: Linear relationship between supplemental glycine and breast weight of experimental birds

4.3.6 Lipidoxidative stability of experimental birds offered diets containing varied SID threonine and supplemental glycine levels

The influence of variedconcentrations of SID Thr and SGly on oxidative stability is displayed in Table 4.21. The TBARS values recorded an interaction (P = 0.01)between the varied concentrations SID Thr and SGly. In particular, when the 0.69 and 0.81% Thr diets were fed to the broilers, the TBARS values decreased ($P < 0.05$) by increasing supplemental Gly inclusion levels. The lowest ($p < 0.05$) TBARS values was recorded at 1.00% Gly inclusion level in diet containing 0.81 and 0.69 % Thr as 0.43 and 0.55 mg malondialdehyde/kg, respectively, while the highest ($p < 0.05$) TBARS value of 0.71mg malondialdehyde/kg was noticed in chicks fed 0.81% Thr diet at 0.20% SGly level. However, considering 0.93% Thr diet, increasing inclusion concentrations of SGlydid not affect ($p > 0.05$) TBARS values. There were no SID Thr and SGly main effects ($p > 0.05$) on the oxidative stability of the relative breast weight of the broilers. The TBARS values recorded for the SID Thr and SGly levels ranged from 0.58 (0.81% Thr) to 0.68 (0.93% Thr) mg malondialdehyde/kg and 0.55 (1.00% Gly) to 0.66 (0.20% Gly) mg malondialdehyde/kg, respectively.

Table 4.21: Oxidative stability of experimental birds fed low protein rationwithdifferent concentrations of SID threonine and glycine supplementation

SID Thr (%)	SGly (%)	TBARS (mg malondialdehyde /kg)
0.69	0.20	0.66[ab]
	0.60	0.65[ab]
	1.00	0.55[bc]
0.81	0.20	0.71[a]
	0.60	0.60[ab]
	1.00	0.43[c]
0.93	0.20	0.66[a]
	0.60	0.68[a]
	1.00	0.69[a]
	SEM	0.11
SGly (%)		
0.20		0.66
0.60		0.65
1.00		0.55
SEM		0.71
SID Thr (%)		
0.69		0.62
0.81		0.58
0.93		0.68
SEM		0.06
Anova		
SGly		0.17
SID Thr		0.35
SGly x SID Thr		0.01

[a-b] Values bearing different letters differ significantly ($P < 0.05$).

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

STUDY 1: Response of broilers to diet containing varied concentration of crude protein and glycine supplementation

5.1.1 Performance of experimental birds

The present experiment investigated the impact of Gly supplementation to varied concentrations of crude protein in diets of broiler chicks at 21 d of age. Performance attributes of experimental birds (1 – 21 d) offered various concentration of CP and supplemental Gly (SGly) revealed that average weight gain (AWG) and FCR were influenced by CP levels or supplemental Gly; whereas, feed intake remained unaffected. From our result, areduction in dietary CPlevel from 22 to 18% led toa corresponding decrease in AWG and increase in FCR. Similar trend was equally recorded for supplemental Gly; where improved growth rate and FCR were observed in birds fed 0.4% SGly diet than those on 0.2% and 0.0% diet group. This implies that birds fed higher CP and SGly diet of 22% and 0.4% level, respectively, showed improved AWG and FCR than those offered diets with 20% CP and 0.2% SGly levels, while those on low CP (18% CP) and SGly (0.0% Gly) diets recorded the least AWG and FCR. Thus the data suggested level of 22% CP and 0.4% Gly appear to be adequate for commercial broilers during the starter phase than 20 and 18% CP, and 0.2 and 0.0% SGly levels. Increased efficiency of dietary CP at higher concentrations might have increased the protein availability, which in turn supported the growth in broilers fed 22% levels of CP in diet. The resultof the present study agrees with the findings of earlier workers who reported similar findings of improved AWG and improved FCRwith adequate CP concentrations (Waldroup, 2000; Si *et al*., 2004a, b;Sterling *et al*., 2005; Kriseldi*et al*., 2018). On the other hand, decreasing CP level in diets beyond 20% to 18% resulted to a decreased performance. Earlier studies showed that incremental decreases in the dietary CP level resulted in low growth rate of broiler chickens (Namroud *et al*., 2008; Hernandez *et al*., 2012;

Wang *et al*., 2020).On the other hand, the present result did not agree with the reports of El-Maksoud *et al*. (2011) and Srilatha*et al*. (2018), who indicated higher growth rate in broiler offeredlowdietary CP levels with additional crystalline EAAs that meet the minimum NRC (1994) specifications than those provided with higherCP diet. Several researchers have confirmed that birdsprovided withreduced CP dietsfortified with EAA shown similar growth rate as those given adequate CP ration (Aletor *et al*., 2000 (23% vs 18%); Ciftci and Ceylan (2004) (21.30% vs19.13%) Namroud *et al*., 2008 (23% vs 21%); Srilatha*et al*., 2018 (21% vs 19%)]. Besides, Hernandez *et al*. (2012) and McGill *et al*. (2012) confirmed that 3% reduction of dietary CP level with sufficient EAA fortication can be achieved without any detrimental effect on performance in broilers. In contrast, several studies (Jiang *et al*., 2005; Waldroup *et al*., 2005; Farkhoy *et al*.,2012) revealed thatsupplementing low CP diet without AAs did not improveAWG and feed:gainin broilers.In the current study, only concentrations of the Met and Lys were sufficiently considered in the low CP diets unlike as observed in some earlier studies that considered additional limiting EAA such as threonine, valine, isoleucine, arginine, and trytophan concentrations. Si *et al*. (2004a) validated that18% CP diet contained lower concentrations of Thr, Val, Ile, Arg, and Trp by 5, 7, 10, 12, and 31%, respectively, compared to rations with CP level of22% CP. This observation suggested that the negative effects of reducing dietary CP levelscould be as a result of lower concentrations of less limiting AA in low CP diets apart from Met and Lys (Bregendahl *et al*., 2002; Si *et al*., 2004b).

Several explanations for the disparities in growth of broilers givenreduced CP diets have been suggested.Such variations include the concentrations of CP and AA supplementation; feed stuffs used, specific amino acid concentrations, and age of bird and strain (Corzo *et al*., 2005). Earlier studies have shown that poor performance of birds was not linked to decreased feed intake compatibility between EAA and Non-EAA (Waldroup*et al*., 2005), branched-chain amino acid interactions (Waldroup*et al*., 2002), or the proportion of tryptophan and broad neutral AAs(Si *et al*., 2004a). Meanwhile, several authors have reported poorer performance of birds fed diets with low dietary crude protein level could be attributed to inadequate N quantity for NEAA production (Nyachoti *et al*., 2006; Awad *et al*., 2017). Broilers consuminggreater quantities of CP in theirration can produce NEAA from surplus EAA in the body. Hence, when broilers are fed diets with low protein concentrations, surplus EAA is decreased, thereby rendering them less accessible for transformation to the NEAA (Waldroup, 2007). Berres *et al*. (2010) indicated that it is important to supply non-essential

N to broilers given low-CP diets, especially at the beginning of growth development, as dispensable AAs may be limited beyond a particular amount of dietary CP.Nevertheless, the productive variables of chickens provided with individual NEAA supplementation other than Gly (Dean *et al*., 2006; Awad *et al.,* 2014a) could have responded negatively in many studies Gly levels may need to be considered explicitly, instead of overall NEAA concentrations, when formulating low-protein feed (McGill *et al*., 2012). Many findings have shown that glycine is the restricting NEAA component and may become inadequate when crude protein concentrations are reduced in diets formulated solely with plant ingredients (Ospina-Rojas *et al*., 2013a; Awad *et al*., 2017). These results are consistent with the fact that practical maize-soyabean diets respond to additional SGly inclusion because of the low level of Gly content in the diet based on vegetable ingredients. ParrandSummers(1991)observedthat providing Gly as a source of dispensable amino N in diet containing approximately 20% CP showed improved growth rate.

The interaction between SGly and CP levels showed a marked improvement in AWG and FCR of the birds as the level of CP reduced in the diet from 22 to 18% with corresponding increase in SGly level from 0.0 to 0.4%. This observation shows an indication of the synergistic relationship between increasing SGly levels and reducing dietary CP concentrations in maintaining optimum performance of broiler chicks. Thus, interaction of 22% CP and 0.4% SGly depicted a relative higher AWG and better FCR among other dietary combinations. The higher need of glycine in high CP level diet (22% CP) may be linked to the role of Gly in nitrogen excretion since it is basically utilized as a component of uric acid synthesis (Corzo *et al*., 2004;Berres *et al*. 2010).Hence, the higher CP diet generally provided at the pre-starter and starter phases of broilers will contribute to a higher demand for excretion of nitrogen and allows more glycine to produce uric acid. This could clarify the rising demand for Gly in diets that contain high amounts of dietary CP (Akinde, 2014b).It is important to realize that glycine improves zootechnical efficiency when complemented with regular CP diets that are appropriate for essential AAs; thus, Gly can be considered a growth booster.This is consistent with the findings of Heger and Pack (1996), which shown that the demand for Gly+Ser increased as the dietary CP concentration increases. In consideration of Gly's function in eliminating surplus N as uric acid, it becomes reasonable to suggest that low CP diets lack strong demand for Gly compared to standard protein ration, where the latter includes several AAswhich tend to surpass the requirement of the broiler chicken (Aftab *et al*., 2006).

Nevertheless, there is increasing understanding that in low CP diets, the demand for Gly is probably higher and a Gly deficit is the primary cause of decreased performance in birds offered diets that are relatively less in CP concentration (Awad *et al*., 2017; Hohmann *et al*., 2019; Wang *et al*., 2020). This corroborated with the present result which depicted an increased performance when SGlylevel was increased to 0.4% in birds consuming 20% and 18% CP diets.This advantage of reduction in dietary CP levels is connected with decreased Gly+Ser abundance in the feed as feedstuffs with protein origin decline(Yuan *et al*., 2012); and increased Gly need in the maintenance of metabolic activities including mucin secretion (Wang *et al*., 2014). Composition of rations with reduced CPlevel include a reduction insources of intact protein such as soybean meal containing greater amount of Gly;thus, becoming necessary to explicitly evaluate Gly concentrationsinstead of overall NEAA levels when preparingminimal CP ration (Waldroup *et al*., 2005; Dean *et al*., 2006). Namroud *et al*. (2008) found that addition ofexcess amounts of crystalline AAs to low intact CP rationincreases the concentrations of blood and excretory ammonia, which can contribute to reduced performance and appetite due to adverse impacts on tissue synthesis.

As obtained in the present result, Waldroup *et al*. (2005) found similar findings that AWG and FCR of birds given 20% CP diets were 6% lower and 3% more than birds consuming the 22% protein ration, respectively. Addition of 0.4% SGly to broilers consuming 20% CP diet culminated in a comparable BW with birds receiving 22% CP diet, but no improvement in FCR was observed when complementing 0.4% SGly to 20% CP diet compared to those groups fed 22% protein ration; unlike the broilers fed 18% CP diet, thouogh there was an improvement in AWG and FCR when SGly increased from 0.0 to 0.4%, but these parameters could not equal to birds fed 20 and 22% CP diets. Such studies could show the value of sustaining sufficient, less restricting concentrations of AA with increased SGly to provide higher dietary Gly+Ser to help restore growth performance of broilers given minimal level of CP in the feed (Si *et al*., 2004a; Waldroup *et al*., 2005).It has been documented that incorporating crystalline Met, Lys, andThr into ration of birds led to a 1.9% decrease in dietary CP relative to incorporating solely DL-Met (Yuan *et al*., 2012). According to Kriseldi*et al*. (2018), fortifying a corn-soybean based dietwithsupplemental EAAs and Glycan successfully achieve a 4%-point reduction in CP content thana similar diet accompanied only by supplemental DL-Met at the starting period of broiler production. Previously, most publications have shown that NRC (1994) recommended Gly+Ser level of 1.25% was inadequate and suggested a level of 1.80% (Corzo *et al*., 2004), 2.32% (Dean *et*

al., 2006), 2.10% (Waguespack *et al*., 2009), 2.00% (Yuan *et al*., 2012), 2.03% (Awad *et al*., 2015) and 2.13% (Kriseldi*et al*., 2018) for lowCPdiettoachieveoptimalgrowthinyoungchicks. Moreover, in the present study, though AWG and FCR was improved when 0.4% SGly wasaddedtothe low CP diet (18% CP) which provided 1.85%total Gly+Ser concentrations, but this improvement could not match to those group fed standard CP diet (22% CP). This could be an indication that levels of Gly+Ser in 18% CP dietwere not sufficient to support optimal growth. Therefore, this current study validates the reports of recent findings that the need of Gly+Ser of birdsis dependent on the degree of CP levelreduction in the feed (Kriseldi *et al*., 2018; Hofmann *et al*., 2019; Wang *et al*., 2020).

5.1.2 Carcass qualities

The varied concentrations of CP and glycine supplementation did not show any influence on most carcass attributes but affected weight of abdominal fat of the experimental birds. The similar mean values obtained in carcass prime cuts of broilers fed different dietary CP levels recorded in our result is in agreement with the report of earlier researchers (Fancher and Jensen, 1989; Srilatha *et al*., 2018). Also, previous findings have reported that lowering dietary levels of CP usually does not influence the relative breast weight of birdprovided the concentration of limiting amino acids were maintained (Rezaei *et al*., 2004; Sterling *et al*., 2002, 2006). According to Kerr and Kidd (1999a, b), a decrease in the CP level with AA incorporation in the rationshowed no influence on breast weight. Considering the liver as the vital organ responsible for lipid metabolism in birds, they are predicted to risebecause of increased lipogenesis in broilers fed decreased quantities of dietary CP due to higher calorie:protein(Swennen *et al*., 2006). However, many authors (Sterling *et al*., 2006; Ardekani and Chamani, 2012) reported no significant effect of dietary protein levels on liver weights and this is in accordance with the result of the current investigation. Deposition of fat in abdominal area significantly increased in broiler fed diet with lower CP (18%) level but was lower in those given high CP (20 and 22%) levels. One significant factor to be considered when examining the impact of low CP diets is the accumulation of abdominal fat. It was indicated that when the concentrations of AA are below the requirement, there is a tendency of increase feed consumption in order to cover up the insufficient AAs, and beisdes, excess energy absorbed would be accumulated as fat in the abdominal region (Bartov, 1979). Many researchers have found similar results for fat deposition (Moran*et al*., 1992;KiddandKerr,1999). Consistent with our results, increased fat

depositions associated with low proteinrations were documented and this could be linked to increasedcalorie:protein proportion in rations with reduced CP concentrations, thateventuallycausesexcess energy being available to that needed in protein synthesis, thereby leading to high rate of lipogenesis (Aletor *et al*., 2000; Namroud *et al*., 2008).Widening the energy and protein ratio was reported to predispose fat deposition in chicken due to excess intake of energy per unit intake of CP (Srilatha*et al*., 2018). Broilers alter feed intake to meet the requirement of CP/AA in diets containing adequate levels of energy. This theoryis in accordance with the results of Corzo *et al*. (2010) who proved that wider energy and protein ratios in low-CP diets increases consumption of dietary energy, which might have deposited as fat after meeting the energy requirement. Contrary to this report, earlier studies reported reduction of CP concentration in the diet was not connected with excess intake of feed; thus no major effect was observed on abdominal fat composition (Awad *et al*., 2014b; Gheisari *et al*., 2015).

An interaction observed between CP and glycine levels on relative breast weight and abdominal fat of the birds revealed that increasing levels of glycine resulted to a corresponding increase in breast meat weight and reduced abdominal fat across all dietary CP concentrations. Relative breast meat and abdominal weight of broilers fed diet containing 22, 20 and 18% CP at 0.4% SGly were similar to those fed 0.2% SGly but were better than those fed diets without supplemental glycine (0.0% SGly). As seen in the present result, it strongly depicts that supplemental glycine is necessary in reduction of CP levels in broiler chicks diet to obtain a higher breast meat with lower abdominal fat deposition. Thus, adequate supplemental Gly may encourage the utilisation of low protein diets by maximizing the use of AA in protein synthesis, making more energy available for N depositionin the breast muscle and less energy to be deposited as fat in the abdominal region (Ospina-Rojas *et al*., 2012; Wang *et al*., 2020). These findings have implications for nutrition in that formulating diets with reducing CP levels containing higher glycine fortification and optimum balance of limiting AA will yield a carcass with more edible meat and less fat. This depicts the significance of Gly in enhancing breast meat yield of chicks given low CP feed, due to the metabolic function of Gly as a precursor in the production of creatine, which has been theprimary source of energy for muscle growth and has the potential to increase nutrient utilisation for muscle development (Ospina-Rojas *et al*., 2013a).

5.1.3 Serum biochemistry

Also, the present study revealed a significant decreased of SUA in birds fed lower dietary CP level than those provided with higher levels of CP. The UA is known as the final product of protein degradations in broilers and many workers have reported the decrease in serum UA due to low CP levels in the ration of birds (Swennen *et al*., 2006; Hernandez *et al*., 2012; Awad *et al*., 2014a). Since the breakdown of excess protein is responsible for UA formation, hence, the observed reduction in UA when lower dietary CP level is provided, is expected and may be linked to the decrease ingestion of CP/AA (Waldroup *et al*., 2005; Namroud *et al*., 2008). According to Corzo *et al*. (2005), decreased concentration of serum UA obtained in birds fed low CP diet could be as result of the shortage of non-essential N synthesis. Hofmann *et al*. (2019) reported that serum UA level reduced substanially as CP concentration declined in diet of broiler chickens at the early stage of growth. Furthermore, increasing level of SGly resulted in a decrease in serum UA concentration, which is a reflection that SGlyincorporation spared variousNEAA and consequentlyincreasedthe utilisation of N (Powell *et al*., 2009; Ospina-Rojas *et al*. 2012). The present result and earlier findings (Jiang *et al*., 2005; Ospina-Rojas *et al*., 2012) revealed that maintaining greater concentrations of Gly+Ser above 1.25% recommended by NRC (1994) is necessary due to that fact that synthesis of UA raises the need for Gly+Ser in broiler chickens fed varied levels of CP. Also, serum triglyceride concentrations increased significantly with lower dietary CP levels, but were not influenced by glycine levels. The diet with 18% CP level resulted in broilers with significant higher serum concentrations of triglyceride than diets with higher CP levels.The higher accumulation of serum triglycerides in birdsfed diets with lower CP levels is linked to the observed corresponding increase in abdominal fat disposition. The current study agrees with the report of earlier authors (Swennen *et al*., 2006; Awad *et al*., 2014b) who demonstrated that decreased level of CP in the diet of birds led to increased plasma triglyceride. The authors further stated that higher blood triglyceride in broiler chicks given reduced dietary crude protein level could be explained as a result of increased energy:proteinratio which might elevate the lipogengesis in the liver.

5.1.4 Apparent crude protein and amino acid ileal digestibility

The coefficient of apparent CP and AAs ileal digestibilities were influenced by different CP levels among the birds. Reducing the levels of CP in the diets had a negative impact on ileal digestibility of CP and AAs of birds. This implies that birds fed higher CP diets showed greater digestibilities of CP and AAs than those fed lower CP diets. This result is in

accordance with the reports of earlier studies which demonstrated that CP digestibility and AAs in the intestinal digesta were increased due to rise in CP levels of the feed (Kamisoyama *et al*., 2009; Boonsinchai *et al*., 2016). This could be linked to the fact that higherlevel of CP in diet of broiler chicks effectively induced the production of cholecystokinin, which facilitated the pancreatic juice secretion (Furuse *et al*.,1999). Thus, since birds fed 22% CP diet group showed higher ileal digestibility of overall AAs than those on reduced CP diet group, it implies that providing adequate dietary CP results in higher magnitude of protein degradation, and consequently increase the amount AA available for uptake in the broiler chicks (Law *et al*., 2015). Moreover, interaction between supplemental Gly and CP levels was observed to be significant for CP and overall apparent ileal AA digestibility and the result showed a synergistic effect of increasing Gly supplementation to varying CP levels in the diets of broiler chicks. At all levels of dietary CP, there was a progressive improvement in the apparent ileal digestibility of DM, CP and overall AAs of broiler chicks with increasing concentration of supplemental Gly. This is in line with the reports of Akinde (2014b) that benefit of Gly supplementation to improving nutritive value of decreasing dietary protein levels could be attributed to improvement in the quality of use of AAs under decreased nitrogen loads by improved intake of Gly.

STUDY 2: Response of broilers to low protein diet with varied levels of methionine and glycine

5.1.5Performance indices

The result of present study indicated that with low protein diet, higher level of SGly (Gly+Ser was seen to improve AWG and FCR of the birds. This was consistent with previous investigationsshowing evidence that SGlyeliminates the reduction in performance encountered in low protein diets (Berres *et al*., 2010; Ospina-Rojas *et al*., 2012; Awad *et al*., 2017). Thus, the significance of lesser protein levels in diet has beenrelated to low dietary Gly + Ser density as protein feedstuffs are reduced, coupled with the higher needs for Gly in maintenance metabolism (Yuan *et al*., 2012). According to Corzo *et al.* (2004), the positive impact of SGly in AA fortified low CP diet reflected more of Gly demand for growth than maintenance related roles. Moreover, Eklund *et al*. (2005) demonstrated that Gly exhibited a vita role in the release of growth hormones; and response to elevated levels of growth hormones is attributed toimproved efficiency of AAutilization. Hence, the observed improvement in feed:gain with increased concentrations of SGly (dietary Gly+Ser) in

reduced dietaryCP may be attributed to the action of Gly as a growth-secreting hormone (Dean *et al*., 2006).As Met level increased to 0.70% in the diet, lowerAWG and feed consumption were recorded as well as increased feed:gain. Thisobservation concurs with earlier reports showing reduction in feed consumption, depressed AWG and poorer FCR of birds fed diets containing excess Met (El-Wahab *et al*., 2015; Sigolo *et al*., 2019). Met has been reported to create negative impact on broilers when fed in excess of its requirement, among the dietary essential AAs (Peng *et al*., 1973). More often, commercial chicken diets consist above the minimum recommendation for Met due to addition of supplemental Met to meet the needs for both Met and TSAA; consequently, Met levels in low CP diets become rather far in surplus of minimum its requirements (Si *et al*., 2004b). Obviously, such diets will be inadequate in Cys, thus formulating diets to meet TSAA requirements coupled with achieving minimum specifications of Met may become unnecessarily escalating the cost of poultry diets and as well promoting possible adverse impactarising from excess levels of Met. This adverse effect of excess Met is attributed to the buildup of serum homocysteine (Sigolo *et al*., 2019).

The interaction detected between Met and SGly concentrations in the ration was significant for feed:gain. In deficient dietary Met concentration (0.30%), feed:gain decreased linearly with SGly levels. This result suggest higher levels of SGly is required to optimize feed:gain in low protein diets containing deficient Met levels. Besides, in absence of a plateau, this linear response of birds for feed:gain is an indication that with lower dietary Met levels, increasing level of SGly could be more than 1.0% added in the diet that supplied 2.32% dietary Gly+Ser concentrationin low protein diet. This agrees with the previous findingswhich observed that the requirement of Gly+Ser in low CP diets seems not to be less than 2.32% for broilers at their earlier stage of growth (Dean *et al*., 2006). In adequate Met (0.5%) diet, a quadratic relationship was observed betweenfeed:gain and increasing SGly levels. The regression equation indicated that increasing Gly supplementation that provided a dietary Gly+Ser level of 2.10% would support the minimum feed:gain. At higher level of dietary Met (0.70%), feed:gain decreased linearly with increasing levels of SGly, thus indicating that feed:gain was optimized at higher level of Gly supplementation in the diet. This improvement in feed:gain could be due to an increased demand for Gly+Ser to aid in the metabolism of surplus TSAA (Powell *et al*., 2011). One possible mechanism by which Gly+Seravailabilityimprove the utilization of Met is through its involvement in facilitating the synthesis of Cys for protein disposition (Powell *et al*., 2009; Hofmann *et al*., 2020).

In Met supplemented diet, the Ser and Gly are considered to be limiting in the synthesis Cys; and with supplus dietary Met level, the transformation of the readily abundant dietary Gly into Ser, would promote an increased rate of Cys production. Supplus Met above requirement would react with Ser to satisfy the requirement of broiler chickens for Cys, being the first AA to be limiting in Met fortified diets (Baker, 2005). Therefore, in the present study,improvedperformance obtained from higher Gly+Ser levels was achieved at Met levels below requirement but not excess Met, justifying the report of Powell *et al*. (2011) who discovered significant improvement in feed:gain when excess Gly was in combination with sub-marginal Met levels than when there is adequate or excess Met concentration. This possibly reveals the metabolic function of SGly in fullfilling the requirement of Cys as a readily accessible precursor during its synthesis. Moreover, the alleviatory effect of Gly on excess methionine has been explained by elevated cystathionine synthesis for which serine is needed as anavailable source of carbon skeleton (Namroud *et al*., 2008).

5.1.6 Pectoral muscle creatine and breast yield

The pectoral muscle creatine and breast weight fed dietary treatments were influenced ($P < 0.05$) by the main effect of SGly and dietary Met levels. Increasing levels of SGly showed quadratic effect on both pectoral creatine and relative breast weights, having a peak maximum response at an estimated Gly+Ser requirement of 2.11 and 2.22%, respectively. This result collaborates the report of Ospina-Rojas *et al* (2013a) and Awad *et al*. (2015), who showedthat Gly+Ser requirement for synthesis of muscle tissue and creatine in broilers at 21 d of age is more critical and higher than 1.25% level of Gly+Ser as recommended by NRC (1994). Moreover, the current study demonstrated that birds fed lower SGly diet had reduced concentration of pectoral creatine compared to those on higher SGly diet. This might be explained by the fact that Gly may have been a limiting factor in the involvement of Arg required for the synthesis of guanidino acetic acid (GAA), which is the immediate precursor of creatine. Because creatine when phosphorylated to phosphocreatine is used to sustain energy homeostasis in the muscles (Wyss and Kaddurah-Daouk1 2000), the increase in pectoral creatine concentration in birds fed diet with adequate dietary Gly levels is an indication ofimproved muscle growth. Furthermore, the increase in pectoral creatine might be related to the increment observed in the relative breast weight in response to higher SGly

because of the significant role of creatine an importantenergy source for muscle tissues (Wyss and Kaddurah-Daouk, 2000; Mitchiels *et al.,* 2012).

Moreover, a two-way interaction between dietary Met and SGly levels were observed for relative breast weight. As SGly levels increased, linear increase in breast weight of broilers given0.30% and 0.50% Met diets was recorded. This is an indication that with deficient and adequate dietary Met, higher glycine supplementation is needed to improve the breast weight of birds when fed low protein diet. However, among birds fed diet with 0.70% Met, a quadratic effect of SGly level was obtained on breast muscle weight, with a maximum response point of 2.06% dietary Gly+Ser level. The improvement in the relative breast weight observed with increasing Gly+Ser level in the diet can be explained by the fact that supplementation of Gly spared Met for the biosynthesis of Cys by increasing the serine availability, thereby increasing the amount of Met for other useful demands such as protein accretion in musclesand improving the efficiency of dietary SAA (Powell *et al*., 2009). To further corroborate this result, Powell *et al*. (2009) confirmed that 2.32% total Gly+Ser level improved marginal and excess TSAA in the diet, which they attributed to higher rate of Cys production arising from increased endogenous Ser formation viaGly supplementation. Hence, it is possible that Gly might be acting as a readily available source of Cys synthesis, by increasing Ser availability (Powell *et al*., 2011).

5.1.7 Serum biochemistry

Increasingdietary Metlevelsup to 0.70% resulted to a higher serum uric acid (SUA)and ammonia concentrations inbroilers compared to 0.3 and 0.5% in the diet. This implies that with increased concentration of dietary Met, there is higher tendency of achieving ahigherSUAand ammonia concentration in the birds, showing an indication of AA imbalance resulting from excess TSAA metabolism. This is corroborated by the reports of previous authors (Xie *et al*. 2004; Donsbough *et al*. 2010) who confirmed higher concentration of SUA of birds as Met concentration increased in the diet. Excess AA intake has been proved to be responsible for their high rate of degradation and thus leading to excretion of N as uric acid as well aspromoting environmental pollution via ammoniaemission (Perry *et al*., 2003; Namroud *et al*., 2008).

Our current investigation shows that a quadratic relationship ($P < 0.05$) exist between increasingSGly levels in the diets and SUA across the treatments. A prediction from the regression equation reveals that an optimum level of 2.10% Gly+Ser was estimated to suppport a minimum SUA concentration, indicating that the greatest response occurred when 0.8% SGly was added to the diets. However, SA concentration of experimental birds was observed to decrease linearly with increasing levels of SGly in the diet. Thepresent investigation indicated that reductions in dietary CP content with higher dietary Gly+Ser level due to Gly supplementation, decreased plasma ammonia and uric acid concentrations of broilers in a manner that may also indicate an improvement in nitrogen utilization (Kriseldi *et al*. 2018). The remarkable linear reduction in concentration of serum ammonia with corresponding decrease in SUA at increasing dietary level of Gly+Ser in the present study is an indication that Gly supplementation positively affects low protein diets by enhancing the efficiency of protein/AA utilization, leading to more decline of dietary EAA specifications. Uric acid is known to be the final substrate of catabolism of protein and provides a clue to the degree of degradation of protein in broiler nutrition. According to Swennen *et al*. (2006), a SUA concentration is inversely proportional to the efficiency of protein utilisation. Furthermore, Powell *et al*. (2009) shown that lower plasma concentration of uric acid observed in birds given low CP diets containing varied TSAA levels with increased Gly fortification, is one possible justification for Gly positive response in improvement of amino acid utilization of broilers fed low protein diet. Thus, considerably lower ammonia and SUA levels with increasing dietary Gly+Ser in chickens fed smaller quantities of CP in their ration is an evidence of decrease in degree of AA degradation in order to secure higher protein for efficient utilization for rapid tissue development. This further explains that diminished protein/AA oxidation results in better dietary CP retention as a corrective mechanism for a decreased protein intake (Namroud *et al*., 2008).

5.1.8 Lipid oxidative stability

The TBA-reactive values measured as mg malondialdehyde/mass in kg of chicken breast were used as a measurement of lipid oxidation within the breast meat. TBARS, an indicator of lipid oxidation showed no differencein birds when offereddiets containing different Met and supplemental Gly levels. The interaction between supplemental Gly and dietary Met concentrations showed thatthe minimumTBARSwas noticed in birds offered the dietary combination of 0.5% Metand 1.0% SGly, indicatingthat in low CP diet with adequate Met

concentration, addition of sufficient supplemental Gly could be a positive influence on oxidative stability of meat in broilers. Gly supplementation also proves to offer a way of making increased PUFA content in the meat more sustainable by inhibiting negative effects on oxidative stability and meat quality during retail storage. The mode of action by which Gly improves meat quality is likely related to its metabolic role as a substrate in glutathione biosynthesis, which increases the activity of glutathione peroxidase, an antioxidant enzyme, thus, glycine works in antioxidative defence (Akinde, 2014a). According to Chen *et al*. (2013), reduced malondialdehyde production may partly be connected to better antioxidant status associated with adequate Met levels in the diet.

Moreover, Met in adequate or excess supply is associated with an improvement in the intracellular antioxidant capacity by meeting the requirement of cysteine which combine with Gly as precursors in glutathione biosynthesis (Melendez-Hevia *et al*., 2009). Glutathione is a peptide that is responsible for mitochondrial detoxification–including free radical neutralization and has sensitivity to charged toxicantsthrough its sulphydryl group action (Friedman, 1994). Some enzymes such as glutathionine peroxidase (GPx) can scavenge produced reactive oxygen species (ROS) that function as antioxidants. Endogenous defense from oxidative damage is provided by enzymes that eliminate free radicals and other reactive species. Increasing GPx production will consequently improve the clearance potential of oxygen-free radicals in broilers (Saleh *et al*., 2018). However, inadequate level of supplemental Gly in low protein diet containing 0.50% Met might have increased TBARS values, thus showing an increase in level of oxidation. This revealed that Gly deficiency becomes a rate limiting step in synthesis of gluthionine due to decrease in the activity of GPx. Consequently, this could result in the decline of broiler meat quality as a result of overproduction of ROS which could cause oxidative damages to several organs including skeletal muscle tissue (Wang *et al*., 2009; Azad *et al*., 2010a,b). According to Lilly *et al*. (2011), the rise in oxidation was presumably as a result of an increase in polyunsaturated fatty acids (linoleic and linolenic acids), which seems more vulnerable to degradation owing to their unsaturated double bonds. This present result suggested that Gly may, through its role as a precursor in the biosynthetic pathway of glutathione, play a role in improving glutathione peroxidase activity, which is a primary component of antioxidant defence systems present in the biological system. Therefore, increasing antioxidant capacity by supplementing Gly at high levels has the potential to manage oxidative stress and reduce loss of exudate and

stabilize fat deposits to deter lipid oxidation of the meat in the birds fed adequate Met concentrations.

STUDY 3: Response of broiler chickens to low protein diet containing varied concentrations of threonine and supplemental glycine

5.1.9 Growth performance

The aim of the current investigations was to evaluate the influence of glycine supplementation in low CP diets containing varied concentrations of SID Thr by broiler chickens (1-21 d). With the main effects, the result demonstrated that birds fed lower dietary SID Thr diets had poorer AWG and feed:gain than those on adequate dietary SID Thr diets. This corroborate the outcome of Dozier *et al*. (2000)who demonstarted that broilers supplied with deficientSID Thr resulted inpoorgrowth due to interruption of the synthesis and utilization of other limiting amino acids.An adequate digestible Thr level is needed in low protein diets to support optimum growth (Al-Hayani, 2017), immune response (Azzam *et al*., 2019) and gastrointestinal mucin production (Kidd and Tillman, 2016), because of its metabolic responsibility as vitalfactor of body protein and displays asignificant functionin increasing the efficiency of utilizationof sulphur amino acid (SAA) and lysine (Kidd, 2000; Ojano and Waldroup, 2002). Thus, the current finding supportedthe result of Al-Sagan*et al*. (2018) who revealed that a marginal dietary deficiency of digestible Thr can result in economic losses due to increased feed conversion efficiency. A quadratic effect of SGly levels was observed for AWGand feed:gain, with an optimum point of 2.16% and 2.14 dietary Gly+Ser, respestively. This result is compatible with the findings of earlier reports (Ospina-Rojas *et al*, 2013b, Awad *et al*., 2017; Belloir *et al*., 2017; Wang *et al*., 2020) investigating the requirement of dietary Gly+Ser in low protein broiler diets suggesting that the need for dietary Gly+Ser is above the recommended level of 1.25% by NRC (1994). This proves that the NRC (1994) recommended Gly+Ser level might be inadequate to meet the needs of growing broiler chicks for maximum growth in low CP diet formulated with vegetable based ingredients. The result of our study also supported the report Ospina-Rojas *et al*. (2013a) who reported an improved AWG and FCR of birds fed low protein diet in a quadratic manner to increasing dieraty levels of Gly+Ser with optimization points of 2.12% and 2.08% respectively for broilers at 21 d of age.

Owing to the fact that Gly can be synthesized from Thr, interaction between the two AAs has been observed and quantified in previous feeding trials (Ospina-Rojas *et al*., 2013a;Siegert *et*

al. 2015a; Chrystal *et al*., 2020). The result of the current study shows that SGly and SID Thr interaction was significant for feed:gain. Higher levels of SGly resulted in a dercreasing linear effect on FCR for birds fed diets with 0.69% and 0.81% SID Thr. The lack of a plateau seen in the decreasing linear response of FCR at increasing SGly levels was an indication that the dietary need of total Gly+Ser might be greater than the highest level of Gly+Ser (2.32%) evaluated in this present study. This shows that higher Gly+Ser requirementis needed to optimize feed:gain in diet with deficient and adequate level of SID Thr. Nevertheless, a quadratic effect of SGly levels was observed on FCR for diets with higher SID Thr concentration (0.93%),with an optimumminimum point of 2.0% Gly+Ser. Among diets with 0.93% SID Thr, broilers fed 0.6% SGly (2.02% Gly+Ser) levels had better feed:gain than chicks given same diets with 1.0% SGly (2.32% Gly+Ser) and 0.2% SGly (1.72% Gly+Ser). Thus, in diets with lower CP and high SID Thr concentrations, marginal Gly supplementation can eliminate an impaired performance of the birds. Hence, the efficacy of total dietary Gly+Ser levelwas greater in diet below the adequate SID Thr concentration than when Thr supplementation resulted in excess dietary SID Thr level (Corzo *et al*., 2009). This was attributed to the catabolic action of Thr aldolase and dehydrogenase enzymes on surplus threonine into glycine, thus, an indication that the requirement of Gly is lower when Thr is sufficiently supplemented (Ospina-Rojas *et al*., 2013b).

Meanwhile, reducing dietary CP with suboptimal SGly levels, higher concentration of Thr (0.98%) is required to avoid any decrease in performance because Thr being a possible precursor of Gly would serve as a vital source of Gly synthesis (Corzo *et al*., 2009;Waldroup *et al*., 2005). This situation is more is likely to occur when formulating diets with all plant based sources especially typical corn-soybean based diets. According to Baker *et al*. (1972), sparing of Gly by Thr supplementation was more pronounced when dietary Thr was in moderate excess. This result coincides with the report of Ospina-Rojas*et al*. (2013b), which stated that glycine deficiency may lead to an increase in the threonine requirement. Thus, our result is in support with the findings of Chrystal *et al*. (2020), who suggested that increased Thr concentration reduces the dietary Gly+Ser requirementin broiler chicks to achieve optimum growth.

5.1.10 Serum biochemical indices

The SUA and SA concentrations of broilers fed low protein diet was observed to decreased in a consistent manner with increasing SGly (dietary Gly+Ser) levels at all levels of SID Thr. This demonstrates a strong indication of improvedN utilization when sufficient level of Gly is supplemeneted in starting broilers diet (Corzo *et al*., 2005; Ospina-Rojas *et al*., 2012; Awad *et al*., 2015). In the present study, diets with low Thr levels resulted in a linear effect on SUA and SA with higher level of Gly supplementation. This proves that efficacy of higher need of dietary Gly+Ser is confirmed in low protein diet with Thr deficiency. This is in support with the claims of earlier studies (Powell *et al*., 2009; Ospina-Rojas *et al*, 2012), which indicated that higher levels of Gly+Ser improves the utilization of all dietic EAA, due to its significant role in SUA synthesis. In this study, the SA level of birds receiving excess SID Thr was also observed to decreased numerically in a linear manner as Gly+Ser concentrations increased in the ration. This could be indication of decreased rate of AA oxidation in order to spare more protein for efficient retention (Powell *et al*., 2009).

According to Namroud *et al*. (2008), high amount of supplemental AAs in diet with reduced CP concentration was the primary cause of high levels of ammonia in the blood due to its high degree of absorption compared to intact protein. In birds, ammonia is very toxic to cells and is metabolically transform to uric acid molecules; hence, any process that would promote the reduction ofammonia concentration was seen as an efficient strategy to decreasing uric acid synthesis, reflecting improved AA utilization. A rise in Thr concentration decreases the need for dietary Gly+Ser to attain desired levels of response thus reducing the need for Gly to be used for UA synthesis through excess SID Thr (Chrystal *et al*., 2020). In low CP diet that is insufficient in Gly+Ser level, excess Thr can be metabolised for production of Gly, thereby decreasing its quantities in the blood and consequently, leading to other dietetic EAA being in supplus to be oxidized as well as increase nitrogen excretion as UA (Hofmann *et al.,* 2019). This is corroborated by the report of Akinde (2014a) that high N intake causes ineffective Thr-to-Gly catabolism as metabolic cost of N reduction.

5.1.11 Relative breast yield and pectoral muscle creatine

The relative breast weight of the birds improved in a linear manner in response to higher SGly levels. The absence of plateau observed in the linear regression between relative breast weight and increasing dietary Gly+Ser reveals that the dietary Gly+Ser need is higher for protein accretion at their early phase of broiler's growth when offered low CP ration. Our result confirms the recent findings of Ospina-Rojas *et al*. (2013b), who also observed an

increasing linear trend in relative breast weight of broilers at 21 d of age to increasing level of dietary Gly+Ser. The authors explained that the increase in breast meat weight might be as a result of the increase in the concentration of the pectorial muscle creatine in response to supplemental Gly. According to previous reports, higher creatine concentration in the breast muscles can increase nutrient utilization for muscle development (Wyss and Kaddurah-Daouk, 2000; Mitchiels *et al*., 2012; Dilger *et al*., 2013). Hence, our study has shown that dietary Gly+Ser higher than adequate level could permit for the use of low CP in diet of starter broilers without undermining protein accretion in the breast muscles which was evidenced by increased pectoralmuscle Cre and relative breast weight obtained in this present experiment.

Increasing SGly levels in the low protein diet of broiler chicks showed a quadratic relationship for pectoral musclescreatine, with an optimal level of 2.16% dietary Gly+Ser estimated to support maximum muscle creatine. This observation from the present result is in close agreement with the finding of Ospina-Rojas *et al*. (2013a), who reported that the requirement of dietary Gly+Ser appears to be higher for Cre synthesis for optimum muscle growth in broilers at this early growth stage. The Cre is a constituent of central importance in the energy metabolism particularly of muscle cells and assists in maintaining energy homeostasis in muscles (James *et al*., 2002; Brosnan *et al*., 2009). Cre helps improve the energy balance in cells and tissues by absorbing high-energy phosphate groups from adenosine triphosphate (ATP) to produce phosphocreatine (PCr) and then removing lower-energy phosphate groups to release ATP when energy need is high (Wyss and Kaddurah-Daouk, 2000). In our study, higher dietary Gly+Ser increased concentration of muscle Cre and because Cre is used to retain energy homeostasis in the muscle, this provides evidence that muscles of birds fed Gly adequate diet were proneto increased muscle performance. Since the endogenous production of Gly is inadequate to satisfy its high metabolic demands and creatine synthesis require a mole each of arginine and Gly with the subsequent incorporation of a methyl group (Bloch and Schoenheimer, 1940; Du Vigneaud *et al*., 1941), consequently, a considerable quantity of SGly would be needed for rapid creatine formation in the muscles.

5.1.12 Lipid oxidative stability

An interaction between SGly and SID Thr levels was significant on lipid oxidative stability and this revealed that birds fed 0.69 and 0.81 SID Thr diets with 1.0% supplemental Gly

(2.32% Gly+Ser) have lower amounts of malondialdehyde. This is an indication that increasing levels of supplemental Gly favourably influenced the oxidative capacity of the birds consuming poor protein rations containing 85 and 100% Thr requirements. Malondialdehyde is produced as an end result of lipid oxidative metabolism and therefore the degree of lipid peroxidation ROS can be measured by the TBARS level as an essential lipid peroxidation marker (Chen *et al*., 2012). Besides, glutathione (GSH; $_{\gamma}$-glutamyl-cysteinylglycine) is regarded to be the most sufficient endogenous intracellular antioxidant available in the animal and is synthesized from its precursor amino acids glycine, cysteine, and glutamic acid (Wu, 2013). The first and major contributor to limitation of GSH synthesis in birds fed low protein diet has been attributed to diminish Gly availability (Xie *et al*., 2017). Glycine deficit indicates a decrease of GSH production and thus influences the oxidative status of the animal and facilitates malondialdehyde prevalence. This defect is rapidly overcome with dietary supplementation with glycine, which increases its intracellular concentrations and that of GSH and also significantly lowers oxidative distress and injury (Senthilkumar *et al*., 2004; Nguyen *et al*., 2013). Thus, Gly works in antioxidative defence as a substrate in the biosynthesis of glutathione (Akinde, 2014a).

This current result proves that with the addition of supplemental Gly in adequate Thr, low protein diet made an influential impact to the antioxidant system of broiler chicks by increasing the intracellular concentration of GSH. This observation concurs with earlier findings presenting Gly asa potential antioxidant (Wu, 2013; Xie *et al*., 2017),as well as reducing oxidative stress in rats and broilers fed low crude protein diets (Takahashi*et al*., 2008). Glutathione protects cells from oxidative stress directly by scavenging reactive oxygen species (ROS) as free radicals and peroxides that are formed in metabolism and by using glutathione-dependent enzymes such as glutathione peroxidase (GPx) and glutathione S-transferase (GST) (Meister 1983; Cappiello *et al*. 2013). For this reason, it is an extremely important endogenous antioxidant and plays a key role in the antioxidant defence system (Enkvetchakul and Bottje, 1995; Aw, 2005).

Another explanation for lower TBARS values which reflects decreased lipid oxidation as observed by increasing the concentration of dietary Gly+Ser in adequate Thr concentration is linked to the ability of increased Gly supplementation to decrease the rate of Thr degradation, thereby increasing the amount of Thr in the diet for other useful reasons, such as production of intestinal protein and anti-oxidatant enzyme activities in broilers (Saleh *et al*., 2018; Ji *et al*., 2019). Liu *et al*. (2017) and Debnath *et al*. (2018) observed that the production of serum

GSH-PX and superoxide dismutase was greater when the Thr was incorporated above the recommended amount. Recent reports indicated that sufficient dietary Thr could significantly increase the development of broiler antioxidant enzymes (Azzam *et al*., 2012; Habte-Tsion*et al*., 2015; Ji *et al*., 2019). Therefore, maintaining sufficient dietary Gly supplementation to sustain Thr levels instead of decreasng its metabolic impact would improve the quality of broiler's meat by decreasing oxidative pressure on molecules of fats and protein; and improving the function of GPx,Superoxide dismutase(SOD) and catalase (Chrystal *et al.,* 2020). This is in accordance with the report of Azzam *et al*. (2019), that sufficient supply of dietary Thr promote antioxidative ability of laying hens. Moreover, it is also possible that higherSGlylevel could indirectly improves oxidative stability by preventing endogenous Thr degradation into Gly because of its role in antioxidative defence and stimulation of antibodies synthesis for increased immunity in broilers under oxdative stress (Azzam *et al*., 2012; Akinde, 2014a; Trevisi *et al*., 2015; Qaisrani *et al*., 2018). The present study has shown that maintaining adequate Thr concentration with higher supplemental Gly in diet of broilers have the potential to confer a greater protection against oxidation and to manage oxidative stress in the bird.

5.2 SUMMARY AND CONCLUSION

Responding to protein and amino acid requirements is one of the biggest expenses associated with feeding poultry, making the transition to low protein broiler diets become indispensable for curbing rapidly increasing feed and production costs. Increased supplementation of glycine to low crude protein diets can be considered as an important step towards combating environmental pollution concerns surrounding poultry production in recent times.This research was conducted to determine the optimal level of crystalline Gly supplementation and requirement of Gly+Serin LCPDwith varying Met and Thr levels for broiler chicks at 21-d old.

In experiment 1, reduction of CP concentrationin maize-soybean based diets from 22 to 18% without supplemental glycine resulted negatively on performance of broilers chicks. Glycine supplementation at 0.4% in 18% CP diet significantly improved body weight gain, feed conversion ratio, ileal amino acid digestibility and reduced abdominal fat deposition. Two other experiments were conducted to determine the total Gly + Ser requirement in a low CP,

corn-soybean diet containing different levels of methionine and threoninewith supplemental glycine. In experiment 2, the requirement of Gly+Ser in low crude protein diets (18% CP) containing deficient or adequate methionine concentrations is a minimum of 2.02% (1.79% Gly_{equi}) while maintaining an optimum level of 2.17% (1.94% Gly_{equi}) in methionine surfeit diet is necessary to support maximum performance of growing broilers at 21 days old. In experiment 3, an optimal level of 2.32% Gly+Ser(2.06% Gly_{equi}) concentration is required in diets containing deficient or adequate SID Thr concentration while a minimum requirement of 2.02%Gly+Ser(1.79% Gly_{equi}) in excess Thr dietsupported maximum growth of broiler chicks. Thus, the result of the last two experiments indicated that aminimum level of 0.8% supplemental glycine was capable of ameliorating the effects ofimbalance methionine or threonine levels on growth of broiler chicks fed LCPD.

Therefore, when feeding broilers on low CP based diets, there is need to supplement crystalline glycine to provide sufficient amount of dietary glycine+serine to achieve optimum growth performance and increase the efficiency of amino acid utilisation. The reduction in performance encountered in broilers fed low CP diets containing varying levels of methionine and threonine were improved with increased crystalline glycine addition. A possible solution to mitigate low growth efficiency of broilers fed a reduced CP ration should be via concurrent incorporations of limiting EAA and supplemental glycine, so as to retain sufficient concentrations of total Gly + Ser in the feed without compromising performance. Thus, such a feeding policy will help to strengthen the viability in production of birds as well as to minimize the cost implication and environmental pressure connected with the excretion of nitrogen.

5.3 RECOMMENDATION

Further research is needed to determine specifically how the broilers are utilizing glycine in these low CP diets, and to determine the effects of additions of crystalline glycine to low CP diets fed to broilers at other stages of growth.Other dietary approaches need to be established in the future in order to gain greater reduction in dietary CP that is below 18% at starter phase of broiler production. Also, it is important to determine the criteria for another limiting non-essential AA apart from glycine.

CONTRIBUTIONS TO KNOWLEDGE

- Glycine (or glycine+serine) becomes growth-limiting nutrient in growing broiler chickens at 1 – 21 days old when offered rations with crude protein concentration less than 20%, especially when formulating exclusively with all-vegetable based ingredients.

- The adoption and incorporation of supplemental glycine to supply sufficient concentration of dietary glycine+serine not less than 2.02% (1.79% Gly_{equi}) can enable considerable reduction in crude protein level to about 18% in ration of broiler chicken from 1 to 21 days without undesirable impacts on growth.

- Sufficient glycine fortification in low crude protein, vegetable based diets has the potential to sparing methionine in diets containing imbalance methionine concentrations for better protein accretion and performanceof growing broiler chicks.

- Supplementation of threonine higher than requirement can spare glycine/glycine+serine requirement in young broiler chickens fed LCPD with marginal glycine/glycine+serine levels.

- A minimum level of 0.8% supplemental glycineis necessaryto provided adequate dietary Gly+Ser concentration needed to support growth when formulating low crude protein diets with recommended methionine (0.5%) and threonine (0.8%) for young broilers.

- Sufficient glycine supplementation of broiler feed withadequate EAAs increases the oxidative stability of broiler carcasses.

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